UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

WASHINGTON, D.C. 20460

OFFICE OF
PREVENTION, PESTICIDES
AND TOXIC SUBSTANCES

MEMORANDUM

Date: 08/21/2006

Subject: Prothioconazole. Petition for Establishment of Tolerances for Use on Barley, Oilseed (Except Sunflower and Safflower) Crop Group, Dried Shelled Pea and Bean (Except Soybean) Crop Subgroup, Peanut, Rice, and Wheat. Summary of Analytical Chemistry and Residue Data. PP#4F6830

DP Numbers:	D303508 & D314517	Decision Number:	341716 & 341717
PC Code:	113961	MRID Numbers:	46246139, 46246141-46246150, 46246201-46246211, 46246213-46246227, 46477701-46477704
40 CFR 180.		Chemical Class:	Fungicide

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This document was originally prepared under contract by Dynamac Corporation (20440 Century Boulevard, Suite 100; Germantown, MD 20874; submitted 05/31/2005). The document has been reviewed by the Health Effects Division (HED) and revised to reflect current Office of Pesticide Programs (OPP) policies.

Executive Summary

Bayer CropScience has proposed, in PP#4F6830, 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. 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 animal commodities:

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

Prothioconazole is a systemic demethylation inhibitor fungicide (Group 3 fungicide) of the triazolinthione chemical class. Prothioconazole has demonstrated protective, curative, and

TABLE C.1.1 Recovery Results from Method Validation of Various Plant Matrices Using the Data-Gathering Analytical Method.[1]

Matrix	Analyte	Spiking Level (ppm)	Sample size (n)	Recoveries Obtained (%)	Mean ± SD [CV] (%)
		0.2	3	91, 91, 92	91 ± 1 [1]
Dried pea and bean crop field trial					
Dried shelled bean	1*H*-1,2,4-triazole	0.04	2	85, 97	91
	Triazolylalanine	0.24	3	79, 79, 90	83 ± 6 [8]
	Triazolylacetic acid	0.08	3	70, 71, 86	76 ± 9 [12]
Dried shelled pea	1*H*-1,2,4-triazole	0.05	6	70, 73, 92, 93, 102, 119	92 ± 18 [20]
		0.50	2	78, 82	80
	Triazolylalanine	0.25	5	80, 84, 85, 86, 87	84 ± 3 [3]
		0.50	2	86, 89	88
	Triazolylacetic acid	0.25	5	70, 71, 72, 72, 82	73 ± 5 [7]
		0.50	2	74, 77	76
Peanut crop field trial					
Peanut nutmeat	1*H*-1,2,4-triazole	0.02	2	86, 95	91
		0.05	3	83, 92, 92	89 ± 5 [6]
		0.50	1	80	NA
	Triazolylalanine	0.50	2	91, 93	92
	Triazolylacetic acid	0.10	2	85, 90	88
		0.50	1	71	NA
Peanut hay	1*H*-1,2,4-triazole	0.02	2	88, 99	98
		0.50	1	100	NA
	Triazolylalanine	0.03	2	73, 77	75
		0.50	2	88, 89	89
	Triazolylacetic acid	0.10	2	71, 89	80
		0.50	1	97	NA
Rice crop field trial					
Rice grain	1*H*-1,2,4-triazole	0.02	1	87	NA
		0.10	3	81, 90, 93	88 ± 6 [7]
	Triazolylalanine	0.10	3	71, 76, 87	78 ± 8 [10]
		0.50	1	88	NA
	Triazolylacetic acid	0.10	3	72, 72, 74	73 ± 1 [2]
		0.50	1	78	NA
Rice straw	1*H*-1,2,4-triazole	0.01	1	71	NA
		0.10	3	76, 80, 91	82 ± 8 [9]
	Triazolylalanine	0.10	4	75, 91, 97, 97	90 ± 10 [12]
	Triazolylacetic acid	0.10	4	76, 76, 77, 91	80 ± 7 [9]

eradicative action against plant diseases caused by ascomycetes, basidiomycetes, and deuteromycetes fungi in many crops, and is intended to be used for the control of fusarium head blight and reduction of deoxynivalenol levels in barley and wheat.

This chemical has been submitted for joint EPA and PMRA review by Bayer CropScience. The crops proposed for joint review include barley, the oilseed crop group, the dried shell and bean subgroup, and wheat. Uses on peanuts and rice are being proposed for the U.S. only.

In conjunction with the subject tolerance petition, Bayer CropScience has submitted an application for Section 3 registration of a 4 lb a.i./gal suspension concentrate (equivalent to a flowable concentrate; FlC) formulation (Proline® 480 SC Fungicide; EPA File Symbol No. 264-IEL). The product is to be applied as broadcast postemergence foliar or soil sprays (application to soil for peanuts only) using ground or aerial equipment at 0.088-0.178 lb ai/A/application (0.100-0.200 kg ai/ha/application). The proposed maximum seasonal rates range 0.285-0.713 lb ai/A (0.320-0.800 kg ai/ha), and the proposed retreatment intervals are 5-21 days. The proposed PHIs range from 7 days for dried shelled peas and beans to 40 days for rice.

The available data from metabolism studies with wheat, peanut, and sugar beet indicate that metabolism of prothioconazole is similar in dissimilar crops. Prothioconazole was not found to be a major component of the residue in plant commodities, at 1.0-7.4% of the total radioactive residues (TRRs) in wheat matrices, peanut hay, and sugar beet tops; prothioconazole was not identified in peanut nutmeat or sugar beet root. Prothioconazole desthio was a major component of the residue, at 9.3-35% of the TRRs in wheat matrices, 24-28% of the TRRs in peanut hay, 6.2% of the TRRs in peanut nutmeat, and 19-58% of the TRRs in sugar beet tops and root. In triazole-label studies, triazolylalanine accounted for 71% of the TRRs in wheat grain, 4.1-25% of the TRRs in wheat forage, hay, and straw, 50% of the TRRs in peanut nutmeat, 29% of the TRRs in sugar beet root, and <2% of the TRRs in peanut hay and sugar beet tops. Triazolylacetic acid accounted for 19% of the TRRs in wheat grain, <5% of the TRRs in wheat forage, hay, and straw, and peanut nutmeat and hay, and was not identified in sugar beet root or tops. Free triazole was not identified in any plant matrix. Based on the results of the confined rotational crop studies, metabolism in rotational crops was similar to that in peanut, sugar beet, and wheat.

The residues of concern for tolerance enforcement and for risk assessment in plant commodities are defined as the sum of prothioconazole and its metabolite prothioconazole desthio, calculated as prothioconazole. Additionally, the contribution of triazole, triazolylalanine (TA), and triazolylacetic acid (TAA) from the use of prothioconazole to the aggregate exposure for human-health risk assessment has been considered (DP322215, 02/07/2006).

The available data indicate that the metabolism of prothioconazole is similar in goats and hens. Prothioconazole was found to be a major residue in liver, kidney (goat only) and fat, at 11-31% of the TRRs and was identified in muscle at 2.5-13% of the TRRs; prothioconazole was found at lower levels in milk and egg (<4% of the TRRs). Desthio-Prothioconazole was a major metabolite in fat and egg, at 15-29% of the TRRs, but was found at lower levels in other tissues and milk (<8% of the TRRs). 4-Hydroxy prothioconazole was found at ~11% of the TRRs in goat liver and at <8.5% of the TRRs in other goat matrices and in hen liver and muscle. Two co-eluting metabolites, JAU6476-O- or S-glucuronide and JAU6476-3-hydroxy-desthio, were found

to be major metabolites, at ~34% of the TRRs in goat kidney and 4.4%-23.7% of the TRRs in goat milk and tissues and hen matrices. In triazole-label studies, 1,2,4-triazole accounted for a significant portion of radioactivity in egg (11% of the TRRs) and hen muscle (19% of the TRRs); 1,2,4-triazole was found at lower levels in hen liver and fat (<2% of the TRRs) but was not detected in goat matrices. Thiocyanate was found to account for a major portion of radioactivity in milk and goat kidney, muscle, and fat, at 9.0-41% of the TRRs; thiocyanate was found at lower levels in goat liver (~2% of the TRRs) and hen matrices (<10% of the TRRs). JAU6476-triazolyl-ethanol was a major metabolite in egg (16% of the TRRs) and hen muscle (28% of the TRRs), was found at lower levels in hen liver and fat (<4% of the TRRs), and was not detected in goat matrices. Additional metabolites found at significant levels were JAU6476-S-methyl, at 20-28% of the TRRs in hen fat (found at <7% of the TRRs in hen matrices and <1% of the TRRs in goat liver), and JAU6476-hydroxy-glucuronide, at 11% of the TRRs in goat fat (<7% of the TRRs in other goat matrices and in hen liver).

The residue of concern for tolerance enforcement in livestock commodities is defined as the sum of prothioconazole, prothioconazole desthio, and conjugates that are converted to prothioconazole or prothioconazole desthio via acid hydrolysis, calculated as prothioconazole. The residue of concern for risk assessment for livestock commodities is defined as the sum of prothioconazole, prothioconazole desthio, 4-hydroxy prothioconazole and conjugates that are converted to prothioconazole or prothioconazole desthio or 4-hydroxy prothioconazole via acid hydrolysis, calculated as prothioconazole. Additionally, for livestock commodities, aggregate exposure from triazole and the triazole derivatives has been considered (DP322215, 02/07/2006). Prothioconazole was included in that assessment.

Crop field trial data have been submitted reflecting the proposed use pattern for the 4 lb a.i./gal FlC formulation; however, confirmatory storage stability data are required to support all crop field trials. Adequate processing data have been submitted for canola, peanut, rice, and wheat, which indicate that a tolerance is needed for rice hulls; additional storage stability data are required to support the processing studies. Adequate cattle feeding studies with prothioconazole have been submitted; a poultry feeding study was not submitted, but based on the results of poultry metabolism study no residues are anticipated in poultry commodities for the currently proposed uses, except poultry liver (which is at the LOQ). A poultry feeding study is required to confirm these findings.

The available rotational crop data indicate that the proposed rotational crop restrictions (30 day PBI) are appropriate; no rotational crop tolerances are needed to support this petition. However, finite residues of triazole and triazole derivatives are found at the proposed PBI. Triazole and triazole derivatives in rotational crops have been considered as part of the aggregate exposure issue (DP322215, 02/07/2006).

There are currently no U.S., Canadian, Mexican, or international Codex tolerances established for prothioconazole.

The petitioner included residue data for 1,2,4-triazole and triazole conjugates triazolylalanine and triazolylacetic acid with the crop field trial, processing, and field rotational crop studies submitted with this petition. The data indicate that quantifiable residues of the triazole

conjugates will occur in primary crop, processed, and field rotational crop commodities following treatment of primary crops with prothioconazole. Radiovalidation data for the method used to collect triazole and triazole conjugates data and completion of the ongoing storage stability study with these compounds are needed to support the residue data.

Regulatory Recommendations and Residue Chemistry Deficiencies

HED has examined the residue chemistry database for the new active ingredient prothioconazole. Pending resolution of the deficiencies noted below, there are no residue chemistry issues that would preclude granting a conditional registration for this fungicide or establishment of tolerances for prothioconazole as follows:

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 prothioconazole desthio [α-(1-chlorocyclopropyl)-α-[(2-chlorophenyl)methyl]-1H-1,2,4- triazole-1-ethanol], calculated as prothioconazole :

Barley, grain	0.35	ppm
Barley, hay	7.0	ppm
Barley, straw	4.0	ppm
Grain, aspirated grain fractions	11	ppm
Pea and bean, dried shelled, except soybean, subgroup 6C	0.90	ppm
Peanut	0.02	ppm
Peanut, hay	6.0	ppm
Rapeseed, seed	0.15	ppm
Rice, grain	0.20	ppm
Rice, straw	1.4	ppm
Rice, hulls	0.90	ppm
Wheat, grain	0.07	ppm
Wheat, forage	6.0	ppm
Wheat, hay	4.5	ppm
Wheat, straw	5.0	ppm

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 prothioconazole desthio [α-(1-chlorocyclopropyl)-α-[(2-chlorophenyl)methyl]-1*H*-1,2,4-triazole-1-ethanol] and conjugates convertible to these two compounds by acid hydrolysis, calculated as prothioconazole

Cattle, fat	0.10	ppm
Cattle, meat	0.02	ppm
Cattle, meat byproducts	0.20	ppm
Goat, fat	0.10	ppm
Goat, meat	0.02	ppm
Goat, meat byproducts	0.20	ppm
Hog, meat byproducts	0.05	ppm
Horse, fat	0.10	ppm
Horse, meat	0.02	ppm
Horse, meat byproducts	0.20	ppm
Milk	0.02	ppm
Poultry, liver	0.02	ppm
Sheep, fat	0.10	ppm
Sheep, meat	0.02	ppm
Sheep, meat byproducts	0.20	ppm

A human health risk assessment will be conducted and described in a separate document.

The following deficiencies in the studies submitted in support of the establishment of tolerances for prothioconazole must be successfully addressed:

The available storage *stability data* are tentatively adequate to support the storage intervals and conditions of samples from the submitted crop field trial, processing, and field rotational crop studies. The final reports of the ongoing storage stability studies with prothioconazole and prothioconazole desthio (interim results for which were reported in MRID 46477701) must be submitted as confirmatory data.

The available poultry metabolism study and analytical method for ruminant commodities are tentatively adequate to establish tolerances for poultry commodities. A *poultry feeding study* and fully validated *analytical method* for poultry commodities are required as conditions of the registration of prothioconazole.

The proposed *enforcement analytical method* for livestock commodities must undergo successful method validation at EPA.

860.1200 Directions for Use

- The applicant has proposed use on an "Oilseed Crop Subgroup" which consists of the members of the Oilseed Crop Group 20 with the exception of safflower seed and sunflower seed. The representative crops of Crop Group 20 are canola and sunflower. Currently, no crop subgroups have been defined by HED for Crop Group 20. The applicant has submitted crop field trial data for canola but not for sunflower. In the absence of crop field trial data for sunflower, the applicant must modify the use directions to remove reference to the Oilseed Crop Subgroup and to delete the following commodities from the label: Indian mustard (Brassica juncea); black mustard (Brassica nigra); flax (Linum usitatissimum); and borage (Borago officinalis).

- The retreatment intervals proposed by the applicant are not in agreement with the retreatment intervals used in the crop field trials for several crops, and the applicant did not propose a retreatment interval for rice. For barley, rice, wheat, and canola and the oilseed crops of rapeseed, Indian rapeseed, field mustard seed, and crambe, the applicant must propose a minimum retreatment interval of 14 days.

- Although the label specifies use of a spray adjuvant for all uses except soil application to peanuts, the only crops for which surfactants were used in the field trials were those in the dried pea/bean crop subgroup. In the absence of data supporting their use, the label must be modified to remove the recommendation regarding spray adjuvants for all crops except chickpea, lentils, and the dried shelled peas and beans subgroup.

- We note that the use directions for barley and wheat specify that the maximum single application rate is 0.178 lb ai/A (200 g ai/ha) and that a maximum of two applications may be made. The maximum seasonal application rate for barley and wheat is 0.293 lb

ai/A (328 g ai/ha) which is less than two times the maximum single application rate. For wheat and barley, the applicant may wish to note on the product label that the maximum seasonal rate would be exceeded if two applications were made at the maximum single application rate.

860.1340 Residue Analytical Methods

- The proposed data collection and enforcement methods for livestock commodities must be validated for poultry commodities.

860.1380 Storage Stability

- The final report of the ongoing storage stability study with prothioconazole and desthio-prothioconazole in plant commodities (interim results for which were reported in MRID 46477701) must be submitted as confirmatory data.

- To support the reported results for 1,2,4-triazole and the triazole conjugates, the final report of the ongoing storage stability study with triazole and triazole conjugates in plant commodities (interim results of which were reported in MRID 46246211) must be submitted.

860.1480 Meat, Milk, Poultry, and Eggs

- The applicant must submit a poultry feeding study with prothioconazole.

860.1650 Submittal of Analytical Reference Standards

- Based on the proposed tolerance expressions and the proposed enforcement methods, analytical reference standards of the following compounds must be supplied and supplies replenished as requested by the Repository:
  - destihio prothioconazole [JAU6476-desthio; (2-(1-chlorocyclopropyl)-1-(2-chlorophenyl)-3-(1*H*-1,2,4-triazol-1-yl)-2-propanol)]
  - prothioconazole sulfonic acid potassium salt [potassium salt of JAU6476 sulfonic acid; 1-[2-(1-chlorocyclopropyl)-3-(2-chlorophenyl)-2-hydroxypropyl]1*H*-1,2,4-triazole sulfonic acid, potassium salt]
  - [triazole-^{15}N-^{13}C]prothioconazole
  - [triazole-^{15}N-^{13}C]JAU6476-desthio
  - [triazole-^{15}N-^{13}C]JAU6476 sulfonic acid

860.1650 Proposed Tolerances

- The proposed tolerance expression for plant commodities should be revised to be calculated in terms of the "combined residues of the fungicide prothioconazole [2-[2-(1-

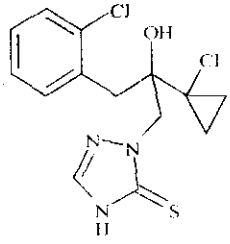
chlorocyclopropyl)-3-(2-chlorophenyl)-2-hydroxypropyl]-1,2-dihydro-3*H*-1,2,4-triazole-3-thione] and prothioconazole desthio [α-(1-chlorocyclopropyl)-α-[(2-chlorophenyl)methyl]-1H-1,2,4-triazole-1-ethanol], calculated as prothioconazole."

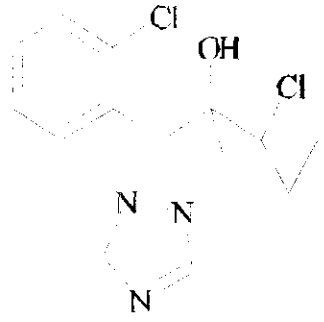
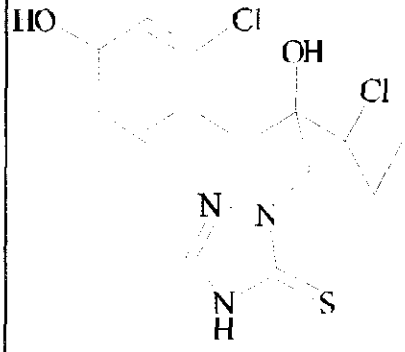
- The proposed tolerance expression for livestock commodities should be revised to be calculated in terms of the "combined residues of the fungicide prothioconazole [2-[2-(1-chlorocyclopropyl)-3-(2-chlorophenyl)-2-hydroxypropyl]-1,2-dihydro-3*H*-1,2,4-triazole-3-thione] and prothioconazole desthio [α-(1-chlorocyclopropyl)-α -[(2-chlorophenyl)methyl]-1*H*-1,2,4-triazole-1-ethanol] and conjugates convertible to these two compounds by acid hydrolysis, calculated as prothioconazole."

- The proposed tolerances should be revised to reflect the correct commodity definitions as specified in Table 9. In addition, revisions to the proposed tolerance levels for certain commodities and deletion of certain tolerances are required, as specified in Table 9.

Background

The subject petition, PP#4F6830, represents the first food/feed uses of prothioconazole proposed in the U.S. or Canada. The chemical structure and nomenclature of prothioconazole and its metabolites to be regulated and the physicochemical properties of prothioconazole are presented in the tables below. The chemical names and structures of prothioconazole and all transformation products identified in plant and livestock commodities are presented in Appendix 1.

Prothioconazole Nomenclature	
Chemical structure	
Common name	Prothioconazole
Company experimental name	JAU6476
IUPAC name	2-[(2RS)-2-(1-chlorocyclopropyl)-3-(2-chlorophenyl)-2-hydroxypropyl]-2H-1,2,4-triazole-3(4H)-thione
CAS name	2-[2-(1-chlorocyclopropyl)-3-(2-chlorophenyl)-2-hydroxypropyl]-1,2-dihydro-3*H*-1,2,4-triazole-3-thione
CAS registry number	178928-70-6
PC Code	113961
End-use product (EP)	Proline® 480 SC (4 lb/gal suspension concentrate)

Prothioconazole Nomenclature	
Chemical structure of prothioconazole desthio	 α-(1-chlorocyclopropyl)-α-[(2-chlorophenyl)methyl]-1*H*-1,2,4-triazole-1-ethanol
Chemical structure of 4-*hydroxy prothioconazole*	 2-[2-(1-chlorocyclopropyl)-3-(2-chloro-4-hydroxyphenyl)-2-hydroxypropyl]-1,2-dihydro-3*H*-1,2,4-triazole-3-thione

Physicochemical Properties of Prothioconazole		
Parameter	Value	Reference
Melting point/range	139.1 to 144.5°C	MRID 46246003
pH	5.8	MRID 46246003
Density	1.36 g/mL	MRID 46246003
Water solubility	mg/L (20°C) pH 4 5 pH 8 300 pH 9 2000	MRID 46246003
Solvent solubility	g/L at RT Acetone >250 Acetonitrile 69 Dichloromethane 88 Dimethylsulfoxide 126 Ethyl acetate >250 n-Heptane <0.1 1-Octanol 58 Polyethylene glycol >250 2-Propanol 87 Xylene 8	MRID 46246003

Physicochemical Properties of Prothioconazole		
Parameter	Value	Reference
Vapor pressure	<<4 x 10^{-7} Pa at 20 or 25°C (calculated from determinations at 70°C)	MRID 46246003
Dissociation constant, pK_a	6.9 (calculated from K_{OW})	MRID 46246003
Octanol/water partition coefficient, Log(K_{OW}) at 20°C	unbuffered water 4.05 pH 4 4.16 pH 7 3.82 pH 9 2.00	MRID 46246003
UV/visible absorption spectrum	Peak maximum at 257 nm	MRID 46246003

860.1200 Directions for Use

Use directions for the U.S. label (EPA File Symbol 264-IEL) are from a draft label dated 3/31/04 for the 4 lb/gal suspension concentrate formulation (equivalent to an FlC formulation), and are summarized in Table 2.

Table 1. Summary of End-Use Products.

Trade Name	Reg. No.	ai (% of formulation)	Formulation Type	Target Crops	Target Pests	Label Date
U.S. Label						
Proline® 480 SC Fungicide	264-IEL	4 lb/gal (41%)	Suspension concentrate	Barley; canola; chickpea; dried shelled peas and beans subgroup; lentils; oilseed crop subgroup; peanut; rice; wheat	Ascomycetes, basidiomycetes, and deuteromycetes diseases	3/31/04 (draft)

Table 2. Summary of Directions for Use of Prothioconazole (U.S. Label).

Trade Name	Applic. Timing, Type, and Equip.	Applic. Rate (lb ai/A) [g ai/ha]	Max. No. Applic. per Season	Max. Seasonal Applic. Rate (lb ai/A) [g ai/ha]	PHI (days)	Use Directions and Limitations
Barley						
Proline® 480 SC Fungicide	Postemergence Broadcast foliar Ground or aerial	0.134-0.178 [150-200]	2	0.293 [328]	32	Apply during Feekes stages 10.3-10.5 (70-100% heads on the main stem fully emerged); applications may be made up to Feekes stage 10.52 (heads in full flower). A 7- to 14-day retreatment interval is proposed. Use of a spray surfactant is recommended.
Proline® 480 SC Fungicide	Postemergence Broadcast foliar Ground or aerial	0.088-0.134 [100-150]	2		32	Apply when earliest disease symptoms appear on leaves or stems. A 7- to 14-day retreatment interval is proposed. Use of a spray surfactant is recommended.
Canola						

Table 2. Summary of Directions for Use of Prothioconazole (U.S. Label).

Trade Name	Applic. Timing, Type, and Equip.	Applic. Rate (lb ai/A) [g ai/ha]	Max. No. Applic. per Season	Max. Seasonal Applic. Rate (lb ai/A) [g ai/ha]	PHI (days)	Use Directions and Limitations
Proline® 480 SC Fungicide	Postemergence Broadcast foliar Ground or aerial	0.134-0.178 [150-200]	2	0.356 [400]	36	Apply during the 20% up to the 50% bloom stage. A 5- to 7-day retreatment interval is proposed. Use of a spray surfactant is recommended.
Chickpea and Lentils						
Proline® 480 SC Fungicide	Postemergence Broadcast foliar Ground or aerial	0.134-0.178 [150-200]	3	0.534 [600]	7	Apply at early flower (lentil) or first sign of disease. A 10- to 14-day retreatment interval is proposed. Use of a spray surfactant is recommended.
Dried Shelled Peas and Beans Subgroup : *Lupinius* spp. (grain, sweet, white, and white sweet lupin); *Phaseolus* spp. (field, kidney, dry lima, navy, pinto, and tepary beans); *Vigna* spp. (adzuki bean, blackeyed pea, catjang, cowpea, Crowder pea, moth bean, mung bean, rice bean, southern pea, and urd bean); dry broad bean; guar; lablab bean; and *Pisum* spp. (pea (including field pea), and pigeon pea)						
Proline® 480 SC Fungicide	Postemergence Broadcast foliar Ground or aerial	0.134-0.178 [150-200]	3	0.534 [600]	7	Apply at first sign of disease. A 5- to 14-day retreatment interval is proposed. Use of a spray surfactant is recommended.
Oilseed Crop Subgroup: Rapeseed (canola varieties only - see specific instructions above for canola); rapeseed (*Brassica napus* and *Brassica rapa*); Indian rapeseed (*Brassica rapa*); Indian mustard (*Brassica juncea*); field mustard (*Brassica rapa*); black mustard (*Brassica nigra*); flax (*Linum usitatissimum*); crambe (*Crambe abyssinica*); and borage (*Borago officinalis*)						
Proline® 480 SC Fungicide	Postemergence Broadcast foliar Ground or aerial	0.134-0.178 [150-200]	2	0.356 [400]	36	Apply during the 20% up to the 50% bloom stage. A 5- to 7-day retreatment interval is proposed. Use of a spray surfactant is recommended.
Peanut						
Proline® 480 SC Fungicide	Postemergence Broadcast soil Ground or aerial	0.178 [200]	4	0.713 [800]	14	Apply as 4 consecutive applications with a 14-day retreatment interval. The feeding of hay or thrashings or grazing of livestock in treated areas is prohibited.
Proline® 480 SC Fungicide	Postemergence Broadcast foliar Ground or aerial	0.156-0.178 [175-200]	4	0.713 [800]	14	A 14- to 21-day retreatment interval is proposed. Use of a spray surfactant is recommended. The feeding of hay or thrashings or grazing of livestock in treated areas is prohibited.
Rice						

Table 2. Summary of Directions for Use of Prothioconazole (U.S. Label).

Trade Name	Applic. Timing, Type, and Equip.	Applic. Rate (lb ai/A) [g ai/ha]	Max. No. Applic. per Season	Max. Seasonal Applic. Rate (lb ai/A) [g ai/ha]	PHI (days)	Use Directions and Limitations
Proline® 480 SC Fungicide	Postemergence Broadcast foliar Ground or aerial	0.143 [160]	2	0.285 [320]	40	Apply at first sign of disease; usually from panicle differentiation to late boot. A second application may be made up to 70% panicle emergence from the boot. A retreatment interval is not specified. Use of a spray surfactant is recommended.
Wheat						
Proline® 480 SC Fungicide	Postemergence Broadcast foliar Ground or aerial	0.134-0.178 [150-200]	2	0.293 [328]	30	Apply during Feekes stages 10.4-10.52 (at least 75% of wheat heads on main stem fully emerged to when 50% of heads on main stem in flower); applications may be made up to Feekes stage 10.52 (heads in full flower). A 7- to 14-day retreatment interval is proposed. Use of a spray surfactant is recommended.
Proline® 480 SC Fungicide	Postemergence Broadcast foliar Ground or aerial	0.134-0.156 [150-175]	2		30	Apply when earliest disease symptoms appear on leaves or stems. A 7- to 14-day retreatment interval is proposed. Use of a spray surfactant is recommended.

The general use directions for the label specify that ground applications are to be made in a minimum of 10 gal/A, and aerial applications are to be made in a minimum of 5 gal/A. For all uses except soil application in peanuts, the label specifies that the lowest labeled rate of a non-ionic spray surfactant should be tank-mixed with the product. For soil application in peanuts, the label notes that the product must be carried by rainfall or irrigation into the root and pod zone of the plants. Application through any type of irrigation system is prohibited. A restricted entry interval of 24 hours is specified.

The label provides mixing procedures for tank mixes, and notes that the product is compatible with most insecticide, fungicide, herbicide, and foliar nutrient products. It states that physical compatibility of product with tank-mix partners should be tested using a jar test before use.

The following rotational crop restrictions are proposed: crops listed on the label may be planted as soon as practical after last application; all other crops may be planted 30 days following last application.

Conclusions. The proposed U.S. label is adequate to allow evaluation of the residue data submitted in support of this petition. Several label amendments are required.

The applicant has proposed use on an "Oilseed Crop Subgroup" which consists of the members of the Oilseed Crop Group 20 with the exception of safflower seed and sunflower seed. The representative crops of Crop Group 20 are canola and sunflower. Currently, no crop subgroups have been defined by HED for Crop Group 20. The applicant has submitted crop field trial data for canola but not for sunflower. In the absence of crop field trial data for sunflower, the applicant must modify the use directions to remove reference to the Oilseed Crop Subgroup and to delete the following commodities from the label: Indian mustard (Brassica juncea); black mustard (Brassica nigra); flax (Linum usitatissimum); and borage (Borago officinalis).

The retreatment intervals proposed by the applicant are not in agreement with the retreatment intervals used in the crop field trials for several crops. For barley, wheat, and canola and the oilseed crops of rapeseed, Indian rapeseed, field mustard seed, and crambe, the applicant must propose a minimum retreatment interval of 14 days. The proposed retreatment intervals for the other crops are supported by the crop field trial data. The applicant did not propose a retreatment interval for rice. The rice crop field trials reflected an application pattern in which the first application was made at panicle differentiation, and the second application was made approximately 14 days later; therefore, the applicant should propose a 14-day retreatment interval for rice.

Although the label specifies use of a spray adjuvant for all uses except soil application to peanuts, the only crops for which surfactants were used in the field trials were those in the dried pea/bean crop subgroup. In the absence of data supporting their use, the label must be modified to remove the recommendation regarding spray adjuvants for all crops except chickpea, lentils, and the dried shelled peas and beans subgroup.

The barley and wheat crop field trials reflect a maximum seasonal rate of 0.293 lb ai/A. HED notes that the use directions for barley and wheat specify that the maximum single application rate is 0.178 lb ai/A (200 g ai/ha) and that a maximum of two applications may be made. The maximum seasonal application rate for barley and wheat is 0.293 lb ai/A (328 g ai/ha) which is less than two times the maximum single application rate. For wheat and barley, the applicant may wish to note on the product label that the maximum seasonal rate would be exceeded if two applications were made at the maximum single application rate.

860.1300 Nature of the Residue - Plants

46246141.der.wpd (Wheat)
46246142.der.wpd (Wheat)
46246143.der.wpd (Wheat)
46246145.der.wpd (Peanut)
46246146.der.wpd (Peanut)
46246147.der.wpd (Sugar beet)
46246148.der.wpd (Sugar beet)

Bayer submitted eight plant metabolism studies to support the proposed uses: (1) a wheat metabolism study reflecting foliar application of [phenyl-^{14}C]-prothioconazole (MRID

46246141); (2) a wheat metabolism study reflecting seed treatment with [phenyl-^{14}C]-prothioconazole (MRID 46246142); (3) a wheat metabolism study reflecting foliar application of [triazole-^{14}C]-prothioconazole (MRID 46246143); (4) a peanut metabolism study reflecting foliar application of [phenyl-^{14}C]-prothioconazole (MRID 46246145); (5) a peanut metabolism study reflecting foliar application of [triazole-^{14}C]-prothioconazole (MRID 46246146); (6) a sugar beet metabolism study reflecting foliar application of [triazole-^{14}C]-prothioconazole (MRID 46246147); (7) a sugar beet metabolism study reflecting foliar application of [phenyl-^{14}C]-prothioconazole (MRID 46246148); and (8) a wheat metabolism study reflecting application of [triazole-^{14}C]-prothioconazole desthio (MRID 46246144), which was not reviewed because it is not useful to support the registration of prothioconazole. Chemical names and structures of prothioconazole and the metabolites identified in the plant metabolism studies are presented in Appendix I.

Wheat - MRID 46246141/46246143:

Phenyl-Label

Bayer CropScience has submitted studies investigating the metabolism of [phenyl-UL-^{14}C]-prothioconazole (specific activity 27.6 mCi/mmol) in wheat as a foliar treatment. The radiolabeled test substances were formulated as emulsifiable concentrate (EC) formulations and applied as two foliar broadcast sprays to wheat plants grown outdoors in planting containers at the beginning of tillering (BBCH 32) and at full flowering (BBCH 65). Applications were made at 0.193 lb a.i./A (216 g a.i./ha) and 0.178 lb a.i./A (199 g a.i./ha), with a 17-day re-treatment interval, for a total seasonal application of 0.371 lb a.i./A (415 g a.i./ha). Forage and hay were harvested 6 and 26 days, respectively, and grain and straw were harvested 48 days following the second application.

The TRRs were 10.45 ppm (forage), 8.90 ppm (hay), 26.74 ppm (straw), and 0.08 ppm (grain) following foliar application of [phenyl-UL-^{14}C]-prothioconazole.
Extraction with organic solvents released the majority of the radioactivity (~61-85% of the TRRs) in all wheat matrices. Cysteine HCl was added to all extracting solvents and to extracts during concentration procedures to prevent oxidative decomposition of prothioconazole. Accelerated solvent extraction (ASE) released an additional ~5-13% of the TRRs (0.02-2.57 ppm) from all matrices. Acid hydrolysis with HCl:dioxane released ~7-8% of the TRRs (0.64-2.18 ppm) in hay and straw. Enzyme hydrolysis of the grain with diastase released an additional ~15% of the TRRs (0.01 ppm). Non-extractable residues remaining following extraction/hydrolysis accounted for <4% of the TRRs (<0.83 ppm) in forage, hay, and straw; and accounted for 18% of the TRRs (0.013 ppm) in grain. Accountabilities were approximately 99-100% for all matrices. Residues were identified primarily by LC-MS, LC-MS/MS, and/or NMR spectroscopy with confirmatory analysis by HPLC and/or TLC co-chromatography. These methods successfully identified the predominant residues in wheat forage, hay, straw, and grain. The stability of prothioconazole and the major metabolite JAU6476-dethio were demonstrated via the co-chromatography of initial (12 – 57 days after harvest) commodity extracts and extracts from crop samples stored frozen for 15 – 18 mo. The stability of minor metabolites was not demonstrated.

Approximately 67-73% of the TRRs were identified in all wheat matrices except in grain where only 34% of the TRRs were identified. Prothioconazole was identified at low levels (1-4% of

the TRRs, <0.01-0.98 ppm) in all wheat matrices. Metabolite JAU6476-desthio was the major identified residue, accounting for 35% of the TRRs (3.7 ppm) in forage, 19% of the TRRs (1.6 ppm) in hay, 22% of the TRRs (6.0 ppm) in straw, and 16% of the TRRs (0.014 ppm) in grain.

All remaining metabolites were identified at <10% of the TRRs. In the phenyl-label study, metabolites JAU6476-α-OH-desthio and JAU6476-triazolinone were identified in all wheat matrices at ~1-9% of the TRRs (<0.01-1.64 ppm). The metabolites JAU6476-3-OH-desthio, JAU6476-4-OH-desthio, JAU6476-6-OH-desthio and JAU6476 sulfonic acid were identified as 1-9% of the TRRs (0.11-2.24 ppm) in forage, hay and straw. In hay, straw and grain, the metabolites JAU6476-α-acetoxy-desthio (along with benzylpropyldiol) and benzylpropyldiol glucoside were identified at <5% of the TRRs (<0.55 ppm). JAU6476 disulfide, JAU6476-OH-desthio (comprised of the 3-OH, 4-OH and/or 6-OH-desthio isomers) and two JAU6476-OH-desthio glucoside isomers were tentatively identified at <6% of the TRRs (≤1.08 ppm) each. The third JAU6476-OH-desthio glucoside isomer was tentatively identified in all matrices except grain at <3% of the TRRs (<0.35 ppm). JAU6476-desthio glucoside was only tentatively identified in hay and straw at <7% of the TRRs (≤1.79 ppm). Characterized radioactivity remaining at the TLC origins accounted for ~12-20% of the TRRs (0.01-3.28 ppm). Unassigned radioactivity was reported as characterized at ~3-10% of the TRRs (<0.01-2.29 ppm). Unknowns accounted for 2-6% of the TRRs (<0.01-0.83 ppm) in each matrix. In grain, ~15% of the TRRs (0.01 ppm) were characterized based on diastase hydrolysis. Another 8% of the TRRs (<0.01 ppm) were extracted by ASE but not analyzed, and 6% of the TRRs (<0.01 ppm) were characterized as polar and aqueous soluble.

Triazole-Label

Bayer CropScience has submitted studies investigating the metabolism of [triazole-3,5-^{14}C]-prothioconazole (specific activity 18.3 mCi/mmol) in wheat as a foliar treatment. The radiolabeled test substances were formulated as emulsifiable concentrate (EC) formulations and applied as two foliar broadcast sprays to wheat plants grown outdoors in planting containers at the beginning of tillering (BBCH 32) and at full flowering (BBCH 65). Applications were made at 0.159 lb a.i./A (178 g a.i./ha) and 0.260 lb a.i./A (292 g a.i./ha), with a 23-day re-treatment interval, for a total seasonal application of 0.420 lb a.i./A (470 g a.i./ha). Wheat forage was harvested 6 days after the second application, 29 days for hay and 64 days for grain and straw.

Total radioactive residues (TRRs) were 7.96 ppm (forage), 11.18 ppm (hay), 7.94 ppm (straw) and 4.97 ppm (grain) following foliar application of [triazole-3,5-^{14}C]-prothioconazole.

Extraction with organic solvents released the majority of the radioactivity (~65-81% of the TRRs) in all wheat matrices. Accelerated solvent extraction (ASE) released an additional ~8-24% of the TRRs (0.61-1.58 ppm) from all matrices. Acid hydrolysis with HCl:methanol and or HCl:dioxane released ~6-11% of the TRRs (0.48-1.05 ppm) in forage, hay and straw. Acid hydrolysis with HCl:dioxane released 1% of the TRRs (0.041 ppm) for grain. Non-extractable residues remaining following extraction/hydrolysis accounted for <6% of the TRRs (<0.45 ppm) in forage, hay, and straw, and 0.1% of the TRRs (<0.01 ppm) in grain. Extraction results were normalized; therefore, accountabilities were 100%. Residues were identified primarily by LC-MS, LC-MS/MS, and/or NMR spectroscopy with confirmatory analysis by HPLC and/or TLC co-chromatography. These methods successfully identified the predominant residues in

wheat forage, hay, straw, and grain. The results of the analysis of some later extracted samples (450 days storage) showed residue profiles which were very similar to the profiles for the samples extracted within 34 days of harvest. No previously unidentified residue was found in the later extracts and the residues identified in the later extracts represented only less than 1 to 3% of the TRRs in the corresponding matrices. No storage stability data were needed for the RACs as the storage duration of the RACs was no more than one month. For the extracts that were stored up to 473 days, residue profiles covering the storage interval indicated no significant changes.

Approximately 57-91% of the TRRs were identified in all wheat matrices. Prothioconazole was identified at low levels (3-7% of the TRRs, 0.38-0.53 ppm) in forage, hay and straw. However, in grain, neither prothioconazole nor any metabolites unique to prothioconazole were identified. Metabolite JAU6476-desthio was identified as 9-19% of the TRRs (0.74-1.50 ppm) in forage, hay and straw. In grain, the predominant metabolites were triazolylalanine (TA) at 71% of the TRRs (3.54 ppm) followed by triazolylacetic acid (TAA) accounting for 19% of the TRRs (0.95 ppm) with triazolylhydroxypropionic acid (THPA) constituting the remaining identified residue at 0.4% of the TRRs (0.02 ppm). The triazole-specific metabolite, TA, was also a major residue in hay (25% of the TRRs; 2.77 ppm), and accounted for 12% of the TRRs (0.95 ppm) in forage and 4% of the TRRs (0.32 ppm) in straw. TAA was identified at <5% of the TRRs (<0.5 ppm) in forage, hay, and straw, and THPA was identified at <8% of the TRRs (<0.85 ppm) in these same matrices. Free triazole or *1H*-1,2,4-triazole was not identified in any wheat matrix.

All remaining metabolites were identified at <10% of the TRRs. In the triazole-label study, JAU6476-α-OH-desthio and JAU6476-triazolinone were identified at 1-9% of the TRRs (0.08-0.78 ppm) in forage, hay and straw. JAU6476-OH-desthio and JAU6476-α-acetoxy-desthio were identified in forage and straw at 2-6% of the TRRs (0.16-0.49 ppm). Triazolyl-ethanol-glucoside, JAU6476-OH-desthio-glucoside isomers, and JAU6476-OH-desthio-malonyl-glucoside isomers were identified in forage, hay and straw each at <4% of the TRRs (<0.30 ppm). Unresolved glucoside isomers were found in forage and hay at <5% of the TRRs (<0.52 ppm). Triazolyl-ethanol (found in straw), JAU6476-desthio-malonyl-glucoside (found in forage), JAU6476-desthio-phenyl-cysteine isomers and JAU6476-diOH-desthio-malonyl-glucoside (both found in forage) were identified each at <3% of the TRRs (<0.18 ppm). Unknowns accounted for 6-22% of the TRRs (0.3-1.9 ppm) in each matrix. However, these consisted of multiple components, each generally <3% of the TRRs (≤0.36 ppm). The remaining radioactivity was characterized as HCl hydrolysates at ~6% of the TRRs (<0.49 ppm).

Based on the results of the wheat metabolism study, the applicant concluded that prothioconazole is initially metabolized in wheat by oxidation and loss of sulfur to form JAU6476-desthio, after which two major metabolic processes occur: (1) hydroxylation of the phenyl ring and or benzylic carbon to form isomers of JAU6476-OH-desthio, JAU6476-diOH desthio, and JAU6476-α-OH-desthio, followed by conjugation to form the corresponding glucosides, malonyl-glucosides and acetate; and (2) release of the triazole moiety to form TA and THPA and further metabolism of the triazole conjugates to form TAA. The applicant noted that the absence of 1,2,4-triazole in any wheat matrix suggested that immediate or very rapid conjugation of released triazole occurred. The following minor metabolic pathways were

reported: formation of JAU6476-triazolinone and JAU6476-desthio-phenyl-cysteine; conjugation of JAU6476-desthio with glucose and malonic acid; oxidation of the sulfur atom of prothioconazole to form JAU6476 sulfonic acid; cleavage of the benzylic group to form triazolyl ethanol and its glucoside; and conjugation of the benzylpropyldiol portion of the remaining molecule.

Wheat - MRID 46246142: Bayer CropScience has submitted a study investigating the metabolism of [phenyl-UL-^{14}C]-prothioconazole (specific activity 2.97 MBq/mg) in wheat as a seed treatment. The radiolabeled test substance was applied at 7.3 µg a.i./seed (equivalent to 18.4 g a.i./kg seed; low-rate) and 37 µg a.i./seed (equivalent to 93.3 g a.i./kg seed; high-rate). Wheat plants were grown from the treated seed in the greenhouse. Forage was harvested at BBCH 41, hay was harvested BBCH 83, and grain and straw were harvested at maturity (57, 110, and 153 days, respectively, after planting).

Total radioactive residues (TRRs) in forage, hay, and straw were determined by combustion followed by liquid scintillation counting (LSC). In wheat matrices harvested following the low-rate seed treatment, TRRs were 0.020 ppm in forage and hay, 0.030 ppm in straw, and 0.008 ppm in grain. In wheat matrices harvested following high-rate seed treatment, TRRs were 0.07 ppm in forage, 0.09 ppm in hay, 0.28 ppm in straw, and 0.01 ppm in grain. Only forage, hay, and straw were subjected to further analysis.

Solvent extraction with acetonitrile/water released the majority of the TRRs (~71.2-85.2%) in wheat matrices from both treatment rates. HED notes that cysteine HCl was added to extracting solvents and to extracts during concentration procedures to prevent oxidative decomposition of prothioconazole. Hydrolysis with dioxane/HCl solubilized an additional 7.8% of the TRRs in straw (high-rate treatment only). Non-extractable residues remaining following extraction/hydrolysis accounted for 17.1-28.8% of the TRRs (0.003-0.006 ppm) in wheat matrices from the low-rate treatment, and for 7.7-25.7% of the TRRs (0.01-0.02 ppm) in wheat matrices from the high-rate treatment. Because TRRs were determined by summing extractable and non-extractable radioactivity, accountabilities ranged from 100-119%. Residues were identified primarily by TLC co-chromatography with some confirmatory analysis by HPLC. These methods successfully identified the predominant residues in wheat forage, hay, straw, and grain following seed treatment. Only extracts and hydrolysates from the high-rate treatment were subjected to analysis for characterization/identification of residues. Extraction and analysis of all samples were conducted within 30 days of harvest.

Approximately 18-33% of the TRRs (0.018-0.092 ppm) were identified in wheat forage, hay, and straw. Prothioconazole was identified at <1% of the TRRs in all matrices (≤0.002 ppm). Metabolite JAU6476-desthio was the major identified residue, accounting for 10.9% of the TRRs (0.01 ppm) in forage and 6.4-6.6% of the TRRs (0.005-0.019 ppm) in hay and straw. Metabolites JAU6476-3-OH-desthio and JAU6476-4-OH-desthio together accounted for 3.8-12.0% of the TRRs (≤0.017 ppm). In addition, JAU6476-OH-glucosides were tentatively identified at 10.6% of the TRRs (0.030 ppm) in straw and were tentatively identified but not quantitated in wheat forage and hay. Remaining identified metabolites, including JAU6476-α-OH-desthio, JAU6476-6-OH-desthio, JAU6476-triazolinone, JAU6476 sulfonic acid,

JAU6476-α-acetoxy-desthio, benzylpropyldiol glucoside, and JAU6476-disulfide were present at ≤3.3% of the TRRs (≤0.008 ppm) each.

Based on the results of the phenyl-label seed treatment wheat metabolism study, it is concluded that prothioconazole was extensively metabolized in wheat via: (1) oxidation and loss of sulfur to form JAU6476-desthio; and (2) hydroxylation of the chlorobenzyl methylene C-atom to form JAU6476-α-hydroxy-desthio and hydroxylation of the chlorobenzyl ring at positions 3, 4, and 6 of JAU6476-desthio to form the hydroxy desthio metabolites. Exchange of oxygen for sulfur, the elimination of the triazole moiety and conjugation of the benzylpropyldiol portion of the remaining molecule, and the formation of glucosides of the monohydroxylated JAU6476-desthio isomers were proposed as minor metabolic reactions.

Wheat Metabolism Summary: The submitted wheat metabolism data are adequate to satisfy data requirements. Based on the results of the wheat metabolism studies, the applicant concluded that prothioconazole is initially metabolized in wheat by oxidation and loss of sulfur to form prothioconazole desthio, after which two major metabolic processes occur: (1) hydroxylation of the phenyl ring and/or benzylic carbon to form isomers of JAU6476-OH-desthio, JAU6476-diOH desthio, and JAU6476-α-OH-desthio, followed by conjugation to form the corresponding glucosides, malonyl-glucosides and acetate; and (2) release of the triazole moiety to form triazolylalanine and THPA and further metabolism of the triazole conjugates to form triazolylacetic acid. The applicant noted that the absence of free triazole in any wheat matrix suggested that immediate or very rapid conjugation of released triazole occurred. The following minor metabolic pathways were reported: formation of JAU6476-triazolinone and JAU6476-desthio-phenyl-cysteine; conjugation of prothioconazole desthio with glucose and malonic acid; oxidation of the sulfur atom of prothioconazole to form JAU6476 sulfonic acid; and cleavage of the benzylic group to form triazolyl-ethanol and its glucoside.

A similar metabolic pathway is proposed for the metabolism of prothioconazole in wheat following application as a seed treatment.

Peanut - MRID 46246145/46246146:

Phenyl-Label

Bayer CropScience has submitted studies investigating the metabolism of [phenyl-UL-^{14}C]-prothioconazole (specific activity 2.77 MBq/mg) in peanut plants grown in a greenhouse. The radiolabeled test substances were formulated as an emulsifiable concentrate (EC) formulation. [Phenyl-UL-^{14}C]-JAU6476 were applied to peanut plants 'Georgia Green' as three foliar spray applications with 20 to 22-days interval at growth stages beginning at pod development (BBCH codes 66, 71 and 75). Each treatment was performed at a rate of approximately 0.267 lb a.i./A (299 g a.i./ha) for a maximum seasonal rate of 0.800 lb a.i./A (897 g a.i./ha). A 5-fold rate exaggeration study was also performed to allow for metabolite identification. Peanut plants were harvested at maturity (BBCH growth stages 89-91) at a pre-harvest interval of 21 days. Nuts were removed and cleaned from adhering soil. The plants (hay and nuts with shells) were allowed to dry for 4-5 days. The hay and nutmeat samples were individually homogenized with liquid nitrogen. All samples were stored at -18°C or below. The experimental work from extraction to first analysis (TLC-profiling) was completed within 17-91 days (peanut hay) and

51-99 days (nutmeat). Nutmeat extracted 355 days after harvest using MSPD, indicated similar metabolic distribution.

The overall distribution of TRRs was achieved by combustion and radioassay by liquid scintillation counting (LSC). Identification and characterization of metabolites were performed by HPLC with photodiode array or variable wavelength UV detector and a flow-through radiodetector. Confirmation of residues was by radio-TLC co-chromatography with authentic reference standards or by mass spectrometry (MS) and when possible ^{1}H-NMR. Homogenized peanut hay was extracted with acetonitrile (ACN)/water, with added cysteine hydrochloride to prevent oxidative decomposition of the parent during extraction. Further extraction was achieved using an accelerated solvent extractor (ASE). The homogenized nutmeat samples were extracted by two separate methods; refluxing with hexane and matrix solid phase dispersion (MSPD)/microwave extractions.

The TRRs found in peanut hay and nutmeat were 107.51 ppm and 0.29 ppm, respectively. Solvent extraction with acetonitrile/water released 77.5% of the TRRs in peanut hay. Hexane reflux and/or MSPD extraction with a series of solvents released approximately 67-74% of the TRRs in nutmeat. Accelerated solvent extraction (ASE) and microwave extraction was useful to release additional radioactivity from the peanut matrices. Non-extractable residues remaining following extraction/hydrolysis accounted for <7% of the TRRs (6.73 ppm) in hay and <13% of the TRRs (<0.05 ppm) in nutmeat. Accountabilities ranged from 100-124%.

Approximately 65 to 74% of the TRRs were identified in peanut matrices for the phenyl-label study. Prothioconazole was identified at about 2% of the TRRs (2.0 ppm) in hay. Metabolite JAU6476-desthio was the major identified residue in hay, accounting for 28.2% of the TRRs (30.37 ppm), and one additional metabolite, JAU6476-desthio-dihydroxyolefin glucosides, was identified at 14.1% of the TRRs (15. ppm). All remaining metabolites were identified at <10% of the TRRs and included JAU6476-3-OH-desthio, JAU6476-4-OH-desthio, JAU6476-dihydroxydiene sulfonic, JAU6476-dihydroxyolefin sulfonic acid, glucoside conjugates of the JAU6476-OH-desthio isomers, JAU6476-desthio-hydroxydienyl-cysteine, JAU6476-triazolinone, JAU6476 sulfonic acid, and JAU6476-disulfide. Neither prothioconazole nor JAU6476-desthio were identified in nutmeat. The majority of the TRRs in nutmeat (42.6-47.8%; 0.13-0.14 ppm) were associated with peanut oil and determined as fatty acids, indicating that prothioconazole may be completely metabolized to CO_2 in plants. Identified metabolites (each found at <10% of the TRRs) included JAU6476-desthio-dihydroxyolefin glucosides, JAU6476-desthio-hydroxydienyl-cysteine, JAU6476-OH-desthio glucosides, and JAU6476 sulfonic acid.

Triazole-Label

Bayer CropScience has submitted studies investigating the metabolism of [triazole-UL-^{14}C]-prothioconazole (specific activity 2.11 MBq/mg) in peanut plants grown in a greenhouse. The radiolabeled test substances were formulated as an emulsifiable concentrate (EC) formulation. [Triazole-UL-^{14}C]-JAU6476 was applied to peanut plants 'Georgia Green' as three foliar spray applications with 20 to 22-days interval at growth stages beginning at pod development (BBCH code 66, 71 and 75). Each application was between 0.365-0.267 lb a.i./A (297-299 g a.i./ha) for a maximum seasonal rate of 0.799 lb a.i./ha (895 g a.i./ha). Peanut plants were harvested at

maturity (BBCH growth stages 89-91) at a pre-harvest interval of 14 days (triazole-label study). Nuts were removed and cleaned from adhering soil. The plants (hay and nuts with shells) were allowed to dry for 4-5 days. The hay and nutmeat samples were individually homogenized with liquid nitrogen. All samples were stored at -18°C or below. The experimental work from extraction to first analysis (TLC-profiling) was completed within 17-91 days (peanut hay) and 51-99 days (nutmeat). The aqueous phase of peanut hay and polar fractions of nutmeat were monitored for stability using different HPLC systems throughout the study. No significant changes in the profiles were observed. Adequate storage stability was demonstrated.

The overall distribution of TRRs was achieved by combustion and radioassay by liquid scintillation counting (LSC). Identification and characterization of metabolites were performed by HPLC with photodiode array or variable wavelength UV detector and a flow-through radiodetector. Confirmation of residues was by radio-TLC co-chromatography with authentic reference standards or by mass spectrometry (MS) and when possible ^{1}H-NMR. Isolation and characterization of triazole metabolites was conducted by incubating heterotrophic plant cell suspension cultures prepared from apples with [^{14}C]-triazole for 7 days. Homogenized peanut hay was extracted with acetonitrile (ACN)/water, with added cysteine hydrochloride to prevent oxidative decomposition of the parent during extraction. Further extraction was achieved using an accelerated solvent extractor (ASE). The homogenized nutmeat samples were extracted by two separate methods; refluxing with hexane and matrix solid phase dispersion (MSPD)/microwave extractions.

The TRRs found in peanut hay and nutmeat were 47.38 ppm and 1.4 ppm, respectively. Solvent extraction with acetonitrile/water released 85% of the TRRs in peanut hay. Hexane reflux and/or MSPD extraction with a series of solvents released approximately 77% of the TRRs in nutmeat. Accelerated solvent extraction (ASE) and microwave extraction was useful to release additional radioactivity from the peanut matrices. Non-extractable residues remaining following extraction/hydrolysis accounted for 5.4% of the TRRs (2.55 ppm) in hay and 1.9% of the TRRs (0.03 ppm) in nutmeat. Accountabilities ranged from 100-102%.

Approximately 80-85% of the TRRs were identified in peanut matrices for the triazole-label study. Prothioconazole was identified at 6.6% of the TRRs (3.11 ppm) in hay. Metabolite JAU6476-desthio was the major identified residue in hay, accounting for 23.6% of the TRRs (11.15 ppm). All remaining metabolites were identified at <10% of the TRRs and included JAU6476-3-OH-desthio; JAU6476-4-OH-desthio; JAU6476-triazolinone; JAU6476 sulfonic acid; JAU6476-desthio-phenyl-cysteine; JAU6476-disulfide; glucoside conjugates of JAU6476-3-OH-desthio, JAU6476-4-OH-desthio, unspecified JAU6476-OH-desthio isomers, JAU6476-desthio-dihydroxyolefin, and JAU6476-desthio-dihydroxydiene; JAU6476-malonyl glucoside isomers; JAU6476-dihydroxydiene sulfonic acid; and JAU6476-dihydroxyolefin sulfonic acid. Triazolyl metabolites, including triazolylalanine (TA), triazole acetic acid (TAA), triazolylhydroxy-propionic acid (THPA), JAU6476-triazolyl-ethanol, and JAU6476-triazolyl-ethanol-glucoside were minor components in peanut hay, each accounting for ≤1.5% of the TRRs (≤0.71 ppm). Prothioconazole was not identified in nutmeat, and JAU6476-desthio was identified at 6.2% of the TRRs (0.09 ppm). Triazolyl metabolites were the major identified residues in nutmeat, with TA accounting for 49.8% of the TRRs (0.70 ppm), and THPA accounting for 24.7% of the TRRs (0.35 ppm). TAA was identified at 1.2% of the TRRs (0.02

ppm) and triazolyl unknowns accounted for 4.3% of the TRRs (0.07 ppm). Radioactivity determined as fatty acids in peanut oil accounted for 3.0% of the TRRs (0.05 ppm) in nutmeat.

JAU6476 was extensively metabolized in peanut by: (1) oxidation and loss of sulfur to form JAU6476-desthio; (2) hydroxylation of the chlorobenzyl ring of JAU6476-desthio at positions 3 and 4 to form the hydroxy desthio metabolites; (3) conjugation of the hydroxylated metabolites; (4) exchange of oxygen for sulfur; and (5) release of the triazole moiety to form triazolylalanine (TA) and triazolylhydroxypropionic acid (THPA). Free triazole (1*H*-1,2,4-triazole) was not detected in any peanut matrix.

Peanut Metabolism Summary: The submitted peanut metabolism data are adequate to satisfy data requirements. Based on the results of the peanut metabolism studies, the applicant concluded that prothioconazole is initially metabolized in peanut by: (1) oxidation and loss of sulfur to form prothioconazole desthio; (2) hydroxylation of the chlorobenzyl ring of prothioconazole desthio at positions 3 and 4 to form the hydroxy desthio metabolites; (3) conjugation of the hydroxylated metabolites; (4) exchange of oxygen for sulfur; and (5) release of the triazole moiety to form triazolylalanine and THPA. Free triazole was not detected in any peanut matrix.

Sugar beet - MRID 46246147/46246148:

Phenyl-Label

Bayer CropScience submitted studies investigating the metabolism of [phenyl-UL-^{14}C]-prothioconazole in sugar beet. The radiolabeled test substances were formulated as suspension concentrates and applied as four foliar broadcast sprays at 14-day re-treatment intervals to sugar beet plants. The rate applied for the phenyl-label study was 0.228 lb a.i./A/application (255 g a.i./ha) for a total of 1.028 lb a.i./A/season (1152 g a.i./ha/season). Sugar beet roots and tops were harvested 7 days following the final application.

Following foliar application of [phenyl-UL-^{14}C]-prothioconazole to sugar beets, total radioactive residues (TRRs), determined by combustion and liquid scintillation counting (LSC), were 4.333 ppm in tops and 0.119 ppm in roots. Solvent extraction with acetonitrile/water (4:1) released the majority of the TRRs in tops (92.9 % of the TRRs) and root (69.9 % of the TRRs). Cysteine HCl was added to all extracting solvents to prevent oxidative decomposition of prothioconazole. Additional radioactivity was released from sugar beet matrices by: reflux with methanol/water (4:1) at 60-70°C for 8-9 hours, accelerated solvent extraction with methanol/water (1:1), and acid and base hydrolysis with 0.1% TFA and *1N* NaOH. Non-extractable residues remaining following extraction/hydrolysis accounted for 1.3% of the TRRs (0.056 ppm) in tops, and 8.4 % of the TRRs (0.010 ppm) in root. The applicant normalized the extraction results for accountabilities of 100%. However, the reported accountability in tops prior to normalization was 96%. Residues were identified and quantitated primarily by HPLC co-chromatography with confirmatory analysis and/or structure elucidation by TLC, LC-MS, and LC-MS/MS. These methods successfully identified the predominant residues in sugar beet tops and roots. The interval of storage from harvest to analysis for both the RAC and extracts was less than 28 days for both studies.

Approximately 65% of the TRRs were identified in sugar beet tops, and 60% of the TRRs were identified in roots. Prothioconazole was identified at 7.4% of the TRRs (0.322 ppm) in tops, but was not identified in roots. Metabolite JAU6476-desthio was the major identified residue in both tops and roots, accounting for 28.8% of the TRRs (1.248 ppm) and 57.6% of the TRRs (0.068 ppm), respectively. JAU6476-triazolinone was identified in both tops and roots, at 2.0-2.4% of the TRRs (0.003-0.088 ppm). JAU6476-desthio-hydroxy-dieneyl- cysteine isomers were identified in sugar beet tops only at 10.5% of the TRRs (0.454 ppm). The following additional minor metabolites were identified in sugar beet tops only: JAU6476-OH-sulfonic acid glucoside isomers (8.1% of the TRRs; 0.351 ppm), a JAU6476-OH-desthio glucoside isomer (5.1% of the TRRs; 0.222 ppm), JAU6476-α-OH-desthio and JAU6476-OH-di-sulfonic acid glucoside (≤1.9% of the TRRs each; #0.083 ppm). Remaining radioactivity in sugar beet matrices was characterized as: (1) multicomponent and minor unknowns, accounting for 31.4% of the TRRs in tops (>13 components, each present at ≤4.3% of the TRRs, ≤0.19 ppm); (2) methanol-extractable residues (10.8% of the TRRs in root); and (3) acid- and base-hydrolysable residues (7.6% of the TRRs in tops and 11.0% of the TRRs in root).

Triazole-Label

Bayer CropScience submitted studies investigating the metabolism of [triazole-UL-^{14}C]-prothioconazole (specific activity 18.6 mCi/mmol [MBq/mg]) in sugar beet. The radiolabeled test substances were formulated as suspension concentrates and applied as four foliar broadcast sprays at 14-day re-treatment intervals to sugar beet plants. The rate applied for the triazole-label study was 0.286 lb a.i./A for a total of 1.032 lb a.i./A/season (1157 g a.i./ha). Sugar beet roots and tops were harvested 7 days following the final application.

Following foliar application of [triazole-UL-^{14}C]-prothioconazole to sugar beets, total radioactive residues (TRRs), determined by combustion and liquid scintillation counting (LSC), were 5.154 ppm in tops and 0.130 ppm in roots. Solvent extraction with acetonitrile/water (4:1) released the majority of the TRRs in tops (90.0% of the TRRs) and root (70.2 % of the TRRs). Cysteine HCl was added to all extracting solvents to prevent oxidative decomposition of prothioconazole. Additional radioactivity was released from sugar beet matrices by: reflux with methanol/water (4:1) at 60-70°C for 8-9 hours, accelerated solvent extraction with methanol/water (1:1), and acid and base hydrolysis with 0.1% TFA and *1N* NaOH. Non-extractable residues remaining following extraction/hydrolysis accounted for 1.9 % of the TRRs (0.099 ppm) in tops, and 6.0% of the TRRs (0.008 ppm) in root. The applicant normalized the extraction results for accountabilities of 100%. However, the reported accountabilities prior to normalization ranged from 85-101%. Residues were identified and quantitated primarily by HPLC co-chromatography with confirmatory analysis and/or structure elucidation by TLC, LC-MS, and LC-MS/MS. These methods successfully identified the predominant residues in sugar beet tops and roots. The interval of storage from harvest to analysis for both the RAC and extracts was less than 28 days for both studies.

Approximately 69% of the TRRs were identified in sugar beet tops, and 61% of the TRRs were identified in roots. Prothioconazole was identified at 5.1% of the TRRs (0.265 ppm) in tops, but was not identified in roots. Metabolite JAU6476-desthio was a major identified residue in both tops and roots, accounting for 19.2% of the TRRs (0.988 ppm) and 25.5% of the TRRs (0.033 ppm), respectively. JAU6476-triazolinone was identified in both tops and roots, at 1.6-2.0% of

the TRRs (0.003-0.105 ppm). In the triazole-label study, the triazole-specific metabolite, triazolylalanine, was the major identified residue in sugar beet roots at 28.9% of the TRRs (0.038 ppm) and was identified in tops at 1.6% of the TRRs (0.084 ppm). In the triazole-label study, JAU6476-desthio-hydroxy-dieneyl- cysteine isomers were identified in both tops and roots, at 9.9% of the TRRs (0.512 ppm) and 5.4% of the TRRs (0.007 ppm), respectively. The following additional minor metabolites were identified in sugar beet tops only: JAU6476-OH-sulfonic acid glucoside isomers (6.1% of the TRRs; 0.316 ppm), a JAU6476-OH-desthio glucoside isomer (6.5% of the TRRs; 0.334 ppm), triazolyl-sulfonic acid-ethanol glucoside and triazolyl-ethanol-glucoside (together at 5.1% of the TRRs; 0.263 ppm), triazolylhydroxy-propionic acid (THPA) and triazolyl-ethanol (3.8-4.0% of the TRRs; 0.194-0.207 ppm), JAU6476 sulfonic acid (4.0% of the TRRs; 0.205 ppm), JAU6476-OH-desthio (1.2% of the TRRs; 0.063 ppm). Remaining radioactivity in sugar beet matrices was characterized as: (1) multicomponent and minor unknowns, accounting for 21.4% of the TRRs in tops (9 components, each present at ≤4.6% of the TRRs, ≤0.24 ppm); (2) methanol-extractable residues (5.3% of the TRRs in tops and 16.1% of the TRRs in root); and (3) acid- and base-hydrolysable residues (2.7% of the TRRs in tops and 7.7% of the TRRs in root). In the triazole-label study, strong anion exchange (SAX) and strong cation exchange (SCX) solid phase extraction cartridges (SPE) eluate/effluents accounted for 3.8% of the TRRs in sugar beet root.

Prothioconazole was extensively metabolized in sugar beet via: (1) oxidation of the sulfur of the triazolinethione ring to the corresponding sulfonic acid and subsequent elimination of the sulfonic acid group to form JAU6476-desthio; and (2) hydroxylation of the phenyl ring or the benzyl carbon to form multiple isomers, with subsequent conjugation with glucose or further reaction to produce JAU6476-desthio-hydroxy-dieneyl-cysteine. Observed in the triazole-label study only was the release of the triazole moiety to form triazolylalanine (TA) and triazolylhydroxypropionic acid (THPA) and elimination of the phenyl ring. Free triazole (1*H*-1,2,4-triazole) was not identified in any of the sugar beet matrices.

Sugar beet metabolism summary: The sugar beet metabolism data are adequate to satisfy data requirements. Based on the results of the sugar beet metabolism studies, the applicant concluded that prothioconazole is extensively metabolized in sugar beet via: (1) oxidation of the sulfur of the triazolinethione ring to the corresponding sulfonic acid and subsequent elimination of the sulfonic acid group to form prothioconazole desthio; (2) hydroxylation of the phenyl ring or the benzyl carbon to form multiple isomers, with subsequent conjugation with glucose or further reaction to produce JAU6476-desthio-hydroxy-dieneyl-cysteine; (3) release of the triazole moiety to form triazolylalanine and THPA; and (4) elimination of the phenyl ring. The applicant noted that free triazole was not identified.

Overall Plant Metabolism Conclusions: The applicant has submitted metabolism studies on three dissimilar crops, wheat, peanut, and sugar beet. The crops selected for the studies are sufficiently representative of the crops for which the applicant is requesting registration: barley, peanut, rice, wheat, the dried shell and bean subgroup, and the oilseed crop group. The application patterns used in the studies are similar to those the applicant is proposing (foliar applications), and bracket the preharvest intervals that are being proposed. The applicant submitted studies reflecting labeling in both rings for all three crops.

The metabolism of JAU6476 was investigated in plants following foliar spray applications of [triazole-UL-^{14}C] JAU6476 or [phenyl-UL-^{14}C] JAU6476 to wheat, peanuts, and sugar beet and seed treatment application of [phenyl-UL-^{14}C] JAU6476 to wheat.

The results of the plant studies showed that JAU6476 was extensively metabolized after the foliar and seed treatment application of [triazole-UL-^{14}C] JAU6476 or [phenyl-UL-^{14}C] JAU6476. The parent compound represented only <1 to 7% of the residues in all matrices. In the phenyl label studies, the major residue found in wheat, peanut, and sugar beet was JAU6476-desthio (6% to 58% of the TRRs in wheat, peanuts, and sugar beet). A second major metabolic process involved hydroxylation followed by conjugation. Since JAU6476 has multiple positions that could potentially undergo hydroxylation, the majority of the remaining metabolites were simply multiple isomers of monohydroxylated JAU6476-desthio and their corresponding glucosides along with JAU6476-hydroxy-diene, dihydroxy-diene, dihydroxy-olefin, and their conjugates. Collectively, these conjugated and/or hydroxylated metabolites compounds represented a major portion (18 to 37%) of the TRRs in the crop matrices and contained both the phenyl and the triazole rings in the molecule. However, none of these hydroxylated metabolites individually reached or exceeded 10% of the TRRs in any target crop matrix. Cleavage of the triazole moiety occurred resulting in the formation of the label-specific metabolite, JAU6476-benzylpropyldiol and its glucoside which represented a minor portion of the TRRs.

A total of 60% to 74% of the residues from the phenyl label foliar studies were identified. An additional 20% to 48% of the TRRs were characterized by extraction and/or chromatographic behaviors. Only 1 to 8% of the residues remained unextracted. The unextrated residues from the wheat seed treatment study ranged from 8% to 26% of the TRRs in the wheat forage, hay, and straw. However, the actual ppm residue levels of unextractable residues ranged from only 0.01 ppm to 0.02 ppm.

The triazole label plant studies showed three major metabolic processes (desulfuration, hydroxylation, and cleavage of the triazole moiety). JAU6476-desthio (6% to 25% maximum levels in wheat, peanuts, and sugar beet) and the label-specific triazole conjugates, triazolylalanine (TA), triazolylacetic acid, and triazolylhydroxypropionic acid (THP) collectively representing a maximum of 29% to 90% of the TRRs in wheat, peanuts, and sugar beet were the major residues found in the triazole label studies. Although the triazole label studies showed greater cleavage of the triazole moiety (compared to the phenyl label), no free triazole was detected in any crop matrix. As was found in the phenyl label studies, numerous minor hydroxylated metabolites and their conjugates comprised the majority of the remainder of the residues. Triazolyl-ethanol and its glucoside and triazolyethanol sulfonic acid glucoside were minor metabolites arising from cleavage of the benzylic group.

A total of 61% to 94% of the residues from the triazole label studies were identified. An additional 6% to 33% of the TRRs were characterized by extraction and/or chromatographic behaviors. The unextractable residues ranged from only <1% to 6% of the TRRs in the plant matrices.

With the exception of the label-specific metabolites, the metabolic profiles were very similar for the target crop studies with both labels. The triazole and phenyl label studies clearly elucidated the metabolic fate of JAU6476 molecule in target crops and were very much complementary to each other. Irrespective of the mode of application (foliar or seed treatment) and the target crop (wheat, peanuts, or sugar beet), the major residues found in all crops were JAU6476-desthio, TA, THP acid, and TAA. The metabolic profiles for the target crop were also similar to the profiles found in the rotational crops. However, the levels of the triazole-based conjugates were much higher in the rotational crops, a finding which was consistent with that expected for confined rotational crop studies with a triazole-based fungicide.

Following the initial metabolism of JAU6476 to JAU6476-desthio (through oxidation of the sulfur to the corresponding sulfonic acid with subsequent elimination of the sulfonic acid group), two major metabolic processes were observed. One major pathway involved the hydroxylation of the phenyl ring and/or benzylic carbon to form multiple isomers of JAU6476-hydroxy-desthio, JAU6476-dihydroxy-desthio, and JAU6476-α-hydroxy-desthio followed by conjugation to form the corresponding glucosides or acetate. The other major pathway involved the cleavage of the H_2C-N bond to release the triazole moiety (and benzylpropyldiol) leading to the formation of TA and THPA and further metabolism of the triazole conjugates to TAA. The fact that no free triazole was found in any target crop matrix suggests an immediate or very rapid conjugation of the released triazole to form the triazole conjugates.

Minor metabolic processes involved the successive reductions of the phenyl ring to form dienes and olefins; formation of JAU6476-triazolinone; and cleavage of the chlorobenzylic group to form triazolyl-ethanol and its glucoside.

Based on these considerations, HED concludes that the submitted studies are adequate to delineate the nature of prothioconazole residues in plants. The residue definition in plant commodities for tolerance enforcement is the sum of prothioconazole and the metabolite prothioconazole desthio, calculated as prothioconazole. The residue definition in plant commodities for risk assessement is the sum of prothioconazole and the metabolite prothioconazole desthio, calculated as prothioconazole. The contribution of triazole derivatives (1,2,4-triazole, triazolylalanine, triazolylacetic acid) from prothioconazole to the aggregate exposure for human-health risk assessment has been considered (DP322215, 02/07/2006).

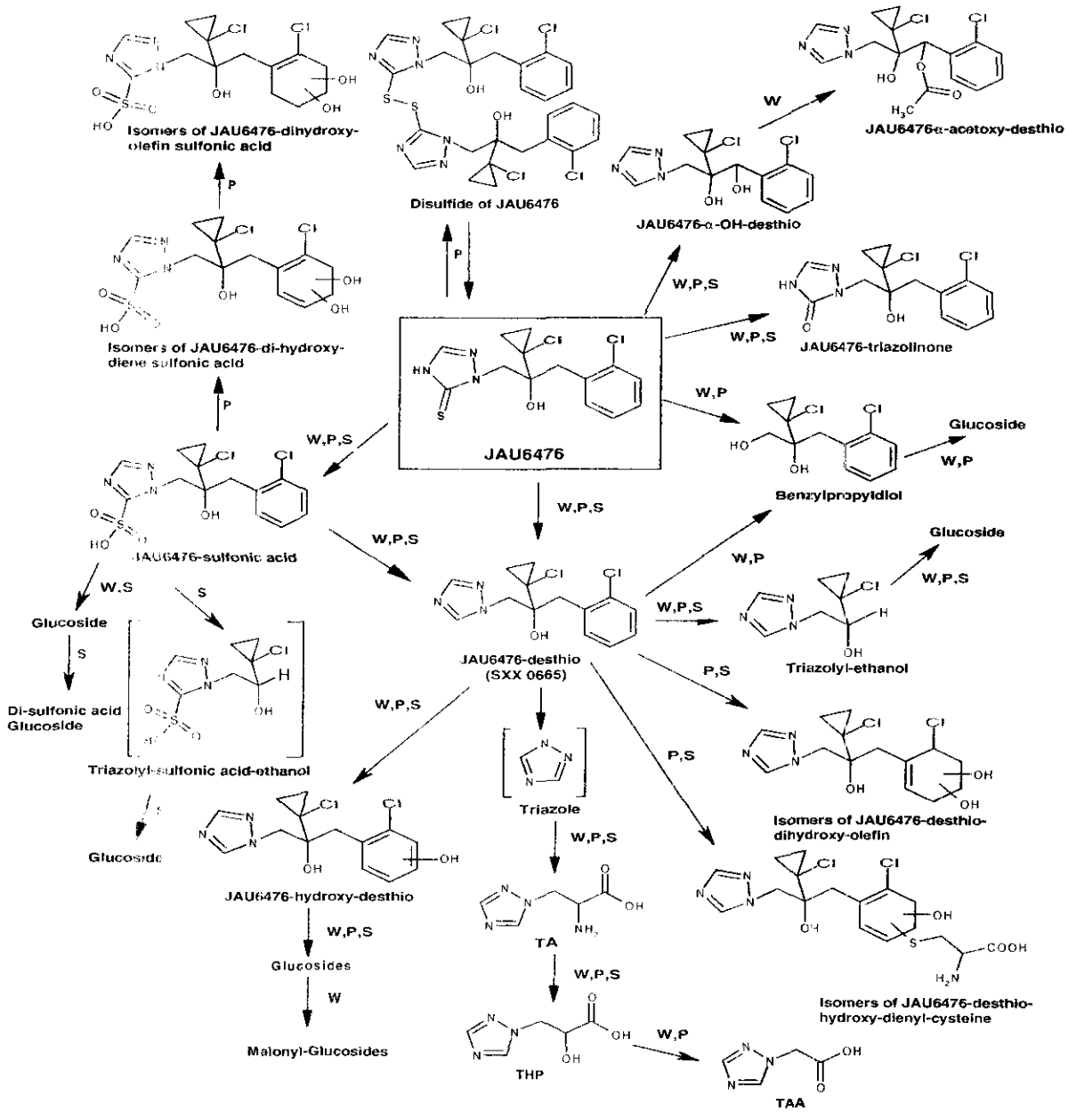


Figure 1. Proposed metabolic profile for [phenyl-UL-^{14}C]-JAU6476 and [triazole-3,5-^{14}C]-JAU6476 in target crops.

8600.1320 Nature of the Residue - Livestock

46246149.der.wpd (Goat)
46246150.der.wpd (Goat)
46246201.der.wpd (Goat)
46246202.der.wpd (Hen)
46246203.der.wpd (Hen)

Bayer submitted five livestock metabolism studies to support the proposed uses: (1) a goat metabolism study reflecting dosing with [triazole-^{14}C]-prothioconazole (MRID 46246149); (2) a goat metabolism study reflecting dosing with [phenyl-^{14}C]-prothioconazole (MRID 46246150); (3) a goat metabolism supplemental study reflecting dosing with [phenyl-^{14}C]-prothioconazole desthio (MRID 46246201); (4) a hen metabolism study reflecting dosing with [phenyl-^{14}C]-prothioconazole (MRID 46246202); and (5) a hen metabolism study reflecting dosing with [triazole-^{14}C]-prothioconazole (MRID 46246203). Chemical names and structures of prothioconazole and its metabolites identified in the livestock metabolism studies are presented in Appendix I.

Goat - MRID 46246149/46246150: Bayer CropScience has submitted studies investigating the metabolism of [phenyl-UL-^{14}C]-prothioconazole (specific activity 0.381 MBq/mg), and [triazole-UL-^{14}C]-prothioconazole (specific activity 369.6 kBq/mg) in lactating goats. The test substances were administered orally to a single goat for each study at 246 ppm (phenyl-label study), and 195 ppm (triazole-label study) in the diet. The goats were dosed once per day for 3 consecutive days. Milk was collected twice daily throughout the studies, and tissues (muscle, fat, liver, and kidney) were collected at sacrifice. Residues were characterized primarily by HPLC analysis, using TLC analyses for confirmation and LC-MS/MS analyses for identification of metabolites. These methods successfully identified the predominant residues in the major extract of each goat matrix. Additionally, adequate storage stability data were submitted demonstrating the stability of the metabolite profile in goat samples and extracts for the duration of the studies.

Phenyl-label study:
The recovered radioactivity accounted for 67.57% of the administered dose. A total of 0.02% of the radioactivity was recovered in milk (0.020-0.071 ppm), while 0.96% was present in the tissues and organs (6.092 ppm in liver, 6.762 ppm in kidney, 0.084-0.106 ppm in muscle, and 0.149-0.172 ppm in fat). Approximately 66% of the total administered dose was excreted in urine and feces. The remaining administered dose was assumed to be absorbed from the intestinal tract prior to excretion. Prothioconazole was monophasically eliminated from plasma with a half-life of 5.3 hours, and the mean residence time (MRT) in plasma was 8.2 hours.

Approximately 29-71% of the TRRs were identified in goat matrices. Prothioconazole was found to be a major residue in liver, kidney, muscle, and fat at 12.94-17.97% of the TRRs (0.012-1.215 ppm), and at 0.89% of the TRRs (<0.001 ppm) in milk. JAU6476-*O*- or *S*-glucuronide and JAU6476-3-hydroxy-desthio were major metabolites in milk and tissues at 34.32% of the TRRs (2.321 ppm) in kidney, and 10.02-14.80% of the TRRs (0.004-0.610 ppm)

in the other matrices. JAU6476-desthio was a major metabolite in fat at 18.98% of the TRRs (0.032 ppm), but <3% of the TRRs (<0.087 ppm) in the other matrices. JAU6476-4-hydroxy was identified at 11.21% of the TRRs (0.683 ppm) in liver, and at <5% of the TRRs (#0.210 ppm) in other goat matrices. Minor metabolites, each at <8% of the TRRs (<0.51 ppm included): JAU6476-4-hydroxy-glucuronide, JAU6476-hydroxy-glucuronide, JAU6476-4-hydroxy-desthio, and JAU6476-*N*-glucuronide. This also included glucuronic acid conjugates of JAU6476-desthio, JAU6476-hydroxy-desthio, JAU6476-dihydroxy-desthio, and JAU6476-hydroxymethoxy-desthio, JAU6476-dihydroxy-diene and JAU6476-desthio-dihydroxy-diene. Unknowns accounted for <8% of the TRRs (<0.182 ppm) in milk, liver, and fat. Accountabilities were normalized to 100%. Non-extractable residues accounted for 16-23% of the TRRs (#0.04 ppm) in milk, muscle, and fat; <3% of the TRRs (0.17 ppm) in kidney; and 17% of the TRRs (1.0 ppm) in liver. The applicant did not attempt to release the non-extractable residues in liver, but it is concluded that an adequate percentage of the TRR was released from liver.

Based on the results of the phenyl-label study, the applicant concluded that prothioconazole is metabolized in goats via several steps: conjugation of the unchanged parent compound with glucuronic acid resulting in an *S*- or *O*-glucuronide; additional glucuronidation of the triazole-thione nitrogen atom of the parent compound to form JAU6476-*N*-glucuronide; hydroxylation of the parent compound to form JAU6476-4-hydroxy and a further hydroxy isomer, followed by conjugation with glucuronic acid; oxidation of the phenyl ring of the parent compound to form JAU6476-dihydroxy-diene; elimination of sulfur to form JAU6476-desthio; further hydroxylation of the chlorophenyl moiety to form JAU6476-3-hydroxy-desthio and JAU6476-4-hydroxy-desthio, followed by conjugation with glucuronic acid; and oxidation of the chlorophenyl moiety of JAU6476-desthio to form JAU6476-desthio-dihydroxy-diene. The presence of JAU6476-dihydroxy-desthio-glucuronides indicated that isomers of JAU6476-dihydroxy-desthio were formed as intermediates. Methylation of JAU6476-hydroxy-desthio-glucuronides to form JAU6476-hydroxymethoxy-desthio-glucuronides occurred to a small extent, as did the glucuronidation of JAU6476-desthio.

Triazole-label study:
The recovered radioactivity accounted for 59.52% of the administered dose. A total of 0.03% of the radioactivity was recovered in milk (0.080-0.249 ppm), while 0.74% was present in the tissues and organs (6.248 ppm in liver, 4.507 ppm in kidney, 0.115-0.142 ppm in muscle, and 0.109-0.213 ppm in fat). Approximately 58% of the total administered dose was excreted in urine and feces. The remaining administered dose was assumed to be absorbed from the intestinal tract prior to excretion. Prothioconazole was monophasically eliminated from plasma with a half-life of 7 hours, and the mean residence time (MRT) in plasma was 10.6 hours.

Approximately 61-84% of the TRRs were identified in goat matrices. Prothioconazole was found to be a major residue in liver, kidney, and fat at 16.12-19.50% of the TRRs (0.028-1.047 ppm), at 3.18% of the TRRs (0.005 ppm) in milk, and at 7.17% of the TRRs (0.008 ppm) in muscle. Thiocyanate accounted for 9.01-41.12% of the TRRs (0.022-0.406 ppm) in milk, kidney, muscle and fat; and at 2.04% of the TRRs (0.128 ppm) in liver. Radioactivity corresponding to 10.76% of the TRRs (0.016 ppm) was attributed to lactose in milk. JAU6476-*S*-glucuronide and JAU6476-3-hydroxy-desthio were major metabolites in kidney, muscle and

fat at 11.92-33.85% of the TRRs (0.016-1.526 ppm), and in milk and liver at <6.1% of the TRRs (<0.38 ppm). JAU6476-desthio was a major metabolite in fat at 15.11% of the TRRs (0.026 ppm), and <5% of the TRRs (<0.31 ppm) in other goat matrices. Other metabolites included JAU6476-4-hydroxy (10.97% of the TRRs; 0.686 ppm) in liver, and <8.5% of the TRRs (<0.164 ppm) in other goat matrices; and JAU6476-hydroxy-glucuronide at 11.15% of the TRRs (0.019 ppm) in fat , but <7% of the TRRs (<0.32 ppm) in other goat matrices. Minor metabolites, each at <7% of the TRRs (<0.41 ppm) included: JAU6476-4-hydroxy-glucuronide, JAU6476-hydroxy sulfate and sulfate conjugate, JAU6476-lactoside, JAU6476-4-hydroxy-desthio, JAU6476-*N*-glucuronide, and JAU6476-*S*-methyl. Unknown metabolites accounted for 5.1-11.0% of the TRRs (<0.32 ppm) in liver and kidney. Accountabilities were normalized to 100%. Non-extractable residues accounted for 16-23% of the TRRs (≤0.04 ppm) in milk, muscle, and fat; <6% of the TRRs (0.237 ppm) in kidney; and ~10% of the TRRs (0.662 ppm) in liver. The non-extractable residues of liver were subjected to microwave extraction which released an additional 4.1% of the TRRs (0.256 ppm).

Based on the results of the submitted goat metabolism studies with prothioconazole, it is concluded that prothioconazole is metabolized in goats via several steps: conjugation of the triazolinethione moiety of the parent compound with glucuronic acid to form the *S*-glucuronide and *N*-glucuronide of the parent; elimination of sulfur to form the metabolite JAU6476-desthio; oxidative hydroxylation of the phenyl moiety in prothioconazole and JAU6476-desthio to monohydroxy, dihydroxy, and dihydroxy-diene compounds, partly followed by conjugation with glucuronic acid; conjugation of the triazolinethione moiety of the parent compound with lactose; conjugation of hydroxylated metabolites of prothioconazole with sulfate; methylation of the triazolinethione moiety of prothioconazole to form JAU6476-*S*-methyl; and cleavage of the parent compound to form thiocyanate.

Goat - (MRID 46246201):

Bayer CropScience has submitted a study investigating the metabolism of [phenyl-UL-^{14}C]JAU6476-desthio (specific activity 0.397 MBq/mg) in a lactating goat; JAU6476-desthio is a metabolite of prothioconazole. The test substance was administered orally to a single goat at 195 ppm in the diet. The goat was dosed once per day for 3 consecutive days. Milk was collected twice daily throughout the study, and tissues (muscle, fat, liver, and kidney) were collected at sacrifice.

Total radioactive residues (TRR) were 0.074-0.31 ppm in milk, 18 ppm in liver, 19 ppm in kidney, 0.23 -0.28 ppm in muscle, and 0.22-0.24 ppm in fat. Radioactivity was highest in liver and kidney and lowest in fat. Residues in milk were generally highest in samples collected 8 hours after dosing, and did not appear to have reached a plateau by the end of the dosing period. A large portion of the administered dose was excreted, with urine and feces accounting for a total of ~74% of the administered dose. Residues were characterized primarily by HPLC analysis, using TLC analyses for confirmation and LC/MS/MS analyses for identification of metabolites. These methods successfully identified the predominant residues in goat matrices, except for liver.

Analysis of the methanol (organic) extracts was completed within 3 months of sample collection for all matrices except milk; analysis of the evening milk extract was completed

within 6 months of sample collection. Additional extractions and analyses of milk and muscle samples were conducted 17-19 months after sample collection to allow for metabolite identification; comparison of the HPLC or HPTLC profiles of the organic extracts with those of the initial extracts indicated that the metabolite profile was stable during storage. The submitted storage stability information and data are adequate to support the goat metabolism study.

Approximately 60-75% TRR were identified in goat matrices. The test substance, JAU6476-desthio, was found to be a major residue in liver and fat, at 31% TRR (5.7 ppm) and 14% TRR (0.032 ppm), respectively. JAU6476-desthio was *not* found in milk and was found in kidney and muscle at <8% TRR. JAU6476-desthio-glucuronide was the major metabolite in kidney, at 24% TRR (4.567 ppm); this metabolite was also found in milk, muscle, and fat, at <7% TRR. Sulfate conjugates of JAU6476-dihydroxy-desthio, JAU6476-hydroxy-methoxy-desthio, and JAU6476-hydroxy-desthio together accounted for 44% TRR in milk; these conjugates were not detected in goat tissues. One diastereomer of JAU6476-desthio-3,4-dihydroxy-dienyl-glucuronide was a major residue in kidney, muscle, and fat, at 13-15% TRR (0.034-2.6 ppm), and was a minor residue in milk and liver (<4% TRR); a second diastereomer of this metabolite was found in all goat matrices, at <9% TRR. JAU6476-4-hydroxy-desthio was a major metabolite in fat, at 15% TRR (0.034 ppm); this metabolite was also found in liver, kidney, and muscle, at <9% TRR. Several additional metabolites were identified in goat matrices, each at <8% TRR: JAU6476-desthio-3,4-dihydroxy-diene in all matrices; glucuronides of JAU6476-dihydroxy-desthio, JAU6476-4,5-dihydroxy-desthio, JAU6476-3-hydroxy-desthio, JAU6476-4-hydroxy-desthio, and JAU6476-hydroxy-methoxy-desthio in all matrices; JAU6476-dihydroxy-desthio in milk, liver, muscle, and fat; JAU6476-4,5-dihydroxy-desthio in milk, liver, and muscle; and JAU6476-3-hydroxy-desthio in liver, kidney, and muscle. Unknown metabolites accounted for a significant portion of the radioactivity in liver and kidney (11.0-14.5% TRR); however, HPLC analyses indicated that individual unknowns were ≤5.1% TRR.

Goat Metabolism Summary:
The goat metabolism data are adequate to satisfy data requirements. Based on the results of the goat metabolism studies, it is concluded that prothioconazole is metabolized in goats via several steps: conjugation of the unchanged parent compound with glucuronic acid resulting in an *S*- or *O*-glucuronide; additional glucuronidation of the triazole-thione nitrogen atom of the parent compound to form JAU6476-*N*-glucuronide; hydroxylation of the parent compound to form prothioconazole-4-hydroxy and a further hydroxy isomer, followed by conjugation with glucuronic acid; oxidation of the phenyl ring of the parent compound to form JAU6476-dihydroxy-diene; elimination of sulfur to form prothioconazole desthio; further hydroxylation of the chlorophenyl moiety to form JAU6476-3-hydroxy-desthio and JAU6476-4-hydroxy-desthio, followed by conjugation with glucuronic acid; oxidation of the chlorophenyl moiety of prothioconazole desthio to form JAU6476-desthio-dihydroxy-diene; conjugation of the triazolinethione moiety of the parent compound with lactose; conjugation of hydroxylated metabolites of prothioconazole with sulfate; methylation of the triazolinethione moiety of prothioconazole to form JAU6476-*S*-methyl; and cleavage of the parent compound to form thiocyanate. The presence of JAU6476-dihydroxy-desthio-glucuronides indicated that isomers of JAU6476-dihydroxy-desthio were formed as intermediates. Methylation of JAU6476-hydroxy-desthio-glucuronides to form JAU6476-hydroxymethoxy-desthio-glucuronides occurred to a small extent, as did the glucuronidation of prothioconazole desthio.

The transfer factor (concentration in milk or tissue (ppm) / concentration in feed (ppm)) was generally greater for the [phenyl-UL-^{14}C]JAU6476-desthio metabolite than for the [phenyl-UL-^{14}C]-prothioconazole over the 3 day study period, as summarized:

Chemical	Liver[1]	Kidney[1]	Muscle[1]	Fat[1]	Milk[1]
Prothioconazole + Prothioconazole desthio + Conjugates					
Prothioconazole	6.4	15	0.12	0.29	0.029
Prothioconazole desthio	29	31	0.072	0.22	0.092
4-Hydroxy prothioconazole + 4-Hydroxy prothioconazole desthio + Conjugates					
Prothioconazole	4.6	2.0	0.024	0.041	0.0032
Prothioconazole desthio	10	11	0.12	0.23	0.077

[1] X 10^{-3}

These factors suggest that the feeding of prothioconazole desthio may yield higher residues in livestock commodities than the feeding of prothioconazole. Given that plant metabolism studies generally show a high conversion of prothioconazole to desthtio prothioconazole, feeding studies with both prothioconazole and prothioconazole desthio are evaluated to provide a conservative estimate of residues in livestock commodities (see below).

Hen - MRID 46246202/46246203:

Phenyl-label study

Six laying hens were administered [phenyl-UL-^{14}C]-JAU6476 (0.732 MBq/mg) orally at a mean dose of 9.7 mg/kg b.w. once a day for three consecutive days (corresponding to 171 ppm in the feed). Excreta was collected every 24 hours. Cages were checked for egg production twice daily, and all eggs collected. Five hours after the final dose (53 hours after the initial dose) the hens were sacrificed. Liver (without bile bladder), kidney, leg muscle, breast muscle, skin, subcutaneous fat and eggs from the ovary and oviduct were dissected from the hens. All samples were assayed for total radioactivity by liquid scintillation counting, either directly or by sample combustion. The identification and characterization of metabolites was achieved by high performance liquid chromatography following solvent extraction of tissues.

In [phenyl-UL-^{14}C]-JAU6476 treated hens, the recovered radioactivity accounted for 79.28% of the administered dose. Approximately 78.42% of the administered dose (%AD) was eliminated in the excreta. A total of 0.011 % AD was recovered in eggs, while 0.85% was estimated to have been present in the tissues and organs. A significant portion of the administered dose was absorbed from the intestinal tract prior to excretion, as indicated by the high residue concentrations in the kidney and liver.

Kidney and liver were found to contain the highest total radioactive residues (TRRs), 4.537 ppm and 4.081 ppm, respectively. TRRs in other tissues were 0.597, 0.433, 0.383, 0.107 and 0.058 ppm for eggs from the ovary and oviduct, subcutaneous fat, skin, leg muscle and breast muscle respectively. The major metabolites identified were the parent JAU6476 in muscle (11.33% of the TRRs; 0.010 ppm), fat (30.33% of the TRRs; 0.137 ppm) and liver (24.76% of the TRRs; 0.995 ppm); JAU6476-desthio in fat (28.96% of the TRRs; 0.130 ppm) and eggs (20.13% of the

TRRs; 0.007 ppm); JAU6476-*S*- methyl in fat (19.56% of the TRRs; 0.088 ppm); a glucuronide conjugate of the parent JAU6476 in muscle (15.50% of the TRRs; 0.014 ppm), liver (11.93% of the TRRs; 0.479 ppm) and eggs (16.98% of the TRRs; 0.006 ppm); and sulfate conjugates of hydroxylated JAU6476-desthio in liver (11.07% of the TRRs; 0.445 ppm). Minor metabolites (< 10% of the TRRs) included JAU6476-desthio-3,4-dihydroxy-dienyl-glucuronide, JAU6476-4-hydroxy, JAU6476-4- hydroxy-desthio, JAU6476-*N*-glucuronide, JAU6476-hydroxy-glucuronides and JAU6476- dihydroxy-diene. Attempts were made to further release the bound liver residues by ultrasonic and microwave extractions in the presence of methanol and acidic methanol. These efforts released an additional 6.4% (0.257 ppm) of the TRRs in the liver, leaving 14.4% (0.579 ppm) of the TRRs as non-extractable residues. Total accountabilities ranged from 97-101%.

Triazole-label study

Six laying hens were treated with [triazole-UL-^{14}C]- JAU6476 (0.702 Mbq/mg) orally at a mean dose of 10.4 mg/kg b.w. once daily for three consecutive days (corresponding to 163 ppm in the feed). Excreta was collected every 24 hours. Cages were checked for egg production twice daily, and all eggs collected. Five hours after the final dose (53 hours after the initial dose) the hens were sacrificed. Liver (without bile bladder), kidney, leg muscle, breast muscle, skin, subcutaneous fat and eggs from the ovary and oviduct were dissected from the hens. All samples were assayed for total radioactivity by liquid scintillation counting, either directly or by sample combustion. The identification and characterization of metabolites was achieved by high performance liquid chromatography following solvent extraction of tissues. Mass spectroscopy and ^{1}H-NMR were used to identify a single metabolite in the triazole-label study.

The recovered radioactivity accounted for 66.37% of the administered dose. A large fraction of the administered dose in the [triazole-UL-^{14}C] JAU6476-treated hens was eliminated in the excreta (65.61%). A total of 0.014% of the radioactivity was recovered in eggs, while 0.75% was present in the tissues and organs. A significant portion of the administered dose was absorbed from the intestinal tract prior to excretion, as indicated by the high residue concentrations in the kidney and liver.

Liver and kidney were found to contain the highest mean residues at 3.447 and 3.381 ppm respectively. Mean residues detected in other tissues represented 0.623, 0.342, 0.308, 0.139 and 0.096 ppm for eggs from the ovary and oviduct, subcutaneous fat, skin, leg muscle and breast muscle respectively. The major metabolites identified were parent JAU6476 in fat (15.9% of the TRRs; 0.046 ppm) and liver (30.7% of the TRRs; 1.085 ppm); JAU6476-desthio in fat (26.8% of the TRRs; 0.078 ppm); JAU6476-*S*-methyl in fat (28.5% of the TRRs; 0.083 ppm); JAU6476-*S*-glucuronide in liver (14.9% of the TRRs; 0.526 ppm) and eggs (23.7% of the TRRs; 0.012 ppm); sulfate conjugates of hydroxylated JAU6476-desthio in liver (13.5% of the TRRs, 0.474 ppm); 1*H*-1,2,4-triazole in muscle (18.7% of the TRRs; 0.023 ppm) and eggs (11.4% of the TRRs; 0.006 ppm); and JAU6476-triazolyl-ethanol in muscle (28.3% of the TRRs; 0.035 ppm) and eggs (15.6% of the TRRs; 0.008 ppm). Minor metabolites (<10% of the TRRs) included JAU6476-4-hydroxy, JAU6476-4-hydroxy-desthio, JAU6476-*N*-glucuronide and thiocyanate. Attempts were made to further release the bound liver residues by ultrasonic and microwave extractions in the presence of methanol and acidic methanol. These efforts

released an additional 5.7% (0.202 ppm) of the TRRs in the liver, leaving 12.7% (0.448 ppm) of the TRRs as non-extractable residues. Accountabilities ranged from 100-102%.

The fact that the highest mean residues were found in the kidney and liver indicates that a significant fraction of the administered dose is absorbed through the intestine prior to excretion.

JAU6476 was extensively metabolized following the oral administration of [phenyl-UL-^{14}C] JAU6476 or [triazole-UL-^{14}C] JAU6476 to laying hens. The major metabolic pathways were:

- conjugation of the unchanged parent compound with glucuronic acid forming an *S*-glucuronide,
- methylation of the sulfur atom to form JAU6476-*S*-methyl,
- desulfuration of JAU6476 yielding JAU6476-desthio followed by hydroxylation and conjugation with sulfate, and
- cleavage of the chlorobenzylic group of JAU6476-desthio to JAU6476-triazolyl-ethanol and release of 1*H*-1,2,4-triazole.

Several minor metabolic processes were also elucidated. These minor pathways were:

- conjugation of JAU6476 to the *N*-glucuronide,
- hydroxylation of JAU6476 followed by glucuronidation,
- cleavage of the triazolinthione moiety of JAU6476 to yield thiocyanate,
- hydroxylations of JAU6476 and JAU6476-desthio and reduction of the phenyl ring to the corresponding dihydroxy-dienes followed by conjugation with glucuronic acid, and
- further hydroxylation of JAU6476-4-hydroxy-desthio followed by methylation and/or sulfate conjugation.

Hen Metabolism Summary: The hen metabolism data are adequate to satisfy data requirements. Based on the study results, the applicant concluded that prothioconazole is metabolized in hens via several steps: conjugation of the unchanged parent compound with glucuronic acid to form an S- (more likely) or an O-glucuronide; methylation of the triazolinethione moiety to form JAU6476-S-methyl; elimination of sulfur to form the metabolite prothioconazole desthio; hydroxylation of the chlorophenyl moiety of the metabolite prothioconazole desthio to form JAU6476-4-hydroxy-desthio and possibly JAU6476-3-hydroxy-desthio, followed by conjugation with sulfate; oxidation of the chlorophenyl moiety of prothioconazole desthio, followed by conjugation with glucuronic acid, to form JAU6476-desthio-3,4-dihydroxy-dienyl-glucuronide; cleavage of the aliphatic carbon chain to form 1,2,4-triazole and JAU6476-triazolylethanol; cleavage of the triazolinethione moiety to form thiocyanate; hydroxylation of the parent compound to form prothioconazole-4-hydroxy; glucuronidation of a triazolinethione nitrogen atom of the parent compound to form JAU6476-N-glucuronide; and, to a small extent, methylation of JAU6476-hydroxy-desthio to form JAU6476-hydroxymethoxy-desthio-glucuronides. The presence of sulfate conjugates of JAU6476-dihydroxy-desthio and JAU6476-hydroxymethoxy-desthio indicated that JAU6476-dihydroxy-desthio and JAU6476-hydroxymethoxy-desthio were formed as intermediates.

Overall Livestock Metabolism Conclusions: The metabolic pathway for JAU6476 was evaluated in livestock following three consecutive daily oral doses of [phenyl-UL-^{14}C] JAU6476 or

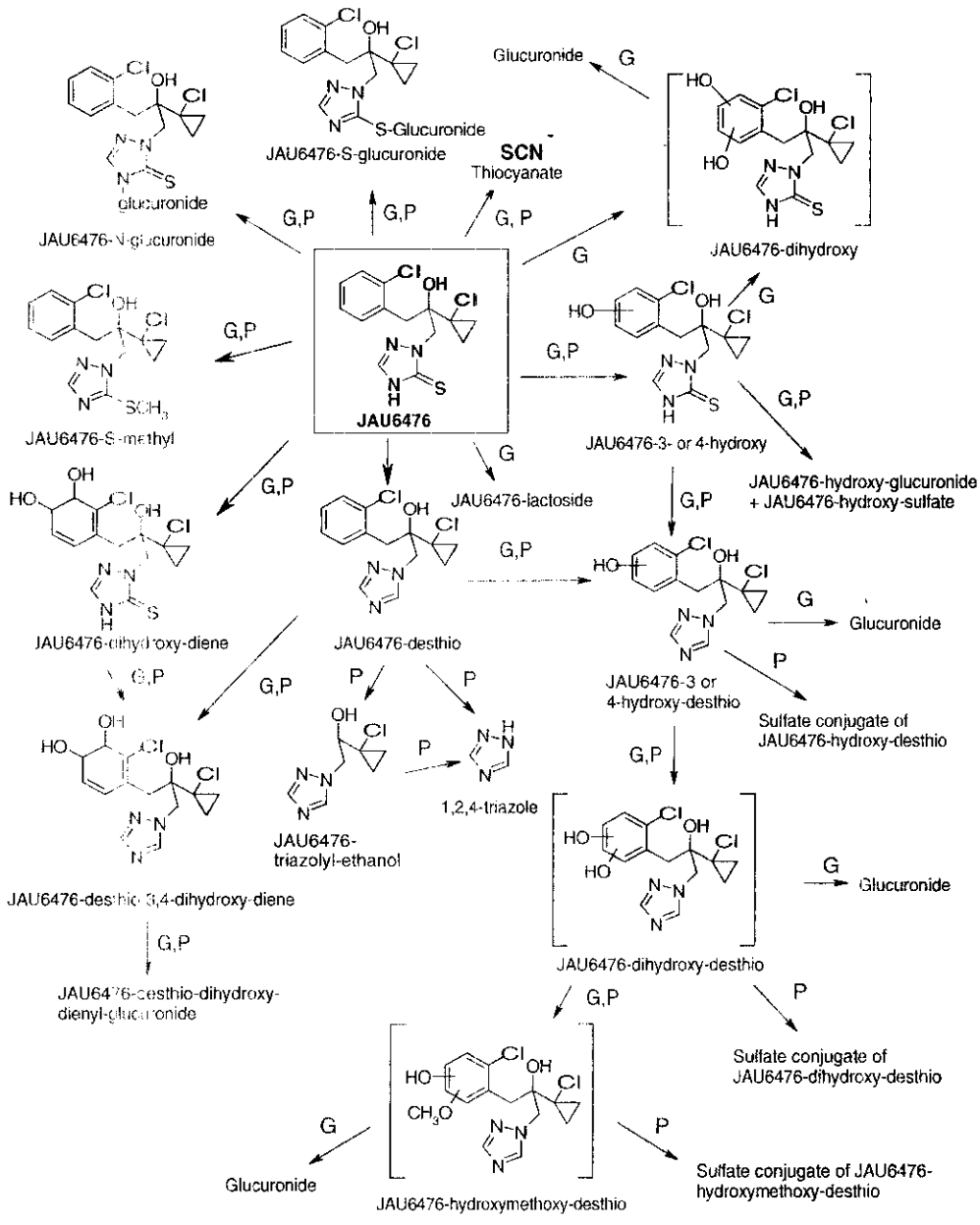
[triazole-UL-^{14}C] JAU6476 to lactating goats and laying hens. Some qualitative and quantitative differences between goats and laying hens were observed. These differences were:

- cleavage of JAU6476 and JAU6476-desthio to release 1,2,4-triazole and triazolylethanol occurred in poultry, but these metabolites were not found in goats (1,2,4-triazole was also found in rats following an oral dose of [triazole-UL-^{14}C] JAU6476),
- methylation of the sulfur atom of the parent compound was a major metabolic process in poultry, but was only a minor pathway in goats,
- cleavage of the triazolinthione ring to yield thiocyanate was a major metabolic process in goats, but was only a minor pathway in poultry, and
- conjugation of JAU6476 with lactose occurred in goats.

With the exception of the above-mentioned differences, the metabolism of JAU6476 was very similar in all livestock. Conjugation of the unchanged parent compound with glucuronic acid forming an S-glucuronide and desulfurization of JAU6476 yielding JAU6476-desthio were major metabolic processes in both poultry and goats. However, the majority of the metabolites found in poultry and goats were products of hydroxylations of JAU6476 and its desthio metabolite (probably through epoxide intermediates) leading to the formation of the corresponding dihydroxy and dihydroxy-dienes.

In goats, the di-hydroxylated metabolites (JAU6476-dihydroxy and JAU6476-desthio-dihydroxy-diene) were further conjugated with glucuronic acid; in poultry, the sulfate and glucuronic acid conjugates of the di-hydroxylated JAU6476-desthio metabolites were formed. Sulfate and glucuronic acid conjugation of JAU6476-3/4-hydroxy and methylation of JAU6476-dihydroxy-desthio occurred in both poultry and goats.

It is concluded that for tolerance enforcement in livestock commodities the residue of concern consists of the sum of prothioconazole, the prothioconazole desthio metabolite, and conjugates that can be converted to either of these two compounds by acid hydrolysis, calculated as prothioconazole. For purposes of risk assessment in livestock commodities, the residue of concern consists of prothioconazole, the prothioconazole desthio metabolite, the 4-hydroxy prothioconazole metabolite, and conjugates that can be converted to any of these three compounds by acid hydroysis. Additionally, contribution of triazole derivatives (in poultry commodities) from the use of prothioconazole to the aggregate exposure for human-health risk assessment has been considered (D322215, 02/07/2006).



P = poultry G = goat

Figure 2. Proposed metabolic pathway for JAU6476 in livestock.

860.1340 Residue Analytical Methods

46246206.der.wpd (Plants; also includes review of MRIDs 46246208 and 46246209)
46246215.de2.wpd (Plants)
46477702.der.wpd (Plants; also includes review of MRID 46477703)
46246204.der.wpd (Livestock; also includes review of MRIDs 46246205 and 46246207)
46477704.der.wpd (Livestock; also includes review of 860.1340 data from MRID 46246201)

Plant commodity methods

Enforcement method: Bayer CropScience has proposed the high performance liquid chromatography (with electrospray ionization) and tandem mass spectrometry (LC-MS/MS) method RPA JA/03/01 for data gathering and the enforcement of maximum residue limits (MRLs) for residues of prothioconazole (JAU6476) and the prothioconazole desthio metabolite (JAU6476-desthio) in plant commodities. The LC-MS/MS method is entitled "An Analytical Method for the Determination of Residues of JAU 6476 and desthio-JAU 6476 in Plant Matrices Using LC/MS/MS."

In the method, crop matrices are extracted with a mixture of methanol, 30% hydrogen peroxide, and 5% aqueous sodium bicarbonate at 65°C for 2 hours. Prothioconazole is converted to both prothioconazole sulfonic acid and prothioconazole desthio because of this oxidative extraction procedure. The prothioconazole desthio metabolite remains unchanged after extraction. The cooled extract is spiked with an isotopically labeled internal standard, cleaned up by C18 solid-phase extraction (SPE), and mixed with either 0.1% formic acid or 1% 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 LOQs reported in the method are 0.02 ppm for canola seed, peanut nutmeat, and wheat grain; and 0.05 ppm for dried peas, wheat forage, wheat hay, and wheat straw. The calculated LODs range from 0.002-0.005 ppm for prothioconazole and prothioconazole desthio in canola seed, dried peas, peanut nutmeat, and wheat (forage, hay, straw, and grain); and from 0.002-0.007 ppm for prothioconazole sulfonic acid in these same commodities.

Concurrent recovery data from the crop field trials, as well as data from the method validation study adequately bracket the expected residue levels. Method validation data demonstrate adequate method recoveries of prothioconazole, prothioconazole sulfonic acid, and prothioconazole desthio at 0.020 ppm (LOQ) and 0.1 ppm, respectively, for canola seed, peanut nutmeat, and wheat grain; and at 0.05 ppm (LOQ) and 1.0 ppm, respectively, for dried peas, wheat forage, wheat hay, and wheat straw. The ranges of recoveries (and CVs) from these matrices are 71-91% (5.4%) for prothioconazole, 76-102% (5.8%) for prothioconazole sulfonic acid, and 85-106% (4.5%) for prothioconazole desthio over all matrices and spiking levels. Adequate extraction efficiency data have been submitted for the method using samples of sugar beet tops and wheat forage. Adequate independent laboratory validation data have been

submitted for the method using samples of peanut nutmeat and wheat forage from the plant metabolism studies. Confirmatory analysis procedures were not conducted for the proposed enforcement method.

The proposed enforcement method for plant commodities has been successfully validated by the EPA ACL. However, the sponsor needs to modify the method to include at least two multiple reaction monitoring (MRM) transitions. A single MS/MS ion transition as used in the current version of the method is no longer considred sufficient for positive confirmation of the analyte residue.

Data collection methods: The proposed LC-MS/MS enforcement method was used to determine residues of prothioconazole and prothioconazole desthio in/on samples of plant commodities from the crop field trial, processing, and field rotational crop studies associated with DP Barcode D303508. The proposed enforcement method was also used to determine residues of prothioconazole and prothioconazole desthio in the majority of samples from storage stability studies reported in MRID 46477701.

Additional data collection methods: An LC-MS/MS method, Method No. 00598, was used to determine residues of prothioconazole and prothioconazole desthio in one storage stability study (MRID 46246139) and in certain samples from another storage stability study (MRID 46477701). Bayer has submitted a description of Method No. 00598 and its modification 00598/M001; the method determines residues of prothioconazole and its metabolite prothioconazole desthio in cereal grain and canola commodities.

For Method No. 00598, cereal grain matrices are extracted with ACN/water containing cysteine HCl; cysteine HCl is added as an antioxidant to prevent degradation of prothioconazole. The extract is partitioned with n-hexane, and the resulting aqueous phase is partitioned with dichloromethane. The dichloromethane phase is concentrated and diluted with ACN and water for LC/MS/MS analysis.

Method No. 00598/M001 includes instructions for the analysis of cereal grain and canola matrices. The extraction procedures are the same as for Method No. 00598 except that internal standards prothioconazole-$^{15}N_3$-$^{13}C_2$ and prothioconazole desthio-$^{15}N_3$-$^{13}C_2$ are added to the final sample extract just prior to LC-MS/MS analysis.

The validated LOQs are 0.01 ppm for each analyte in cereal grain and canola seed and 0.05 ppm for each analyte in all other matrices. The calculated LODs ranged 0.0008-0.0304 ppm.

Method validation data for Method No. 00598 demonstrated adequate method recoveries of prothioconazole and prothioconazole desthio at 0.01 ppm (LOQ) and 0.10 ppm for barley and wheat grain, and at 0.05 ppm (LOQ), 0.50 ppm, and 5.0 ppm for barley and wheat forage and straw. Recovery ranges (and CVs) from these matrices were 67-112% (9.1%) for prothioconazole and 72-104% (7.1%) for prothioconazole desthio. Method validation data for Method No. 00598/M001 demonstrated adequate method recoveries of prothioconazole and prothioconazole desthio at 0.01 and 0.10 ppm for barley and wheat grain and canola seed, and at 0.05, 0.50, and 5.0 ppm for barley and wheat forage and straw and canola forage, straw, and

pod. Recovery ranges (and CVs) from these matrices were 65-118% (10.2%) for prothioconazole and 64-98% (6.5%) for prothioconazole desthio.

Based on the method validation data, the method is adequate for data collection purposes. However, HED notes that in conjunction with the storage stability study reported in MRID 46477701, the applicant concluded that Method No. 00598 was not adequate for the determination of weathered residues of prothioconazole.

Bayer CropScience has submitted a high performance liquid chromatography (with electrospray ionization) and tandem mass spectrometry (LC-MS/MS) data gathering method for the determination of residues of 1*H*-1,2,4-triazole and the triazole conjugates triazolylalanine and triazolylacetic acid in plant commodities. The method was not submitted as a separate study but was submitted as an appendix to the crop field trial, processing, and limited field rotational crop studies submitted under DP Barcode D303508. The LC-MS/MS method, entitled "Working Residue Analytical Method for the Determination of Triazole, Triazole Alanine, and Triazole Acetic Acid Residues in Dried Pea, Dried Bean, Rice, Barley, Wheat, Canola, Peanut, Mustard Greens, and Turnip Matrices," was used to determine residues of 1,2,4-triazole, triazolylalanine, and triazolylacetic acid in/on samples of plant commodities from the crop field trial, processing, and field rotational crop studies associated with DP Barcode D303508.

In the method, crop matrices are extracted with aqueous methanol, and three separate aliquots of the extract are removed for determination of each of the three analytes. Isotopically labeled internal standard is added to each aliquot. For 1*H*-1,2,4-triazole, the aliquot is mixed with dansyl chloride to form the dansyl derivative of 1*H*-1,2,4-triazole, which is partitioned into ethyl acetate and then redissolved in acetonitrile (ACN)/water (1:1, v:v) for LC-MS/MS analysis. For triazolylalanine, the aliquot is cleaned up by solid-phase extraction (SPE), derivatized to the butyl ester using butanolic HCl, and then further derivatized using heptafluorobutyric anhydride (HFBA). The mixture is redissolved in ACN/water (1:1, v:v) for LC-MS/MS analysis. For determination of triazolylacetic acid, the aliquot is cleaned up by SPE, derivatized to the butyl ester using butanolic HCl, and then redissolved in ACN/water (1:1, v:v) for LC-MS/MS analysis. In the crop field trial, processing, and field rotational crop studies, the LOQ has been determined from the lowest spiking level with adequate recovery. Validated LOQs range from 0.01-0.05 ppm for 1*H*-1,2,4-triazole and 0.01-1.5 ppm for triazolylalanine and triazolylacetic acid. The calculated LODs range from 0.001 ppm to values that are greater than the reported LOQs for certain matrices. When the calculated LOD exceeded the reported LOQ, the LODs were set at the LOQ value.

Method validation and concurrent method recovery data for the method demonstrated generally acceptable accuracy/precision for barley (grain, hay, and straw), canola (seed, meal, and refined oil), mustard greens, dried shelled bean, dried shelled pea, peanut (nutmeat, hay, meal, refined oil, dry roasted peanuts, and peanut butter), rice (grain, straw, polished grain, bran, and hulls), turnip (top and root), and wheat (forage, hay, grain, straw, aspirated grain fractions, bran, flour, germ, middlings, and shorts). The spiking levels for these commodities range from 0.01-0.5 ppm for 1*H*-1,2,4-triazole, 0.01-4.5 ppm for triazolylalanine, and 0.01-0.8 ppm for triazolylacetic acid. Recovery ranges (and SD) from these matrices were 59-119% (11%) for

1*H*-1,2,4-triazole, 64-126% (10%) for triazolylalanine, and 67-119% (11%) for triazolylacetic acid.

The spiking levels and samples used in method validation are sufficiently representative of the expected residue levels for the plant commodities. Extraction efficiency has not been demonstrated for plant matrices at this time. The method is not being proposed for enforcement purposes, and as such, independent laboratory validation data is not required.

Conclusions: An HPLC-MS/MS method was developed and proposed for data gathering and enforcement purposes. In plant matrices, an oxidative extraction procedure converts prothioconazole residues to a mixture of desthio prothioconazole and prothioconazole sulfonic acid. The results are reported as prothioconazole equivalents. The method fulfilled the requirements with regards to specificity, accuracy and precision at the respective method limit of quantitation. Acceptable recoveries (70 to 120%) were obtained in plant matrices. Adequate extraction efficiencies were demonstrated using radiolabeled wheat forage, and sugarbeet tops analyzed with the enforcement method. The method has undergone a successful tolerance method validation by EPA ACL.

Based on information reported by the applicant, RAB3 concludes that Method No. 00598 and its modification 00598/M001 should not be used for data collection purposes.

Livestock commodity methods

Enforcement method: Bayer CropScience has proposed a high performance liquid chromatography (with electrospray ionization) and tandem mass spectrometry (LC-MS/MS) method for the data gathering and enforcement of maximum residue limits for residues of prothioconazole, prothioconazole desthio (JAU6476-desthio), prothioconazole-4-hydroxy (JAU6476-4-hydroxy), and conjugates that may be converted to these compounds by acid hydrolysis, in milk and cattle tissues.

Briefly, samples of bovine liver, kidney, and muscle are extracted with acetonitrile (ACN)/water and 25% aqueous L-cysteine HCl. An internal standard solution is added to the extract. The internal standard solution consists of a mixture of [triazole-$^{15}N_3$-$^{13}C_2$]-prothioconazole, [triazole-$^{15}N_3$-$^{13}C_2$]-prothioconazole desthio, and [triazole-$^{15}N_3$-$^{13}C_2$]-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 directly. 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 range from

0.0007-0.0021 ppm for milk, 0.001-0.0019 ppm for skim milk, 0.0021-0.0035 ppm for cream, 0.0006-0.001 ppm for muscle, 0.0005-0.0029 ppm for liver, 0.0021-0.0025 ppm for kidney, and 0.0041-0.0115 ppm for fat.

Method validation data (and concurrent recovery data from the livestock feeding study) for the proposed enforcement method has demonstrated adequate method recoveries of prothioconazole, prothioconazole desthio, and prothioconazole-4-hydroxy at 0.005 ppm (LOQ) and 0.010 ppm for milk; 0.010 ppm (LOQ) for skim milk, cream, and muscle; 0.010 ppm (LOQ) and 0.60 ppm for liver; 0.010 ppm (LOQ), 0.050 ppm, and 0.80 ppm for kidney; and 0.050 ppm (LOQ) and 0.080 ppm for fat. The range of recoveries (and CVs) are 77-115% (9.1%) for prothioconazole, 91-117% (6.0%) for prothioconazole desthio, and 63-117% (14.7%) for prothioconazole-4-hydroxy over all matrices and spiking levels. The spiking levels and samples used in method validation and concurrent method recovery are adequate to bracket expected residue levels in milk and livestock tissues for residues of prothioconazole, prothioconazole desthio, prothioconazole-4-hydroxy, and conjugates that may be converted to these compounds by acid hydrolysis.

Adequate extraction efficiency data have been submitted for the method using samples of goat milk, muscle, liver, and fat. Adequate independent laboratory validation data have been submitted for the method using samples of cattle milk and liver. Confirmatory analysis procedures have not been conducted for the proposed enforcement method.

The proposed enforcement method has been validated by EPA ACL. Acceptable recoveries were achieved for cattle liver fortified at 0.010 mg/kg with each of prothioconazole, prothioconazole desthio, and 4-hydroxy prothioconazole. Acceptable recoveries were also achieved for milk fortified with each of these three compounds at 0.005 mg/kg. However, the method needs to be modified to include at least two multiple reaction monitoring (MRM) transitions. A single MS/MS ion transition as used in the current version of the method is no longer considred sufficient for positive confirmation of the analyte residue.

Data collection methods: The proposed enforcement method was used for the determination of residues of prothioconazole, prothioconazole desthio, and prothioconazole-4-hydroxy in milk and cattle tissues from the prothioconazole dairy cattle feeding study.

Conclusions: An HPLC-MS/MS method was developed and proposed for data gathering and enforcement purposes. In livestock matrices, extracts are acid hydrolysed and residues are reported as prothioconazole equivalents (prothioconazole, prothioconazole desthio, prothioconazole-4-hydroxy). The method fulfilled the requirements with regards to specificity, accuracy and precision at the respective method limit of quantitation. Acceptable recoveries (70 to 120%) were obtained in animal matrices. Adequate extraction efficiencies were demonstrated using radiolabeled goat milk, goat liver goat muscle and goat fat analyzed with the enforcement method. The proposed enforcement method has undergone a successful tolerance method validation by EPA ACL.

860.1360 Multiresidue Methods

46246210.der.wpd

Bayer CropScience has submitted multiresidue method data for prothioconazole, the metabolites JAU6476-desthio and JAU6476-4-hydroxy, and the triazole-related compounds triazole, triazolylalanine, and triazolylacetic acid. The test substances were analyzed according to the FDA Multi-Residue Method Test guidelines in PAM Vol. I (dated 1/94). Prothioconazole, JAU6476-desthio, JAU6476-4-hydroxy, triazole and triazolylacetic acid were tested through Protocols A and C. As a result of Protocol C testing, prothioconazole, JAU6476-desthio, and JAU6476-4-hydroxy were tested through Protocol F. JAU6476-4-hydroxy and triazolylacetic acid were tested through Protocol B. Based on the results of the Protocol F testing, testing under Protocols D and E was not required for prothioconazole, and testing under Protocol E was not required for JAU6476-4-hydroxy. Because the test substances are not substituted ureas, no testing under Protocol G was required. A suitable solvent for triazolylalanine could not be found; therefore, testing of this compound could not be conducted.

Sensitivity for triazolylacetic acid was poor using Protocol A, and no response was obtained for the other test compounds. Protocol C testing indicated that further testing using Protocols D, E, and F was not required for triazolylacetic acid and triazole. Triazolylacetic acid and JAU6476-4-hydroxy could not be adequately recovered under Protocol B. Prothioconazole and JAU6476-4-hydroxy were not adequately recovered using the Florisil column cleanup steps of Protocol F, and JAU6476-4-hydroxy did not yield adequate chromatography using Protocol D; thus, no further testing of these compounds was conducted. JAU6476-desthio could not be adequately recovered under Protocols D or E, using wheat hay. Recovery of JAU6476-desthio was variable (66-100%) under Protocol F, using ground beef.

Conclusions: The multiresidue test data will be forwarded to FDA for further evaluation. Based on the results of the testing, the multiresidue methods are not appropriate for determining prothioconazole residues of concern, or for determining residues of triazole, triazolylalanine, or triazolylacetic acid.

860.1380 Storage Stability

46477701.der.wpd (also includes review of 860.1380 data in MRID 46246219)
46246139.der.wpd

Plant commodities

Bayer has submitted the results of three storage stability studies with prothioconazole and the metabolite prothioconazole desthio in plant commodities (MRID 46477701) as well as the results of a storage stability study with prothioconazole and the desthio metabolite in wheat commodities (MRID 46246139).

MRID 46477701: This submission reported results from three separate storage stability studies with prothioconazole and its desthio metabolite in plant commodities. The first study (which

will be referred to herein as Study 1) was initiated January 2001 at Battelle-AgriFood Laboratories. We note that partial results for this study were submitted as an appendix to the wheat crop field trial study submitted under DP Barcode D303508 (in MRID 46246219). In Study 1, samples of untreated canola seed, mustard greens, tomato, turnip root, and wheat forage, straw, hay, and grain were fortified with a mixed standard of prothioconazole and prothioconazole desthio (in a 1:1 ratio) at a total of 0.200 ppm expressed as parent equivalents. Samples were stored frozen (<-10°C) for up to ~35 months. Only limited information pertaining to sample preparation prior to storage was submitted. At the 3-month storage interval, samples were analyzed at Battelle, and at the final storage interval, samples were analyzed by Bayer. No zero-time analyses were conducted.

Because Study 1 did not include any zero-time analyses, Bayer initiated a second storage stability study (Study 2) in August 2004. In Study 2, samples of untreated canola seed, canola oil, mustard greens, tomato, tomato paste, turnip root, and wheat forage, grain, straw, bran and flour were separately spiked with prothioconazole or prothioconazole desthio at 0.250 ppm. Samples were stored frozen (<-15°C) for up to 12.7 months. The interim results of Study 2 were submitted, and the applicant has volunteered to submit interim data on analyses planned for up to 45 months.

To address the stability of weathered prothioconazole residues, Bayer also reanalyzed certain crop field trial samples from the first study after 30 to 46 months of frozen storage (Study 3). Samples of canola seed, barley hay, grain, and straw, dried peas, wheat forage, and wheat hay and straw from crop field trials submitted in conjunction with DP Barcode D303508 (MRIDs 46246215 and 46246219-46246221) were used. These samples were originally analyzed at Battelle within 3 months of collection and were reanalyzed at Bayer after 18-32 months of frozen storage because it was determined that the original analytical methods did not adequately extract weathered residues. For Study 3, Bayer reanalyzed these samples 12-15 months after the initial analyses at Bayer.

Samples that were analyzed at Battelle (canola seed, mustard greens, tomato, turnip root, and wheat forage, hay, straw, and grain from the ~3-month storage interval from Study 1, and samples of canola seed, barley hay, straw, and grain, and wheat forage, hay, and straw from the ~1- to 3-month storage interval from Study 3) were analyzed for combined residues of prothioconazole and prothioconazole desthio using LC-MS/MS Method 00598 or its modification Method 00598/M001. Samples that were analyzed at Bayer (canola seed, mustard greens, tomato, turnip root, and wheat forage, hay, straw, and grain from the ~35-month storage interval from Study 1, all samples from Study 2, and samples of canola seed, barley hay, straw, and grain, dried peas, and wheat forage, hay, and straw from the ~30- to 42-month storage interval from Study 3) were analyzed for total prothioconazole-derived residues (prothioconazole and the metabolite prothioconazole desthio) using the proposed enforcement method, LC-MS/MS method RPA JA/03/01. Because the applicant has indicated that LC-MS/MS method JA/03/01 is the preferred method for analysis of crop samples, sample results that were generated using Method No. 00598 or 00598/M001 will not be used to evaluate storage stability. Adequate concurrent method recovery data were submitted for both methods.

Based on the reported results from Study 1, combined residues of prothioconazole and prothioconazole desthio appear to be stable in/on wheat forage, hay, and straw stored frozen for up to ~35 months. Combined residues of prothioconazole and prothioconazole desthio were found to decline during frozen storage for ~35 months by ~18% in/on canola seed, ~13% in/on mustard greens, ~20% in/on tomato, ~17% in/on turnip root, and ~32% in/on wheat grain.

Based on the reported results from Study 2, JAU6476 was found to be stable for 12.5 to 12.7 months in canola oil (14% decomposition), canola seed (20% decomposition), mustard green (22% decomposition), tomato fruit (14% decomposition), turnip roots (0% decomposition), wheat flour (12% decomposition), wheat forage (16% decomposition), wheat grain (27% decomposition), and wheat straw (15% decomposition). JAU6476 showed 33% and 36% decomposition in tomato paste and wheat bran, respectively. However, the JAU6476 plant metabolism studies in three dissimilar crops have shown that JAU6476 is expected to contribute only 0 to 7% (0 to 20% normalized) of the total residues measured in the field crop residue studies. Therefore, the apparent slight instability of JAU6476 in tomato paste and wheat bran would not be expected to have any significant effect on the total JAU6476 (JAU6476 plus JAU6476-desthio) residue levels measured in the field crop residue studies. JAU6476-desthio, the major residue anticipated in crop matrices, was found to be stable in all matrices after 12.5 to 12.7 months of freezer storage. Percent decomposition of JAU6476-desthio was equal to or less than 5% in all matrices. JAU6476-desthio would be expected to contribute 6 to 58% (80 to 100% normalized) of the residues measured in the JAU6476 field crop residue trials.

Based on the reported results from Study 3, weathered total prothioconazole-derived residues appear to be stable in/on barley hay, straw, and grain stored frozen for ~13 months beyond initial analyses at Bayer, in/on dried peas stored frozen for 12 months beyond initial analyses, and in/on wheat hay and straw stored frozen for ~15 months beyond initial analyses. Residues were found to decline ~14% in/on canola seed stored frozen for ~12 months beyond initial analyses at Bayer and ~13% in/on wheat forage stored frozen for ~15 months beyond initial analyses. Initial analyses at Bayer were conducted 29-32 months after sample collection for barley, canola, and wheat commodities and 18 months after sample collection for dried peas.

MRID 46246139: Bayer submitted the results of storage stability studies with prothioconazole and prothioconazole desthio. Untreated samples of wheat forage, grain, hay, and straw were separately spiked with prothioconazole and prothioconazole desthio and stored frozen (<-18°C) for up to ~36 months (1088 days; wheat grain) and ~37 months (1126-1128 days; wheat forage and straw). The tested commodities were spiked by spraying the samples with the fortification solution; samples were then homogenized prior to frozen storage. The actual spiking level was determined by analyzing 0-day samples. The results of the storage stability study indicate that under these conditions, residues of prothioconazole are stable in/on wheat forage, grain, and straw for up to ~4, ~6.5, and ~19 months, respectively, before a decrease of $\geq$30% is observed; at the final storage interval, residues had decreased by an average of 50%, 38%, and 31% in forage, grain, and straw, respectively. Residues of prothioconazole desthio were found to be stable for ~36 months in/on wheat grain and ~37 months in/on wheat forage and straw.

Samples of wheat forage, grain, and straw were analyzed for residues of prothioconazole and prothioconazole desthio using LC-MS/MS Method No. 00598. The reported LOQ was 0.01

ppm for wheat grain and 0.05 ppm for wheat forage and straw. Based on acceptable concurrent method recovery data, this method appears to be adequate for data collection. However, the applicant reported in a separate submission (MRID 46477701) that this method was found to be inadequate for determination of weathered residues of prothioconazole.

Storage intervals and conditions of samples from the submitted studies: The storage intervals and conditions of samples from the submitted crop field trial, processing, and field rotational crop studies are presented in Table 3. The reported storage duration represents the interval from sample collection to analysis.

Table 3. Summary of Storage Intervals and Conditions.		
Matrix	Storage Temp. (°C)	Actual Storage Duration
		Total Prothioconazole-Derived Residues
Crop Field Trials; MRIDs 46246215-46246217 and 46246219-46246221		
Barley grain	-30.0 to -4.8	824-1234 days (27.1-40.6 months)
Barley hay	-30.0 to -4.8	859-1269 days (28.2-41.7 months)
Barley straw	-30.0 to -4.8	825-1240 days (27.1-40.8 months
Bean, dried shelled	-24 to -22	490-536 days (16.1-17.6 months)
Canola seed	-30.0 to -4.8	867-1265 days (28.5-41.6 months)
Pea, dried shelled	-24 to -22	494-542 days (16.2-17.8 months)
Peanut nutmeat	-30.0 to -4.8	1175-1214 days (38.6-39.9 months)
Peanut hay	-30.0 to -4.8	1173-1212 days (38.6-39.8 months)
Rice grain	-30.0 to -4.8	1135-1240 days (37.3-40.8 months)
Rice straw	-30.0 to -4.8	1120-1226 days (36.8-40.3 months)
Wheat hay	-30.0 to -4.8	871-1221 days (28.6-40.1 months)
Wheat grain	-30.0 to -4.8	873-1214 days (28.7-39.9 months)
Wheat straw	-30.0 to -4.8	854-1203 days (28.1-39.5 months)
Wheat forage	-30.0 to -4.8	181-469 days (6.0-15.4 months)
Processing Studies; MRIDs 46246218 and 46246222-46246224		
Canola seed	-30.0 to -4.8	1261 days (41 months)
Canola meal and refined oil		918 days (30 months)
Peanut nutmeat	-30.0 to -4.8	1090 days (36 months)
Peanut meal, refined oil, dry roasted peanuts, and peanut butter		911 days (31 months)
Rice grain	<-5	1222 days (40 months)
Rice polished grain, bran, and hulls		902 days (30 months)
Wheat grain	<-5	1285 days (42 months)
Wheat aspirated grain fractions, bran, germ, flour, middlings, and shorts		909 days (30 months)

Field Rotational Crop Study; MRID 46246227		
Mustard greens	-30.0 to -4.8	1135-1263 days (37.3-41.5 months)
Turnip tops	-30.0 to -4.8	1136-1243 days (37.3-40.8 months)
Turnip roots	-30.0 to -4.8	1136-1243 days (37.3-40.8 months)
Wheat forage	-30.0 to -4.8	952-1002 days (31.3-32.9 months)
Wheat hay	-30.0 to -4.8	893-943 days (29.3-31.0 months)
Wheat grain	-30.0 to -4.8	869-919 days (28.6-30.2 months)
Wheat straw	-30.0 to -4.8	861-911 days (28.3-29.9 months)

Conclusions: The available storage stability data are tentatively adequate to support the storage intervals and conditions of samples from the submitted crop field trial, processing, and field rotational crop studies. The final reports of the ongoing storage stability studies with prothioconazole and prothioconazole desthio (interim results for which were reported in MRID 46477701) must be submitted as confirmatory data.

For the storage stability data reported in MRID 46477701 for prothioconazole and prothioconazole desthio, HED concludes that the ongoing study (Storage Stability Study 2) will provide the most information about any actual decline of prothioconazole or prothioconazole desthio residues in crop matrices because it includes more than one sampling interval. The results of the other two studies reported in that submission only reflect one sampling interval. The applicant chose the tested matrices in Studies 1 and 2 of that submission to be representative of five diverse crops [an oilseed (canola), a non-oily grain (wheat), a leafy vegetable (mustard greens), a root crop (turnip), and a fruiting vegetable (tomato)] as well as the processed commodities of three crops [an oilseed, a fruiting vegetable, and a non-oily grain]. Even though JAU6476 appears to be slightly unstable in two matrices (tomato paste, wheat bran), the overall impact on the crop residues will not be significant. However, based on OPPTS 860.1380, the Agency will consider corrections on a case-by-case basis, taking into account factors such as the absolute (ppm) and relative (% ROC) residue levels of the component that is unstable in storage. Therefore, correction for dissipation of prothioconazole-derived residues during freezer storage will not be necessary at this time.

Because the applicant has reported that Method No. 00598 is not adequate for determination of weathered residues of prothioconazole, the results of the storage stability study reported in MRID 46246139 will not be used to evaluate the stability of prothioconazole residues in wheat commodities during frozen storage.

Animal commodities

The storage intervals for all matrices from the cattle feeding studies, except fat from the prothioconazole feeding study, were reported to be <30 days. Because samples were stored frozen prior to analysis and analyzed within 30 days of collection, supporting storage stability data are not needed for milk and tissues except fat. Fat samples from the lowest feeding level (0.5-fold) were stored frozen for up to 86 days prior to analysis. For the 1.4-fold and 4.7-fold feeding levels, samples were stored for 43 and 37 days, respectively. A supporting storage stability study indicated that residues of prothioconazole, prothioconazole desthio, and prothioconazole-4-hydroxy were stable for 27 days (<30% decline). After 89 days in storage, low concurrent recoveries of prothioconazole-4-hydroxy residues were observed at the 0.5-fold feeding level indicating a 33% apparent decline. The 1.4-fold dose group (29.5 ppm) was closest to the anticipated dietary burden (21 ppm).

Conclusions: The applicant will repeat a storage stability study in fat samples for prothioconazole and the prothioconazole-4-hydroxy metabolite for a period of 45 days at the 1.4-fold and 4.7-fold feeding level as confirmatory data. A report will be submitted to EPA. Therefore, correction for dissipation of prothioconazole-derived residues and the prothioconazole4-OH in fat during freezer storage will not be necessary at this time.

860.1400 Water, Fish, and Irrigated Crops

There are no proposed uses that are relevant to this guideline topic.

860.1460 Food Handling

There are no proposed uses that are relevant to this guideline topic.

860.1480 Meat, Milk, Poultry, and Eggs

46246213.der.wpd (Cattle)
46246214.der.wpd (Cattle)

The applicant submitted two cattle feeding studies with the subject petition, one in which cattle were dosed with prothioconazole (MRID 46246213) and one in which cattle were dosed with prothioconazole desthio (MRID 46246214). The second study was considered supplemental. The maximum theoretical dietary burden of prothioconazole to livestock is presented in Table 4.

Table 4. Calculation of Maximum Dietary Burdens of Prothioconazole to Livestock.

				Maximum % of Diet				% of Diet Used				Dietary Burden, ppm			
Crop	Commodity	Residue	%DM	Beef	Dairy	Poultry	Swine	Beef	Dairy	Poultry	Swine	Beef	Dairy	Poultry	Swine
Grain	Aspirated grain fractions	11.025	85	20	20	--	20	20	20	--	20	2.59	2.59	0.00	2.21
Wheat	forage	6.987	25	25	60	--	--	25	60	--	--	6.99	16.77	0.00	0.00
Barley	Hay	6.59	88	25	60	--	--	25	20	--	--	1.87	1.50	0.00	0.00
Peanut	Hay	4.458	85	25	50	--	--	25	0	--	--	1.31	0.00	0.00	0.00
Wheat	Hay	3.571	88	25	60	--	--	5	0	--	--	0.20	0.00	0.00	0.00
Wheat	Straw	1.96	88	10	10	--	--	0	0	--	--	0.00	0.00	0.00	0.00
Barley	Straw	1.871	89	10	10	--	--	0	0	--	--	0.00	0.00	0.00	0.00
Rice	Straw	1.277	90	10	10	--	--	0	0	--	--	0.00	0.00	0.00	0.00
Rice	Hulls	0.8404	90	10	10	15	--	0	0	15	--	0.00	0.00	0.13	0.00
Cowpea	Seed	0.684	88	20	20	10	50	0	0	10	50	0.00	0.00	0.07	0.34
Pea, field	Seed	0.684	90	20	20	20	20	0	0	20	20	0.00	0.00	0.14	0.14
Rice	Bran	0.222	90	15	15	25	15	0	0	25	10	0.00	0.00	0.06	0.02
Rice	Grain	0.222	88	40	40	60	65	0	0	30	0	0.00	0.00	0.07	0.00
Barley	Grain	0.158	88	50	40	75	80	0	0	0	0	0.00	0.00	0.00	0.00
Peanut	Meal	0.158	85	15	15	25	15	0	0	0	0	0.00	0.00	0.00	0.00
Wheat	Milled byproducts	0.108	88	40	50	50	50	0	0	0	0	0.00	0.00	0.00	0.00
Canola	Meal	0.097	88	15	15	15	15	0	0	0	0	0.00	0.00	0.00	0.00
Wheat	Grain	0.061	89	50	40	80	80	0	0	0	0	0.00	0.00	0.00	0.00
							Total	100	100	100	100	12.97	20.86	0.45	2.71

Cattle - MRID 46246213: Prothioconazole was administered orally (via gelatin capsules) to three groups of dairy cattle (3 cows per group) once daily for 29 consecutive days. Dosing was made at levels equivalent to 9.9, 29.5, and 98.4 ppm in the feed. The dosing levels correspond to 0.5-fold, 1.4-fold, and 4.7-fold the maximum theoretical dietary burden. Milk and tissue samples were analyzed using the proposed enforcement method for animal commodities, which was an LC-MS/MS method (Bayer Report No. 200537). This method determined residues of prothioconazole, prothioconazole desthio, and prothioconazole-4-hydroxy, plus any metabolites hydrolysable to these compounds. Tissue samples (except fat) were extracted with acetonitrile (ACN)/water and aqueous L-cysteine HCl. Fat samples were first extracted with n-hexane, then ACN, L-cysteine HCl and acetone. All samples (including milk and cream) were hydrolyzed with aqueous HCl, then partitioned with methylene chloride and acetone before LC-MS/MS analysis. The method was adequate for data collection based on acceptable concurrent method recovery data. The storage intervals for all matrices except fat were reported to be <30 days; therefore no storage stability data is needed for these matrices. Fat samples from the lowest feeding level (0.5-fold) were stored frozen for up to 86 days prior to analysis. For the 1.4-fold and 4.7-fold feeding levels, samples were stored for 43 and 37 days, respectively. A supporting storage stability study indicated that residues of prothioconazole, prothioconazole desthio, and prothioconazole-4-hydroxy were stable for 27 days (<30% decline). After 89 days in storage, residues of prothioconazole-4-hydroxy showed a 33% decline. The 1.4-fold dose group (29.5 ppm) was closest to the anticipated dietary burden (21 ppm). Confirmatory data will be

generated to confirm the stability of the prothioconazole-4-hydroxy in fat for a duration of 45 days.

The maximum residues of prothioconazole, prothioconazole desthio, and prothioconazole-4-hydroxy in milk and tissues are listed in Table 5 below. Because low residue levels were observed in samples from the mid and high dose groups, milk and muscle samples from the low dose group (9.9 ppm) were not analyzed.

Table 5. Maximum Residues of Prothioconazole (A), Prothioconazole desthio (B), and Prothioconazole-4-hydroxy (C), as prothioconazole equivalents, by Feeding Level Following Dosing of Dairy Cattle with Prothioconazole for 29 Days.

Matrix	Residues (ppm)								
	9.9 ppm			29.5 ppm			98.4 ppm		
	A	B	C	A	B	C	A	B	C
Milk (day 29)	--	--	--	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005
Skim milk	--	--	--	--	--	--	<0.01	<0.01	<0.01
Cream	--	--	--	--	--	--	<0.01	<0.01	<0.01
Fat	<0.05	<0.05	<0.05	<0.05 (0.019)[1]	<0.05 (≈0.003)[1]	<0.05 (≈0.006)[1]	0.062	<0.05	<0.05
Kidney	0.062	<0.01	0.017	0.176	<0.01	0.063	0.79	0.011	0.356
Liver	0.063	<0.01	0.054	0.120	0.011	0.181	0.467	0.030	0.518
Muscle	--	--	--	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

[1] At/near the limit of *detection*; in fat, 0.012 ppm for prothioconazole, 0.005 ppm for prothioconazole desthio, 0.008 ppm for prothioconazole-4-hydroxy.

Quantifiable residues (of prothioconazole, 0.005 - 0.006 ppm) were observed in only two samples of milk (over the entire dosing period) from the highest dosing level. However, detectable residues of prothioconazole were observed in several samples. Based on these residues, it appeared that residues had reached a plateau within the first week of dosing.

Cattle – MRID 46246214:

Bayer CropScience has submitted a supplemental dairy cattle feeding study with *prothioconazole-desthio, a metabolite of prothioconazole*. Three groups of dairy cattle (3 cows per group) were dosed orally with prothioconazole-desthio at levels equivalent to 5.1, 29, and 125 ppm in the feed. The dosing levels correspond 0.24x, 1.4x, and 6.0x the anticipated dietary burden (Table 4 above). Cattle were dosed once a day for 29 consecutive days. Cows were milked twice daily, and composited daily samples from Study Days 1, 4, 6, 8, 11, 13, 15, 18, 20, 22, 25, 27, 28, and 29 (mid and high dose groups only) were collected for analysis from each cow. Cattle were sacrificed within 17 hours of the last dose, and samples of composite fat (omental and perirenal), liver, kidneys, and composite muscle (loin, elbow and flank) were collected.

Milk and tissues samples were analyzed using an LC/MS/MS method (Method No. 00655 and its modification 00655/M001). This method determines residues of prothioconazole desthio , prothioconazole desthio-3-hydroxy, and prothioconazole desthio-4-hydroxy and compounds that may be converted to these compounds by acid hydrolysis. The validated LOQ was 0.004 ppm for each analyte in milk and 0.010 ppm for each analyte in tissues; the calculated LODs ranged 0.0001-0.0004 ppm. The method is adequate for data collection based on acceptable concurrent method recovery data.

Table 6 summarizes the maximum concentrations of prothioconazole desthio (B), prothioconazole desthio-4-hydroxy (D), and desthio-3-hydroxy (E) found in milk (29 days) and tissues. The total residue appears to have reached a plateau in whole milk by day 10.

Table 6. Maximum Residues of Prothioconazole desthio (B), Prothioconazole desthio-4-hydroxy (D), and Prothioconazole desthio-3-hydroxy (E), as prothioconazole equivalents, by Feeding Level Following Dosing of Dairy Cattle with Prothioconazole desthio for 29 Days.

Matrix	Residues (ppm)								
	5.1 ppm			29.0 ppm			125 ppm		
	B	D	E	B	D	E	B	D	E
Whole Milk (day 29)	--	--	--	<0.004	<0.004	<0.004	<0.004	0.0043	0.0115
Fat	<0.01	<0.01	<0.01	0.011	<0.01	<0.01	0.091	0.024	0.030
Kidney	<0.01	0.019	<0.01	0.033	0.085	0.064	0.237	0.383	0.477
Liver	0.030	<0.01	0.013	0.178	0.037	0.055	1.19	0.171	0.300
Muscle	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.013

[1] Residues of each analyte are expressed as prothioconazole desthio equivalents. The LOQ for each analyte was 0.004 ppm in milk and 0.01 ppm in tissues.

The concentration of prothioconazole desthio (B) at the 29 ppm feeding level is less than or equal to the concentration of prothioconazole from the prothioconazole feeding study at 29 ppm for all commodities except liver. For liver, the concentration of prothioconazole desthio from the 29 ppm prothioconazole desthio feeding study is slightly higher than the combined concentration of prothioconazole and prothioconazole desthio from the 29 ppm prothioconazole feeding study, 0.18 ppm versus 0.13 ppm. The metabolites 4-hydroxy desthio prothiconazole and 3-hydroxy prothioconazole desthio from the prothioconazole desthio feeding study do not contribute significantly to the residue in the prothioconazole desthio feeding study for milk, fat, and muscle. For kidney, these metabolites contribute significantly (0.15 ppm combined versus 0.033 ppm prothioconazole desthio), but the combined residue (0.18 ppm) compares to the 0.18 ppm prothioconazole from the prothioconazole feeding study at 29 ppm. For liver, these metabolites are about 50% of the prothioconazole desthio (from the prothioconazole desthio feeding study at 29 ppm). The combined residue of prothioconazole desthio, 4-hydroxy prothioconazole desthio, and 3-hydroxy prothioconazole (0.27 ppm) is less than the combined residue of prothioconazole, prothioconazole desthio, and 4-hydroxy prothioconazole (0.31 ppm) from the prothioconazole feeding study at 29 ppm.

Conclusions: The submitted cattle study data are adequate to satisfy livestock feeding study data requirements for ruminants.

The feeding study data indicate that tolerances are needed for the combined residues of prothioconazole and prothioconazole desthio in cattle, goat, hog, horse, and sheep commodities. Generally, the prothioconazole and prothioconazole desthio feeding studies will provide similar estimates of the tolerances for livestock commodities.

Using the results of the prothioconazole feeding study and defining the residue for tolerance purposes as the sum of prothioconazole, prothioconazole desthio, and metabolites that can be hydrolyzed to these compounds, calculated as prothioconazole, HED calculates that tolerances should be set at the combined LOQs for milk (0.02 ppm, using LOQs for skim milk and cream); at the combined LOQs for the fat (0.10 ppm) and muscle (0.02 ppm) of cattle, goats, horses, and sheep; at 0.20 ppm for the meat byproducts of cattle, goats, horses, and sheep; and at 0.05 ppm for the meat byproducts of hogs. The available data indicate that a tolerance is not needed for milk fat or the fat and meat of hogs.

Poultry: The applicant did not submit a poultry feeding study with the subject petition but submitted a request for a waiver from the requirements for a poultry feeding study.

The applicant used a value of 0.455 ppm as the maximum theoretical dietary burden for poultry, based on a diet consisting of 15% rice hulls, 60% rice grain, and 25% barley grain (and using "anticipated" tolerance values of 1.5, 0.3, and 0.2 ppm, respectively). This agrees with the 0.45 ppm calculated in Table 4 above, resulting from the feeding of rice and pea commodities. To determine the residues that would be found by the proposed enforcement method for livestock commodities, it can be assumed that residues of JAU6476-glucuronide (N-, S- or O-glucuronides) would be converted to prothioconazole. Under that assumption, the combined residues for prothioconazole and prothioconazole desthio from the metabolism studies (171 ppm and 163 ppm in diet, or about 360X) were: liver, 1.7 and 1.8 ppm; egg, 0.014 and 0.017 ppm; muscle, 0.031 and 0.018 ppm; fat, 0.29 and 0.14 ppm. The residues at a feeding level of 0.455 ppm can be estimated to be: liver, 0.005 ppm; egg, 0.00005 ppm; muscle, 0.00009 ppm; fat, 0.0008 ppm. The enforcement analytical method for livestock commodities has not been validated for poultry items. Assuming that the method has a LOQ of 0.01 ppm for each of the two analytes for poultry commodities (combined LOQ 0.02 ppm, based on an LOQ of 0.01 ppm for each analyte in cattle liver), no residue would be anticipated at the defined feeding level in any of the poultry commodities, except liver. Residues appeared *not* to have obtained a plateau in eggs, as the TRR in the oviduct egg was about 10X that in the eggs. Factoring in a 10X increase in residue in the egg would not lead to a prediction of detectable residues in eggs at a feeding level of 0.45 ppm prothioconazole in the diet

Under the currently proposed uses and defining the residue for tolerance purposes as the sum of prothioconazole, prothioconazole desthio, and metabolites that can be hydrolyzed to these compounds, calculated as prothioconazole, HED *provisionally* concludes that residues are unlikely in poultry commodities except liver and that, therefore, poultry commodity tolerances are not needed, except liver. A conditional tolerance of 0.02 ppm should be proposed for poultry liver, based on the validated LOQ of 0.01 ppm for each of the analytes in cattle liver.

The extreme extrapolation required (360X) and the short interval of the poultry metabolism study (3 days) make the conclusions on the need for poultry tolerances tentative. Therefore, a poultry feeding study and fully validated analytical method for poultry commodities are required as conditions of the registration of prothioconazole.

860.1500 Crop Field Trials

46246215.der.wpd (Canola)
46246216.der.wpd (Rice)
46246217.der.wpd (Peanut)
46246219.der.wpd (Wheat)
46246220.der.wpd (Barley)
46246221.der.wpd (Dried pea and bean)

Table 7. Summary of Residues from the Crop Field Trials with Prothioconazole.

Crop Matrix	Applic. Rate (lb ai/A) [kg ai/ha]	PHI (days)	Residues (ppm)						
			n	Min.	Max.	HAFT[1]	Median	Mean	Std. Dev.
BARLEY (proposed use = 0.293 lb ai/A [0.328 kg ai/ha] total application rate, 30-day PHI)									
Total Prothioconazole-Derived Residues									
Barley grain	0.286-0.309 [0.321-0.348]	30-71	49	<0.02	0.158	0.151	0.022	0.040	0.041
Barley hay	0.286-0.309 [0.321-0.348]	12-16	49	0.317	6.59	5.95	1.22	1.72	1.39
Barley straw	0.286-0.309 [0.321-0.348]	30-71	50	<0.05	1.871	1.65	0.304	0.554	0.510
1,2,4-Triazole Residues									
Barley grain	0.286-0.309 [0.321-0.348]	30-71	50	<0.01	<0.01	<0.01	0.005	0.005	0.0
Barley hay	0.286-0.309 [0.321-0.348]	12-16	49	<0.01	<0.01	<0.01	0.005	0.005	0.0
Barley straw	0.286-0.309 [0.321-0.348]	30-71	50	<0.01	<0.01	<0.01	0.005	0.005	0.0
Triazole Conjugate Residues									
Barley grain	0.286-0.309 [0.321-0.348]	30-71	50	<0.10	0.915	0.909	0.239	0.300	0.215
Barley hay	0.286-0.309 [0.321-0.348]	12-16	49	<0.05	0.547	0.445	0.135	0.134	0.104
Barley straw	0.286-0.309 [0.321-0.348]	30-71	50	<0.1	0.385	0.359	0.05	0.090	0.093
CANOLA (proposed use = 0.356 lb ai/A [0.400 kg ai/ha] total application rate, 36-day PHI)									
Total Prothioconazole-Derived Residues									
Canola seed	0.35-0.37 [0.39-0.42]	36-83	44	<0.020	0.097	0.086	0.010	0.015	0.0169
1,2,4-Triazole Residues									

Table 7. Summary of Residues from the Crop Field Trials with Prothioconazole.									
Crop Matrix	Applic. Rate (lb ai/A) [kg ai/ha]	PHI (days)	Residues (ppm)						
			n	Min.	Max.	HAFT[1]	Median	Mean	Std. Dev.
Canola seed	0.35-0.37 [0.39-0.42]	36-83	44	<0.020	<0.020	<0.020	0.010	0.010	0
Triazole Conjugate Residues									
Canola seed	0.35-0.37 [0.39-0.42]	36-83	44	0.064	0.848	0.716	0.311	0.321	0.124
DRIED PEA AND BEAN (proposed use = 0.534 lb ai/A [0.600 kg ai/ha] total application rate, 7-day PHI)									
Total Prothioconazole-Derived Residues									
Pea, dried shelled	0.530-0.549 [0.595-0.615]	7-8	26	<0.05	0.684	0.661	0.025	0.156	0.219
Bean, dried shelled	0.534-0.580 [0.598-0.650]	7-8	20	<0.05	0.288	0.243	0.025	0.062	0.072
1,2,4-Triazole Residues									
Pea, dried shelled	0.530-0.549 [0.595-0.615]	7-8	26	<0.01	0.011	0.01	0.005	0.005	0.001
Bean, dried shelled	0.534-0.580 [0.598-0.650]	7-8	20	<0.01	<0.01	<0.01	0.005	0.005	0.0
Triazole Conjugate Residues									
Pea, dried shelled	0.530-0.549 [0.595-0.615]	7-8	26	<0.05	0.789	0.775	0.085	0.177	0.213
Bean, dried shelled	0.534-0.580 [0.598-0.650]	7-8	20	<0.02	0.311	0.249	0.045	0.080	0.093
PEANUT (proposed use = 0.713 lb ai/A [0.800 kg ai/ha] total application rate, 14-day PHI)									
Total Prothioconazole-Derived Residues									
Peanut nutmeat	0.707-0.734 [0.792-0.823]	13-15	24	<0.02	<0.02	<0.02	0.01	0.01	0.0
Peanut hay	0.707-0.734 [0.792-0.823]	13-15	24	0.989	4.458	3.630	2.657	2.612	0.884
1,2,4-Triazole Residues									
Peanut nutmeat	0.707-0.734 [0.792-0.823]	13-15	24	<0.02	0.02	<0.02	0.01	0.01	0.0
Peanut hay	0.707-0.734 [0.792-0.823]	13-15	24	<0.02	<0.02	<0.02	0.01	0.01	0.0
Triazole Conjugate Residues									
Peanut nutmeat	0.707-0.734 [0.792-0.823]	13-15	24	0.162	3.903	3.390	0.827	1.158	1.127
Peanut hay	0.707-0.734 [0.792-0.823]	13-15	24	<0.10	1.278	1.244	0.176	0.323	0.361
RICE (proposed use = 0.285 lb ai/A [0.320 kg ai/ha] total application rate, 40-day PHI)									
Total Prothioconazole-Derived Residues									

Table 7. Summary of Residues from the Crop Field Trials with Prothioconazole.									
Crop Matrix	Applic. Rate (lb ai/A) [kg ai/ha]	PHI (days)	Residues (ppm)						
			n	Min.	Max.	HAFT[1]	Median	Mean	Std. Dev.
Rice, grain	0.34-0.37 [0.38-0.41]	40-67	32	<0.02	0.222	0.191	0.01	0.031	0.048
Rice, straw	0.34-0.37 [0.38-0.41]	40-67	32	<0.05	1.277	1.189	0.432	0.464	0.319
1,2,4-Triazole Residues									
Rice, grain	0.34-0.37 [0.38-0.41]	40-67	32	<0.01	<0.01	<0.01	0.005	0.005	0.0
Rice, straw	0.34-0.37 [0.38-0.41]	40-67	32	<0.01	<0.01	<0.01	0.005	0.005	0.0
Triazole Conjugate Residues									
Rice, grain	0.34-0.37 [0.38-0.41]	40-67	32	<0.05	0.571	0.553	0.025	0.103	0.148
Rice, straw	0.34-0.37 [0.38-0.41]	40-67	32	<0.05	0.506	0.478	0.025	0.088	0.122
WHEAT (proposed use = 0.293 lb ai/A [0.328 kg ai/ha] total application rate, 30-day PHI)									
Total Prothioconazole-derived Residues									
Wheat hay	0.281-0.313 [2] [0.315-0.350]	12-17	66	0.288	3.571	3.543	1.269	1.420	0.970
Wheat grain	0.281-0.313 [2] [0.315-0.350]	10; 30-57	66	<0.02	0.061	0.045	0.010	0.014	0.011
Wheat straw	0.281-0.313 [2] [0.315-0.350]	10; 30-57	64	0.106	1.96	1.899	0.350	0.577	0.471
Wheat forage	0.286-0.299 [0.320-0.336]	7	46	0.061	6.987	5.842	1.352	1.401	1.268
1,2,4-Triazole Residues									
Wheat hay	0.281-0.313 [2] [0.315-0.350]	12-17	66	<0.01	<0.01	<0.01	0.005	0.005	0.0
Wheat grain	0.281-0.313 [2] [0.315-0.350]	10; 30-57	66	<0.01	<0.01	<0.01	0.005	0.005	0.0
Wheat straw	0.281-0.313 [2] [0.315-0.350]	10; 30-57	66	<0.01	<0.01	<0.01	0.005	0.005	0.0
Wheat forage	0.286-0.299 [0.320-0.336]	7	46	<0.01	<0.01	<0.01	0.005	0.005	0.0
Triazole Conjugate Residues									
Wheat hay	0.281-0.313 [2] [0.315-0.350]	12-17	66	0.018	0.665	0.631	0.204	0.220	0.124
Wheat grain	0.281-0.313 [2] [0.315-0.350]	10; 30-57	66	0.098	1.76	1.76	0.460	0.534	0.320
Wheat straw	0.281-0.313 [2] [0.315-0.350]	10; 30-57	64	<0.025	0.495	0.449	0.063	0.095	0.104

Table 7. Summary of Residues from the Crop Field Trials with Prothioconazole.									
Crop Matrix	Applic. Rate (lb ai/A) [kg ai/ha]	PHI (days)	Residues (ppm)						
			n	Min.	Max.	HAFT[1]	Median	Mean	Std. Dev.
Wheat forage	0.286-0.299 [0.320-0.336]	7	46	<0.01	0.175	0.173	0.038	0.050	0.042

[1] HAFT = Highest Average Field Trial.

[2] In one field trial, the total application rate was 0.375 lb ai/A (0.420 kg ai/ha); we note that this trial did not include maximum residues for any of the metabolites.

Barley: Bayer CropScience has submitted field trial data on barley from field trials conducted in the U.S. and Canada. A total of 25 five trials were conducted in Regions 1 (PA; 1 trial), 5 (ND; 2 trials, ON; 1 trial), 5B (QC; 1 trial), 7 (ND; 3 trials, and SK; 1 trial), 9 (AZ; 1 trial), 10 (AZ; 1 trial), 11 (ID and OR; 2 trials) and 14 (AB; 4 trials, MB; 4 trials, and SK; 4 trials) during the 2000-2001 growing season. The number and locations of field trials are in accordance with OPPTS Guideline 860.1500 and Directive 98-02; Section 9.

At each test location, two broadcast foliar applications of the 4 lb/gal (480 g/L) suspension concentrate formulation (flowable concentrate; FC) were made to barley at ~0.11-0.18 lb a.i./A (~0.123-0.202 kg a.i./ha) at an average 12-day retreatment interval, for a total seasonal application rate of ~0.29 lb a.i./A (~0.33 kg a.i./ha). Applications were made in ~5-43 gal/A (~45-407 L/ha) of water using ground equipment. An adjuvant was not added to the spray mixture for any applications. Barley hay was cut at 23 test sites, 12-16 days after treatment, and was left in the field for 1-14 days prior to collection of barley hay. Samples of barley grain and straw were harvested at 23 test sites 30-71 days after the last application. At two locations, additional samples were collected to determine residue decline. In the decline trial performed in Region 7 (Northwood, ND), samples were harvested 8, 13, 22, and 28 days after treatment for barley hay and 32, 37, 44, and 47 days after treatment for barley grain and straw. In the decline trial performed in Region 5 (Branchton, ON), samples were harvested 9, 14, 21, and 29 days after treatment for barley hay and 36, 39, 45, and 49 days after treatment for barley grain and straw.

Samples were analyzed for total prothioconazole and the metabolite prothioconazole desthio using LC-MS/MS method RPA JA/03/01. The validated LOQs for the total combined prothioconazole and prothioconazole desthio residues were 0.02 ppm for barley grain and 0.05 ppm for barley hay and straw. The method is adequate for data collection for barley grain, hay and straw based on acceptable concurrent method recovery data and method validation data. Samples were analyzed for residues of 1*H*-1,2,4-triazole and the triazole conjugates triazolylalanine and triazolylacetic acid using an LC-MS/MS method (Bayer Report No. G200598). The validated LOQ for 1*H*-1,2,4-triazole was 0.01 ppm for barley grain, hay and straw, and the validated LOQs for the triazole conjugates were 0.10 ppm for barley grain and straw, and 0.05 ppm for hay. The methods are adequate for data collection for barley matrices based on acceptable concurrent method recovery data.

In barley matrices harvested 30-71 days (12-16 days for hay), total combined prothioconazole and prothioconazole desthio residues were 0.158 ppm, 6.59 ppm and 1.87 ppm, respectively,

in/on barley grain, hay, and straw. Residues of 1*H*-1,2,4-triazole were less than the LOQ (<0.01 ppm) in/on barley grain, hay, and straw; and 0.915 ppm, 0.547 ppm and 0.385 ppm, respectively, in/on barley grain, hay, and straw for the triazole conjugates. Total combined prothioconazole and prothioconazole desthio residues did not increase with increasing sampling intervals in barley grain, hay, and straw, and residues of the triazole conjugates did not increase in grain, but increased slightly with increasing sampling intervals in samples from one trial each for hay and straw.

The maximum storage intervals of crop samples from harvest to analysis for total combined residues of prothioconazole and prothioconazole desthio were 1234 days (40.6 months) for barley grain and 1269 days (41.7 months) for barley hay and straw. The degree of loss of combined prothioconazole and prothioconazole desthio residues (determined as prothioconazole sulfonic acid and prothioconazole desthio) and prothioconazole desthio residues is not expected to exceed 30% after 42 months in barley grain, hay and straw.

Canola: Bayer CropScience has submitted field trial data on canola from field trials conducted in the U.S. and Canada. A total of 22 trials were conducted in Regions 2 (GA; 1 trial), 5 (ND; 1 trial, and ON; 1 trial), 7 (ND; 1 trial, and SK; 1 trial), 11 (ID; 3 trials), and 14 (AB; 4 trials, MB; 5 trials, and SK; 5 trials) during the 2000 growing season. The number and locations of field trials were in accordance with OPPTS Guideline 860.1500 and Directive 98-02; Section 9.

At each test location, two broadcast foliar applications of the 4 lb/gal (480 g/L) suspension concentrate formulation (flowable concentrate; FC) were made to canola at 0.17-0.19 lb a.i./A (0.19-0.21 kg a.i./ha) at an average 16-day retreatment interval (7-44 days), for a total seasonal application rate of 0.35-0.37 lb a.i./A (0.39-0.42 kg a.i./ha). Applications were made in ~11-42 gal/A (106-395 L/ha) of water using ground equipment. An adjuvant was not added to the spray mixture for any applications. Samples of canola were harvested at 20 test sites, 36-83 days after the last application. Two locations (Ashton, ID and Branchton, ON) were designated for residue decline studies. Samples were harvested 50, 54, 59, and 64 days after treatment in the decline trial performed in ID (region 11). In the ON trial (region 5), all samples were cut inadvertently on day 41. Seed samples from this site were collected on the day of harvest, and 5, 10, and 15 days after harvest.

Samples were analyzed for combined prothioconazole and prothioconazole desthio residues (determined as prothioconazole sulfonic acid and prothioconazole desthio) using LC-MS/MS method RPA JA/03/01. The validated LOQ for combined prothioconazole and prothioconazole desthio residues (designated "total prothioconazole-derived residues" in the method) was 0.02 ppm for canola seed. Samples were analyzed for residues of 1*H*-1,2,4-triazole, and the triazole conjugates triazolylalanine and triazolylacetic acid using an LC-MS/MS method (Bayer Report No. G200598). The validated LOQs were 0.02 ppm for 1*H*-1,2,4-triazole and 0.025 ppm for the triazole conjugates for canola seed. The methods were adequate for data collection based on acceptable concurrent method recovery data.

The results from the canola field trials indicated that the maximum residues of prothioconazole in/on canola seed harvested 36-83 days following the last of two broadcast foliar applications

were 0.097 ppm for the total combined residues of prothioconazole and prothioconazole desthio, <0.02 ppm for 1*H*-1,2,4-triazole, and 0.848 ppm for the triazole conjugates.

In the residue decline trial conducted in ID, total prothioconazole-derived residues and residues of 1*H*-1,2.4-triazole were less than the LOQ (<0.02 ppm each) at all sampling intervals. Residues of the triazole conjugates did not increase with increasing sampling intervals.

The maximum storage interval of canola seed samples from harvest to analysis for total prothioconazole-derived residues was 1265 days (41.6 months). Prothioconazole-derived residues and prothioconazole desthio residues are stable up to 12.7 months (interim report) in canola matrices. The degree of loss of prothioconazole-derived residues and prothioconazole desthio residues is not expected to exceed 30% after 41.6 months.

Dried shelled pea and bean, group 6C: Bayer CropScience has submitted field trial data on dried peas and beans. A total of 23 field trials were conducted during the 2002 growing season in the U.S. and Canada. Thirteen trials were conducted on dried peas in Regions 5 (MN and ON; 2 trials), 11 (ID; 1 trial, OR; 3 trials, and WA; 1 trial), and 14 (AB; 2 trials, MB; 1 trial, and SK; 3 trials), and 10 trials were conducted on dried beans in Regions 5 (IL, IN, KS, and ON; 4 trials), 7 (ND;1 trial), 7A (AB; 1 trial), 8 (TX; 1 trial), 9 (MT; 1 trial), 10 (CA; 1 trial), and 11 (WA; 1 trial). The number and locations of field trials are in accordance with OPPTS Guideline 860.1500 and Directive 98-02; Section 9.

At each test location, three broadcast foliar applications of the 4 lb/gal (480 g/L) suspension concentrate formulation (flowable concentrate; FlC) were made at ~0.180 lb a.i./A (~ 0.200 kg a.i./ha) at 9- to 15-day retreatment intervals, for a total seasonal application rate of ~0.54 lb a.i./A (~0.60 kg a.i./ha). Applications were made in ~10-33 gal/A of water using ground equipment. A non-ionic surfactant was added to the spray mixture for all applications. An additional plot at each trial was treated with three applications at a target rate of ~0.134 lb a.i./A (~ 0.150 kg a.i./ha); however, the applicant stated that the results from this application were not used because they did not support the desired product label application rate. Samples of dried shelled peas and beans were harvested 7-8 days after the last application from all test sites. It should be noted that in three of the pea field trials and five of the bean field trials, the pea and bean plants were cut and allowed to dry in the field for 2-8 days prior to collection. At two locations for dried peas and one location for dried beans, additional samples were collected to determine residue decline. Samples were harvested, at both locations, 0, 3-4, 7, 14-15, and 21-22 days after treatment for dried peas and 0, 7, 14, and 21 days after treatment for dried beans.

Samples were analyzed for combined prothioconazole and prothioconazole desthio residues (determined as prothioconazole sulfonic acid and prothioconazole desthio and designated "total prothioconazole-derived residues" in the method) using LC-MS/MS method RPA JA/03/01. The validated LOQ for combined prothioconazole and prothioconazole desthio residues was 0.05 ppm for dried peas and dried beans. Samples were analyzed for residues of 1*H*-1,2,4-triazole and the triazole conjugates triazolylalanine and triazolylacetic acid using an LC-MS/MS method (Bayer Report No. G200598). The validated LOQs were 0.01 ppm for 1*H*-1,2,4-triazole for dried peas and beans, 0.02 ppm for the triazole conjugates for dried beans,

and 0.05 ppm for the triazole conjugates for dried peas. The methods are adequate for data collection based on acceptable concurrent method recovery data.

The results from the pea and bean field trials show that the maximum residues of prothioconazole in/on dried peas and beans harvested 7-8 days following the last of three broadcast foliar applications at a total seasonal rate of 0.530-0.580 lb a.i./A (0.595-0.650 kg a.i./ha) were 0.684 ppm in/on dried peas and 0.288 ppm in/on dried beans for the combined residue of prothioconazole and desthio prothioconazle ("total prothioconazole-derived residues"); 0.011 ppm in/on dried peas and less than the LOQ (<0.01 ppm) in/on dried beans for 1*H*-1,2,4-triazole; and 0.789 ppm in/on dried peas and 0.311 ppm in/on dried beans for the triazole conjugates.

In the residue decline trials, residues of 1*H*-1,2,4-triazole were less than the method LOQ (<0.01 ppm) at all sampling intervals in dried peas and beans. The combined residues of prothioconazole and prothioconazole desthio did not increase with increasing sampling intervals in the dried bean trial and in one dried pea trial; in the other dried pea trial, residues increased slightly with increasing sampling intervals (from an average of 0.31 ppm at the 7-day PHI to an average of 0.34 ppm at the 21-day PHI). Residues of the triazole conjugates did not increase with increasing sampling intervals in dried peas, but increased slightly in dried beans with increasing sampling intervals.

The maximum storage interval of crop samples from harvest to analysis for total prothioconazole-derived residues was 542 days (17.8 months) for dried beans and peas. The degree of loss of prothioconazole-derived residues and prothioconazole desthio residues is not expected to exceed 30% after 17.8 months in dried beans and peas.

Peanut: Bayer CropScience has submitted field trial data on peanuts. Twelve trials were conducted in Regions 2 (AL; 1 trial, GA; 3 trials, NC; 3 trials, and VA; 1 trial), 3 (FL; 1 trial), 6 (TX; 2 trials), and 8 (OK; 1 trial) during the 2000 growing season. The number and locations of field trials are in accordance with OPPTS Guideline 860.1500 and Directive 98-02; Section 9.

At each test location, four broadcast foliar applications of the 4 lb/gal (480 g/L) suspension concentrate formulation (flowable concentrate; FlC) were made to peanuts at ~0.18 lb a.i./A (~0.20 kg a.i./ha) at 12- to 14-day retreatment intervals, for a total seasonal application rate of ~0.72 lb a.i./A (~0.80 kg a.i./ha). Applications were made in ~13-37 gal/A (~119-349 L/ha) of water using ground equipment. An adjuvant was not added to the spray mixture for any applications. Peanut plants were dug up at all test sites 13-15 days after treatment, and were left in the field for 2-8 days prior to collection of peanuts and peanut hay. In one field trial (GA), additional samples were dug up at 7, 14, 21, and 28 days following the last application to evaluate residue decline.

Samples were analyzed for the combined residues of prothioconazole and prothioconazole desthio (determined as prothioconazole sulfonic acid and prothioconazole desthio and designated "total prothioconazole-derived residues" in the analytical method) using LC-MS/MS method RPA JA/03/01. The validated LOQs for total prothioconazole-derived

residues were 0.02 ppm for peanut nutmeat and 0.05 ppm for hay. Samples were analyzed for residues of 1*H*-1,2,4-triazole and the triazole conjugates triazolylalanine and triazolylacetic acid using an LC-MS/MS method (Bayer Report No. G200598). The validated LOQ for 1*H*-1,2,4-triazole was 0.02 ppm for peanut nutmeat and hay, and the validated LOQs for the triazole conjugates were 0.125 ppm for peanut nutmeat and 0.10 ppm for hay. The methods were adequate for data collection based on acceptable concurrent method recovery data.

The results from the peanut field trials indicated that the maximum residues of prothioconazole in/on peanut matrices harvested 13-15 days following the last of four broadcast foliar applications at a total seasonal rate of 0.707-0.734 lb a.i./A (0.792-0.823 kg a.i./ha) were <0.02 ppm in/on nutmeat and 4.458 ppm in/on hay for the combined residue of prothioconazole and prothioconazole desthio; 0.02 ppm in/on nutmeat and less than the LOQ (<0.02 ppm) in/on hay for 1*H*-1,2,4-triazole; and 3.903 ppm in/on nutmeat and 1.278 ppm in/on hay for the triazole conjugates.

In the residue decline trial, residues of 1*H*-1,2,4-triazole were less than the method LOQ (<0.02 ppm) at all sampling intervals for peanut nutmeat and hay, and total prothioconazole-derived residues were less than the method LOQ (<0.02 ppm) at all sampling intervals for nutmeat. The average total combined residue of prothioconazole and prothioconazole desthio in hay increased slightly from the 7-day sampling interval to the 14-day sampling interval and then decreased by the 28-day sampling interval. Residues of the triazole conjugates increased slightly in nutmeat (from an average of 0.868 ppm to an average of 0.964 ppm) with increasing sampling intervals; a greater increase was observed in peanut hay (from an average of 0.117 ppm to an average of 0.355 ppm).

The maximum storage interval of crop samples from harvest to analysis for total prothioconazole-derived residues was 1214 days (39.9 months) for peanut nutmeat and hay. The combined residues of prothioconazole and prothioconazole desthio (determined as prothioconazole sulfonic acid and prothioconazole desthio) and prothioconazole desthio residues are stable up to 12.7 months (interim report). The degree of loss of combined prothioconazole and prothioconazole desthio residues ("prothioconazole-derived residues") and prothioconazole desthio residues is not expected to exceed 30% after 39.9 months.

Rice: Bayer CropScience has submitted field trial data on rice. A total of 16 trials were conducted in Regions 4 (LA; 6 trials, AR; 4 trials, and MS; 1 trial), 5 (MI; 1 trial), 6 (TX; 2 trials), and 10 (CA; 2 trials) during the 2000 growing season. The number and locations of field trials are in accordance with OPPTS Guideline 860.1500 and Directive 98-02; Section 9.

At each test location, two broadcast foliar applications of the 4 lb/gal (480 g/L) suspension concentrate formulation (flowable concentrate; FlC) were made to rice at ~0.18 lb a.i./A (~0.20 kg a.i./ha) at 13- to 16-day retreatment intervals, for a total seasonal application rate of ~0.36 lb a.i./A (~0.40 kg a.i./ha). Applications were made in ~12-23 gal/A of water using ground equipment. An adjuvant was not added to the spray mixture for any applications. Samples of rice were harvested at 14 test sites 40-67 days after the last application. At two locations, additional samples were collected to determine residue decline. Samples were harvested 49, 55, 58, and 65 days after treatment for the decline trial conducted in Benoit, MS

(Region 4), and 64, 69, 74, and 80 days after treatment for the decline trial conducted in Glen, CA (Region 10).

Samples were analyzed for the combined residue of prothioconazole and prothioconazole desthio (determined as prothioconazole sulfonic acid and desthio prothiocoanzole and designated "total prothioconazole-derived residues" in the method) using LC-MS/MS method RPA JA/03/01. The validated LOQs for the combined residue of prothioconazole and prothioconazole desthio were 0.02 ppm for rice grain and 0.05 ppm for rice straw. Samples were analyzed for residues of 1*H*-1,2,4-triazole and the triazole conjugates triazolylalanine and triazolylacetic acid using an LC-MS/MS method (Bayer Report No. G200598). The validated LOQs were 0.01 ppm for 1*H*-1,2,4-triazole and 0.05 ppm for the triazole conjugates for rice grain and straw. The methods are adequate for data collection based on acceptable concurrent method recovery data.

The results from the rice field trials showed that the combined residue of prothioconazole and prothioconazole desthio (" total prothioconazole-derived residues") in/on rice matrices harvested 40-67 days following the last of two broadcast foliar applications were 0.222 ppm in/on rice grain and 1.277 ppm in/on rice straw. Residues of 1*H*-1,2,4-triazole were less than the LOQ (<0.01 ppm) in/on rice grain and straw. Maximum residues of the triazole conjugates were 0.571 ppm (rice grain) and 0.506 ppm (rice straw).

In the residue decline trials, total combined residues of prothioconazole and prothioconazole desthio in/on rice grain were <LOQ (0.02 ppm) in one trial, and did not increase with increasing sampling intervals in/on rice grain in the other trial. For rice straw, total combined residues of prothioconazole and prothioconazole desthio increased slightly with increasing sampling intervals in one trial. In the other trial, residues in/on straw increased slightly at the middle sampling intervals, and then decreased at the final sampling interval. Residues of the triazole conjugates in/on rice grain were <LOQ (<0.05 ppm) for one trial, and increased slightly in rice grain with increasing sampling intervals in the other trial. For rice straw, residues increased slightly with increasing sampling intervals in one trial, while residues did not increase in the other trial. Residues of 1*H*-1,2,4-triazole in/on rice grain and straw from both trials were less than the method LOQs (<0.02 ppm for total prothioconazole-derived residues, <0.05 ppm for the triazole conjugates, and <0.01 ppm for 1*H*-1,2,4-triazole) at all sampling intervals.

The maximum storage interval of crop samples from harvest to analysis for total prothioconazole-derived residues was 1240 days (40.8 months) for rice grain and straw. The degree of loss of the combined residue of prothioconazole and prothioconazole desthio ("prothioconazole-derived residues") and prothioconazole desthio residues is not expected to exceed 30% after 40.8 months in rice grain and straw.

Wheat: Bayer CropScience has submitted field trial data on wheat from trials conducted in the U.S. and Canada. A total of 54 trials were conducted in Regions 2 (GA and NC; 2 trials), 4 (MS; 2 trials), 5 (IN; 1 trial, KS; 2 trials, NE; 2 trials, and ON; 2 trials), 6 (TX; 2 trials), 7 (AB; 1 trial, ND; 5 trials, SD; 2 trials, and SK; 3 trials), 7A (AB; 2 trials), 8 (OK; 3 trials and TX; 7 trials), 11 (OR; 2 trials), and 14 (AB; 6 trials, MB; 6 trials, and SK; 4 trials) during the 2000

growing season. The number and locations of field trials are in accordance with OPPTS Guideline 860.1500 and Directive 98-02; Section 9.

At each test location, two broadcast foliar applications of the 4 lb/gal (480 g/L) suspension concentrate formulation (flowable concentrate; FlC) were made to wheat. The first application was made at 0.108-0.120 lb a.i./A (0.122-0.135 kg a.i./ha) followed by a second application at 0.170-0.199 lb a.i./A (0.190-0.223 kg a.i./ha) with a 5- to 18-day retreatment interval, for a total seasonal application rate of ~0.29 lb a.i./A (~0.33 kg a.i./ha). In one field trial conducted in IN, the first application was made at 0.185 lb a.i./A (0.207 kg a.i./ha) followed by a second application at 0.190 lb a.i./A (0.213 kg a.i./ha) with a 14-day retreatment interval, for a total seasonal application rate of 0.375 lb a.i./A (0.420 kg a.i./ha). Applications were made in 11-45 gal/A of water using ground equipment. An adjuvant was not added to the spray mixture for any applications.

For 33 trials, including two decline trials, two treatment plots (designated as FORAG and HGRST) were used. The timing of the application varied for the two treatment plots. In the FORAG plot the second application was made 1 day prior to the first cutting of forage and in the HGRST plot the second application was made at full flowering. Wheat forage from the FORAG plots was harvested one day after treatment, but these samples were never analyzed or reported. Wheat hay from the HGRST plots was cut 12-17 days after treatment and was left in the field for 0-14 days prior to collection of wheat hay. Samples of wheat grain and straw from the HGRST plots were harvested at earliest commercial harvest, 30-57 days after the last application, except in one trial in which samples were harvested 10 days after second application.

For 21 trials, one treatment plot (designated as TRTD) was used; the second application was made 7 days prior to the first cutting of the forage. Only wheat forage was harvested from these trials.

At two locations (ND and NE), additional samples were collected to determine residue decline. The samples were harvested at both locations 0, 1, 7, and 14 days after treatment for wheat forage at 6 or 7, 14, 20 or 21, and 28 days after treatment for wheat hay, and at 35 or 36, 39 or 40, 44 or 46, and 49 or 50 days after treatment for wheat grain and straw.

Samples were analyzed for total combined residues of prothioconazole and prothioconazole desthio (designated "prothioconazole-derived residues" in the method) using LC-MS/MS method RPA JA/03/01. The validated LOQs for the total prothioconazole-derived residues were 0.02 ppm for wheat grain and 0.05 ppm for wheat forage, hay, and straw. The method is adequate for data collection for wheat grain hay, forage and straw based on acceptable concurrent method recovery data and method validation data. Samples were analyzed for residues of 1*H*-1,2,4-triazole and the triazole conjugates triazolylalanine and triazolylacetic acid using an LC-MS/MS method (Bayer Report No. G200598). The validated LOQ for 1*H*-1,2,4-triazole and triazolylalanine was 0.01 ppm for wheat forage, hay, grain, and straw; and the validated LOQs for triazolylacetic acid were 0.01 ppm for wheat forage, hay, and grain and 0.025 ppm for wheat straw. The method is adequate for data collection in wheat matrices based on acceptable concurrent method recovery data.

The results from the wheat field trials show that in wheat matrices harvested 10-57 days (12-17 days for hay) following the last of two broadcast foliar applications at a total seasonal rate of 0.281-0.375 lb a.i./A (0.315-0.420 kg a.i./ha), the maximum residues of prothioconazole were 0.061 ppm, 1.96 ppm, and 3.571 ppm, respectively, in/on wheat grain, straw, and hay for the combined residue of prothioconazole and prothioconazole desthio; less than the LOQ (<0.01 ppm) in/on wheat grain, straw, and hay for 1*H*-1,2,4-triazole; and 0.495 ppm, 0.665 ppm, and 1.76 ppm, respectively, in/on wheat straw, hay, and grain for the triazole conjugates. In wheat forage harvested 7 days following the last of two broadcast foliar applications at a total seasonal rate of 0.286-0.299 lb a.i./A (0.320-0.336 kg a.i./ha), the maximum residues were 6.987 ppm for the combined residue of prothioconazole and prothioconazole desthio ("total prothioconazole-derived residues"), less than the LOQ (<0.01 ppm) for 1*H*-1,2,4-triazole, and 0.175 ppm for the triazole conjugates.

In the residue decline trials, residues of 1*H*-1,2,4-triazole at all sampling intervals were less than the method LOQ (<0.01 ppm) in/on wheat hay, grain, straw, and forage for both trials (NE and ND). Total prothioconazole-derived residues did not increase in any wheat matrix with increasing sampling intervals, and residues of the triazole conjugates increased slightly in samples of wheat forage from both trials and in wheat straw from one trial but did not increase in wheat hay or grain with increasing sampling intervals.

The maximum storage intervals of crop samples from harvest to analysis for total prothioconazole-derived residues were 469 days (15.4 months) for wheat forage, 1214 days (39.9 months) for wheat grain, 1221 days (40.1 months) for wheat hay, and 1203 days (39.5 months) for wheat straw. The combined residues of prothioconazole and prothioconazole desthio ("prothioconazole-derived residues") are relatively stable up to 1 year (interim report) in wheat matrices. Corrections due to apparent dissipation of combined prothioconazole and prothioconazole desthio residues in samples stored beyond a year are not necessary due to the low absolute (ppm) and % residue levels in wheat matrices. Residues of prothioconazole desthio are stable for up to 1 year and the degree of loss is not expected to exceed 30% after 40.1 months.

Conclusions: The submitted crop field trial residue data are adequate to satisfy data requirements. As stated under Directions for Use (860.1200), the applicant has proposed use on an "Oilseed Crop Subgroup" which consists of the members of the Oilseed Crop Group 20 with the exception of safflower seed and sunflower seed. The representative crops of Crop Group 20 are canola and sunflower. Currently, no crop subgroups have been defined by HED for Crop Group 20. The applicant has submitted crop field trial data for canola but not for sunflower. The available crop field trial data will support use of prothioconazole on the following oilseed commodities: rapeseed, canola, Indian rapeseed, field mustard seed, and crambe.

The submitted crop field trial data support the following tolerances for the combined residues of prothioconazole and its desthio metabolite: barley grain at 0.35ppm; barley hay at 7.0 ppm; barley straw at 4.0 ppm; dried shelled pea and bean, except soybean, subgroup 6C, at 0.90 ppm; peanut at 0.02 ppm; peanut hay at 6.0 ppm, rapeseed seed at 0.15 ppm; rice grain at 0.20

ppm; rice straw at 1.4 ppm; wheat grain at 0.07 ppm; wheat forage at 6.0 ppm; wheat hay at 4.5 ppm; and wheat straw 5.0 ppm. The tolerance values were determined using a statistical calculation with the available field trial data (See Appendix 2).

Residue data for wheat aspirated grain fractions were included with the processing study (see 860.1520). The residue data indicate that total prothioconazole-derived residues concentrate in aspirated grain fractions. Based on a processing factor of 245x and a HAFT residue of 0.045 ppm for wheat grain, the expected residues in wheat aspirated grain fractions following treatment at 1x would be 11.0 ppm. Therefore, a tolerance for aspirated grain fractions is needed to support the proposed uses. Because the applicant is not proposing use of prothioconazole on field corn, sorghum, or soybeans, the residue data from wheat are used to determine the tolerance level for aspirated grain fractions; these data indicate that a tolerance of 11 ppm would be appropriate.

The residue data for aspirated grain fractions indicate that residues of 1,2,4-triazole and the triazole conjugates do not concentrate in wheat aspirated grain fractions.

860.1520 Processed Food and Feed

46246218.der.wpd (Wheat)
46246222.der.wpd (Rice)
46246223.der.wpd (Peanut)
46246224.der.wpd (Canola)

Table 8. Summary of Processing Factors for Prothioconazole.

RAC	Processed Commodity	Average Processing Factor		
		Sum of prothioconazole and prothioconazole desthio	1,2,4-Triazole	Triazole Conjugate Residues
Canola	Meal	<0.7x	NC [1]	2.9x
	Refined oil	<0.7x	NC	<0.02x
Peanut	Meal	>7.9x	>1.9x	1.9x
	Refined oil	NC	NC	<0.01x
	Dry roasted peanuts	NC	>12.5x	0.5x
	Peanut butter	NC	>11.9x	0.6x
Rice	Polished Grain	<0.1x	NC	0.5x
	Bran	0.6x	NC	6.9x
	Hulls	4.4x	NC	0.3x
Wheat	Aspirated grain fractions	245x	NC	0.3x
	Bran	2.4x	NC	3.1x
	Flour	<0.4x	NC	0.5x
	Germ	2.0x	NC	3.6x
	Middlings	0.6x	NC	0.6x
	Shorts	1.0x	NC	1.5x

[1] NC = Not calculated. The processing factor could not be calculated because residues were below the LOQ in both the RAC and the processed fraction.

Canola: Bayer CropScience has submitted a processing study with canola. In a single test conducted in Ontario, Canada during 2000, the 4 lb/gal (480 g/L) suspension concentrate formulation (flowable concentrate; FlC), was applied to canola plants beginning at the two-leaf stage as two broadcast foliar applications with a 27-day retreatment interval at 0.912-0.919 lb a.i./A/application (1.02-1.03 kg a.i./ha/application), for a total application rate of 1.83 lb a.i./A (2.05 kg a.i./ha; ~5 times the field trial application rate). Canola plants were cut at maturity 47 days after the last treatment and were allowed to dry in the field for 5 days prior to collection of canola seed from treated and control plots. Samples of canola seed were collected, and the remaining bulk samples were processed into meal and refined oil using simulated commercial procedures.

Samples were analyzed for total prothioconazole-derived residues (prothioconazole and the metabolite prothioconazole desthio) using the LC-MS/MS method RPA JA/03/01. The validated LOQ for total prothioconazole-derived residues was 0.02 ppm for canola seed and processed commodities. Samples were analyzed for residues of 1*H*-1,2,4-triazole and the triazole conjugates triazolylalanine and triazolylacetic acid using an LC-MS/MS method with

modifications. The validated LOQs for 1*H*-1,2,4-triazole were 0.02 ppm for canola seed and meal, and 0.01 ppm for canola oil. The validated LOQs for the triazole conjugates were 0.02 ppm for canola seed and oil, and 0.20 ppm for canola meal. The methods are adequate for data collection based on acceptable concurrent method recovery data.

The maximum storage intervals from collection to analysis for total prothioconazole-derived residues were 1261 days (41 months) for canola seed and 918 days (30 months) for processed canola commodities. Prothioconazole-derived residues and prothioconazole desthio residues are stable up to 1 year (interim report) in canola matrices. The degree of loss of prothioconazole-derived residues and prothioconazole desthio residues is not expected to exceed 30% after 41 months.

Total prothioconazole-derived residues did not concentrate in meal or refined oil (<0.7-fold each). Residues of the triazole conjugates concentrated in canola meal (2.9-fold), but not in refined oil (<0.02-fold). Processing factors could not be calculated for 1*H*-1,2,4-triazole in meal and refined oil, as residues were below the LOQ in these commodities.

Peanut: Bayer CropScience has submitted a processing study with peanut. In a single test conducted in GA during 2000, the 4 lb/gal (480 g/L) suspension concentrate formulation (flowable concentrate; FlC), was applied to peanut plants during pod development as four broadcast foliar applications with 13- to 15-day re-treatment intervals at 0.899-0.901 lb a.i./A (1.01 kg a.i./ha), for a total application rate of 3.60 lb a.i./A (4.03 kg a.i./ha; ~5 times the field trial application rate). Peanuts were dug up 14 days after the last treatment and were left to dry in the field for 7 days prior to sample collection. Sub-samples of nutmeat were reserved, and the remaining bulk samples were processed into meal, refined oil, dry roasted peanuts, and peanut butter using simulated commercial procedures.

Samples were analyzed for total prothioconazole-derived residues (prothioconazole and the metabolite prothioconazole desthio), 1*H*-1,2,4-triazole and the triazole conjugates triazolylalanine and triazolylacetic acid using an LC-MS/MS method. The validated LOQ for total prothioconazole-derived residues was 0.02 ppm for peanuts and processed commodities. The validated LOQs for 1*H*-1,2,4-triazole were 0.01 ppm for peanut nutmeat, meal, and refined oil and 0.05 ppm for dry roasted peanuts and peanut butter. The validated LOQs for the triazole conjugates were 0.01 ppm for refined oil, 0.05 ppm for peanut nutmeat, dry roasted peanuts, and peanut butter, and 1.5 ppm for peanut meal. The methods are adequate for data collection based on acceptable concurrent method recovery data.

The maximum storage intervals from collection to analysis for total prothioconazole-derived residues were 1090 days (36 months) for nutmeat and 911 days (31 months) for processed peanut commodities. Prothioconazole-derived residues and prothioconazole desthio residues are stable up to 12.7 months (interim report). The degree of loss of prothioconazole-derived residues and prothioconazole desthio residues is not expected to exceed 30% after 36 months.

Total prothioconazole-derived residues concentrated >7.9-fold (peanut meal). Processing factors could not be calculated for refined oil, dry roasted peanuts, or peanut butter because residues were below the LOQ in these commodities. Residues of 1*H*-1,2,4-triazole

concentrated >1.9-fold (meal), >12.5-fold (dry roasted peanuts), and >11.9-fold (peanut butter). A processing factor could not be calculated for refined oil because residues were below the LOQ in this commodity. Residues of the triazole conjugates concentrated 1.9-fold (peanut meal), and did not concentrate in refined oil, dry roasted peanuts or peanut butter (<0.01-fold, 0.5-fold and 0.6-fold, respectively).

Rice: Bayer CropScience has submitted a processing study with rice. In a single test conducted in MS during 2000, the 4 lb/gal (480 g/L) suspension concentrate formulation (flowable concentrate; FlC), was applied to rice plants during panicle formation as two broadcast foliar applications with a 13-day re-treatment interval at 1.07 lb a.i./A (1.20 kg a.i./ha) for the first application and 0.75 lb a.i./A (0.84 kg a.i./ha) for the second application, for a total application rate of 1.82 lb a.i./A (2.04 kg a.i./ha; ~5 times the field trial application rate). Rice grain was harvested at maturity 49 days after the last treatment. Sub-samples of rice grain (RAC) were collected, and the remaining bulk samples were processed into polished grain, bran, and hulls using simulated commercial procedures.

Samples were analyzed for total prothioconazole-derived residues (prothioconazole and the metabolite prothioconazole desthio), 1*H*-1,2,4-triazole and the triazole conjugates triazolylalanine and triazolylacetic acid using an LC-MS/MS method. The validated LOQs for prothioconazole-derived residues were 0.02 ppm for rice grain, polished grain, and bran, and 0.01 ppm for rice hulls. The validated LOQ for 1*H*-1,2,4-triazole was 0.01 ppm, and the validated LOQs for the triazole conjugates were 0.05 ppm for rice grain, polished grain, and hulls, and 0.75 ppm for rice bran. The methods are adequate for data collection based on acceptable concurrent method recovery data.

The maximum storage intervals from collection to analysis for total prothioconazole-derived residues were 1222 days (40 months) for rice grain and 902 days (30 months) for processed rice commodities. Prothioconazole-derived residues are stable up to 1 year (interim report) in rice matrices. The degree of loss of prothioconazole-derived residues and prothioconazole desthio residues is not expected to exceed 30% after 40 months in rice and its processed commodities.

Total prothioconazole-derived residues concentrated in rice hulls (4.4-fold) but not in polished grain or bran (<0.1-fold and 0.6-fold, respectively). Residues of the triazole conjugates concentrated in bran (6.9-fold), but did not concentrate in polished rice or hulls (0.5-fold and 0.3-fold, respectively). Residues of 1*H*-1,2,4-triazole were below the LOQ (<0.01 ppm) in polished rice, bran, and hulls. The reported processing factors did not exceed the theoretical concentration factors of 5.0-fold for rice hulls and 7.7-fold for rice bran.

Wheat: Bayer CropScience has submitted a processing study with wheat. In a single test conducted in KS during 2000, the 4 lb/gal (480 g/L) suspension concentrate formulation (flowable concentrate; FlC) was applied to wheat plants during flowering as two broadcast foliar applications with an 11-day retreatment interval at 0.564 lb a.i./A (0.632 kg a.i./ha) for the first application and 0.903 lb a.i./A (1.01 kg a.i./ha) for the second application, for a total application rate of 1.467 lb a.i./A (1.64 kg a.i./ha; ~5.6 times the field trial application rate). Wheat grain was harvested at maturity 47 days after the last treatment. Samples of wheat grain

(RAC) were collected, and the remaining bulk samples were processed into aspirated grain fractions, bran, middlings, shorts, flour, and germ using simulated commercial procedures.

Samples were analyzed for total prothioconazole-derived residues (prothioconazole and the metabolite prothioconazole desthio), 1*H*-1,2,4-triazole and the triazole conjugates triazolylalanine and triazolylacetic acid using an LC-MS/MS method. The validated LOQs for prothioconazole-derived residues were 0.02 ppm for wheat grain, bran, flour, middlings, and shorts, and 0.25 ppm for aspirated grain fractions. The validated LOQ for 1*H*-1,2,4-triazole was 0.01 ppm for wheat grain and all processed commodities, and the validated LOQs for the triazole conjugates were 0.01 ppm for wheat grain, 0.20 ppm for aspirated grain fractions and bran, 0.30 ppm for wheat germ and shorts, and 0.25 ppm for middlings. The methods are adequate for data collection based on acceptable method validation and concurrent recovery data.

The maximum storage intervals from collection to analysis for total prothioconazole-derived residues were 1285 days (42 months) for wheat grain and 909 days (30 months) for processed wheat commodities. Prothioconazole-derived residues are relatively stable up to 1 year (interim report) in wheat matrices. Corrections due to apparent dissipation of prothioconazole-derived residues in samples stored beyond a year are not necessary due to the low absolute (ppm) and % residue levels in wheat matrices. Residues of prothioconazole desthio are stable for up to 1 year and the degree of loss is not expected to exceed 30% after 42 months.

Residues of prothioconazole in/on wheat grain (RAC) from the processing study were 0.051 ppm for total prothioconazole-derived residues, less than the LOQ (<0.01 ppm) for 1*H*-1,2,4-triazole, and 1.33 ppm for residues of the triazole conjugates (average of triplicate analyses for each). Total prothioconazole-derived residues concentrated in aspirated grain fractions (245-fold), bran (2.4-fold), and germ (twofold). There was no concentration of residues in flour, middlings, and shorts (<0.4-fold, 0.6-fold, and 1-fold, respectively). Residues of the triazole conjugates concentrated in bran (3.1-fold), germ (3.6-fold), and shorts (1.5-fold), but did not concentrate in aspirated grain fractions, flour, and middlings (0.3-fold, 0.5-fold, and 0.6-fold). Residues of 1*H*-1,2,4-triazole were below the LOQ (<0.01 ppm) in all processed wheat commodities.

The reported processing factors do not exceed the theoretical concentration factors of 7.7-fold for wheat bran, 1.4-fold for wheat flour, and 8.3-fold for wheat shorts.

Conclusions: The submitted processing data for canola, peanut, rice, and wheat are adequate to satisfy data requirements. The processing data indicate that total prothioconazole-derived residues concentrate >7.9x in peanut meal, 4.4x in rice hulls, 2.4x in wheat bran, and 2.0x in wheat germ. Because total prothioconazole-derived residues were below the LOQ in/on all peanut nutmeat samples from the crop field trials, the actual residues observed in peanut meal in the processing study will be used to determine expected residues. Total prothioconazole-derived residues averaged 0.159 ppm in peanut meal in the processing study. When this value is corrected for the exaggeration rate of the study, 5x, expected residues in peanut meal following treatment at 1x are calculated to be 0.032 ppm. Because the tolerance for peanut

nutmeat will be established at the LOQ (0.02 ppm) and because expected residues in peanut meal are less than 2x the LOQ for peanut nutmeat, a tolerance for peanut meal is not needed.

Based on a processing factor of 4.4x for rice hulls and a HAFT residue of 0.191 ppm for rice grain, the expected residues in rice hulls following treatment at 1x would be 0.840 ppm. Because the expected residues are greater than the proposed tolerance of 0.60 ppm for rice grain, a tolerance for rice hulls is needed; a tolerance of 0.90 ppm is appropriate.

Based on processing factors of 2.4x for wheat bran and 2.0x for wheat germ and a HAFT residue of 0.045 ppm for wheat grain, the expected residues in wheat bran and germ following treatment at 1x would be 0.11 and 0.09 ppm, respectively. Because the germ residue is less than the recommended tolerance of 0.10 ppm for wheat grain and because the bran residue is only slightly greater than the recommended tolerance for wheat grain, tolerances are not appropriate for germ and bran.

The HAFT total prothioconazole-derived residues in barley grain are 0.151 ppm. Based on the 2.4x processing factor for wheat bran, the expected residues in barley bran following treatment at 1x would be 0.36 ppm. Because expected residues are not significantly greater than the proposed tolerance of 0.35 ppm for barley grain, a tolerance for barley bran is not needed.

The processing data indicate that residues of 1,2,4-triazole may concentrate in peanut meal (>1.9x), dry roasted peanuts (>12.5x), and peanut butter (>11.9x). Because 1,2,4-triazole residues were below the LOQ in/on all but one peanut nutmeat sample from the crop field trials, the actual residues observed in peanut meal in the processing study should be used to determine expected residues. Residues of 1,2,4-triazole averaged 0.019, 0.125, and 0.119 ppm in peanut meal, dry roasted peanuts, and peanut butter, respectively. When these values are corrected for the exaggeration rate of the study, 5x, expected residues of 1,2,4-triazole in peanut meal, dry roasted peanuts, and peanut butter following treatment at 1x are calculated to be 0.004, 0.025, and 0.024 ppm, respectively.

The processing data indicate that residues of the triazole conjugates may concentrate in canola meal (2.9x), peanut meal (1.9x), rice bran (6.9x), wheat bran (3.1x), wheat germ (3.6x), and wheat shorts (1.5x). The processing factor for canola meal exceeds the theoretical concentration factor of 1.9x; therefore, the theoretical concentration factor will be used to determine expected residues. Based on these processing factors and HAFT residues of 0.716, 3.390, 0.553, and 1.76 ppm for canola seed, peanut, rice grain, and wheat grain, respectively, the expected residues of triazole conjugates in processed commodities following treatment at 1x would be: 1.36 ppm in canola meal; 6.44 ppm in peanut meal; 3.82 ppm in rice bran; 5.46 ppm in wheat bran; 6.34 ppm in wheat germ; and 2.64 ppm in wheat shorts.

860.1650 Submittal of Analytical Reference Standards

As of 3/9/05, an analytical reference standard for prothioconazole is available in the National Pesticide Standards Repository. No standards for the metabolite (prothioconazole desthio) included in the tolerance expression or for the internal standards used in the enforcement method are available. Based on the proposed tolerance expressions and the proposed

enforcement methods, analytical reference standards of the following compounds must be supplied and supplies replenished as requested by the Repository:

- prothioconazole desthio [JAU6476-desthio; (2-(1-chlorocyclopropyl)-1-(2-chlorophenyl)-3-(1H-1,2,4-triazol-1-yl)-2-propanol)]
- prothioconazole sulfonic acid potassium salt [potassium salt of JAU6476 sulfonic acid; 1-[2-(1-chlorocyclopropyl)-3-(2-chlorophenyl)-2-hydroxypropyl]1H-1,2,4-triazole sulfonic acid, potassium salt]
- [triazole-^{15}N-^{13}C]-prothioconazole
- [triazole-^{15}N-^{13}C]JAU6476-desthio
- [triazole-^{15}N-^{13}C]JAU6476 sulfonic acid

The reference standards should be sent to the Analytical Chemistry Lab, which is located at Fort Meade, to the attention of either Theresa Cole or Frederic Siegelman at the following address:

USEPA
National Pesticide Standards Repository/Analytical Chemistry Branch/OPP
701 Mapes Road
Fort George G. Meade, MD 20755-5350

(Note that the mail will be returned if the extended zip code is not used.)

860.1850 Confined Accumulation in Rotational Crops

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Bayer submitted two confined rotational crop studies to support the proposed uses, one conducted using [phenyl-^{14}C]-prothioconazole (MRID 46246225) and one conducted using [triazole-^{14}C]-prothioconazole (MRID 46246226).

MRID 46246225/46246226:
Phenyl-label study
Bayer CropScience has submitted a confined rotational crop study with [phenyl-UL-^{14}C]-prothioconazole (specific activity 3.31 MBq/mg) in rotated crops. The radiolabeled test substance was dissolved in acetonitrile (ACN) and applied to bare sandy loam soil in a single planting container at 0.52 lb a.i./A (582 g a.i./ha). Rotational Swiss chard, turnip, and spring wheat were planted at plantback intervals (PBIs) of 28, 146, and 269 days.

Total radioactive residues (TRRs), expressed as JAU6476 equivalents were determined by combustion and/or liquid scintillation counting (LSC). Aliquots of each raw agricultural commodities (RACs) were extracted using acetonitrile (ACN/water; 4:1), containing 1 mg/mL of cysteine HCl, followed by accelerated solvent extraction (ASE) and/or acidic extraction using dioxane/2N HCl (9:1). As needed, extracts were cleaned up by solid phase extraction (SPE) prior to analysis by thin-layer chromatography (TLC) and/or high performance liquid chromatography (HPLC). Metabolites were identified by co-chromatography with authentic

reference compounds as well as by spectroscopic methods (mass spectroscopy or NMR). Samples were stored for a maximum of 57 days between harvest and HPLC analysis.

The TRRs were variable from the first rotation to the second and third rotations, as there was no clear pattern of increasing or decreasing TRRs over time. TRRs from the first rotation to the second and third rotations were as follows: wheat forage (0.021 to 0.062 to 0.040 ppm); wheat hay (0.114 to 0.135 to 0.160 ppm); wheat straw (0.450 to 0.307 to 0.312 ppm); wheat grain (0.007 ppm at 28 days; others not determined); Swiss chard (0.039 to 0.053 to 0.021 ppm); turnip tops (0.046 to 0.028 to 0.036 ppm); and turnip roots (0.043 to 0.031 to 0.015 ppm). Because of the low radioactivity levels in wheat grain, samples from the 146- and 269-day PBIs were not analyzed.

The majority of the TRRs (61-87% of the TRRs) were released from all rotational crop commodities with ACN/water, with the exception of wheat grain. ACN/water released only 23% of the TRRs from wheat grain. Accelerated solvent extraction with ACN/water released an additional 4 to 8% of the TRRs from wheat hay, straw, and grain. Acid hydrolysis with HCl/dioxane released approximately 9 to 21% of the TRRs from wheat hay and straw. Non-extractable residues remaining following extraction/hydrolysis accounted for less than <39% of the TRRs (#0.029 ppm) in rotational crop matrices. Total accountabilities ranged from 99.1-143%.

Total identified residues ranged from 34 to 77% of the TRRs (0.011-0.304 ppm) in rotated crop commodities, except for wheat grain. The highest absolute residues identified were in wheat straw (0.179-0.304 ppm) at all PBIs. Only 5% of the TRRs were identified in wheat grain (0.003 ppm). Prothioconazole was detected at very low levels (<1% of the TRRs; <0.005 ppm) only in samples of 146-day PBI Swiss chard, 28-day PBI turnip root, 146-day PBI turnip top, and 28- and 146-day PBI wheat straw.

JAU6476-desthio was detected in all rotational crop commodities at all PBIs analyzed, and was found to be a major metabolite (present at >10% of the TRRs; 0.003 -0.016 ppm) in the following rotational crop commodities: 28- and 146-day PBI Swiss chard, 28-, 146-, and 269-day PBI turnip root, 28- and 269-day PBI turnip top, 28-day PBI wheat forage, and 28- and 146-day PBI wheat hay. JAU6476 sulfonic acid was found to be a major metabolite in 28-day PBI wheat hay (0.013 ppm) and 269-day PBI wheat straw (0.04 ppm). Glucosides of JAU6476-desthio-dihydroxy-olefin (two isomers) were detected in all rotational crop commodities except 28-day PBI wheat grain, and one or both isomers were found to be major metabolites in 28-day PBI Swiss chard (0.005 ppm), 28-day PBI turnip root (0.005 ppm), all rotations of turnip top (0.004-0.006 ppm), 146- and 269-day PBI wheat forage (<0.01 ppm), all rotations of wheat hay (0.012-0.029 ppm), and 269-day PBI wheat straw (0.033 ppm). Up to three isomers of the glucoside of JAU6476-hydroxy-desthio were also detected in all rotational crop commodities, except 28-day PBI wheat grain and 269-day PBI wheat forage, and at least one of the isomers accounted for significant radioactivity in 146-day PBI Swiss chard (0.006 ppm), 28-day PBI turnip root and top (≤0.006 ppm), and 146-day PBI wheat straw (0.031 ppm).

Additional metabolites identified in rotational crops, each at <10% of the TRRs, were

JAU6476-triazolinone, JAU6476-3-hydroxy-desthio, JAU6476-4-hydroxy-desthio, JAU6476-6-hydroxy-desthio, JAU6476-α-hydroxy-desthio, JAU6476-α-acetoxy-desthio, JAU6476-benzylpropyldiol and its glucoside, and JAU6476-disulfide.

Triazole-label study

Bayer CropScience has submitted a confined rotational crop study with [triazole-3,5-^{14}C]-prothioconazole (specific activity 18.6 mCi/mmole) in rotated crops. The radiolabeled test substance was mixed with formulation blank and applied to bare sandy loam soil in a single planting container as four applications, with a 14-day retreatment interval, at ~0.18 lb a.i./A/application (~204 g a.i./ha), for a total rate of 0.727 lb a.i./A (815 g a.i./ha). Rotational Swiss chard, turnip, and spring wheat were planted at PBIs of 30, 125, and 366 days.

TRRs, expressed as JAU6476 equivalents were determined by combustion and/or LSC. Aliquots of each RAC were extracted using methanol and/or acetonitrile (ACN/water; 4:1), containing 1 mg/mL of cysteine HCl. If 10% or more of the TRRs in a matrix remained unextracted, ASE followed by reflux with MeOH/2N HCl (1:1) and/or dioxane/2N HCl (4:1) was performed. Identification of metabolites was achieved using reverse phase HPLC. Polar residues from sample extracts with retention times between 11 min and 15 min were further separated by ion-pair chromatography into two peaks (TA and THPA/TAA). The THPA and TAA mixture was separated by esterification followed by analysis using a third reverse phase HPLC system. Samples were stored for a maximum of 47 days between harvest and HPLC analysis.

The TRRs were variable from the first rotation to the second and third rotations, as there was no clear pattern of increasing or decreasing TRRs over time. TRRs from the first rotation to the second and third rotations were as follows: wheat forage (0.251 to 0.575 to 0.439 ppm); wheat hay (2.224 to 2.580 to 2.016 ppm); wheat straw (1.695 to 1.361 to 1.597 ppm); wheat grain (3.806 to 4.136 to 5.875 ppm); swiss chard (0.188 to 0.047 to 0.129 ppm); turnip tops (0.131 to 0.507 to 0.084 ppm); and turnip roots (0.059 to 0.442 to 0.061 ppm). TRRs were highest in wheat grain, hay, and straw.

Extraction with ACN/water (Swiss chard and turnip root and top) or ACN/water and MeOH (wheat forage, hay, straw, and grain) released the majority of the TRRs (70-98% of the TRRs). Accelerated solvent extraction with ACN/water at 50°C and 100°C released an additional ~3 to 26% of the TRRs from all wheat matrices, and subsequent ASE with water released ~1 to 5% of the TRRs in wheat hay, straw, and grain. Acid hydrolysis with HCl/dioxane or HCl released ~1 to 5% of the TRRs in wheat hay, straw, and grain. Non-extractable residues remaining following extraction/hydrolysis accounted for <1 to 6% of the TRRs (0.002-0.076 ppm) in rotational crop matrices. Total accountabilities ranged from 97-102%.

Total identified residues ranged from 72 to 99% of the TRRs (0.034-5.43 ppm) in rotated crop commodities. Prothioconazole was not detected in any rotational crop commodity. Triazolylalanine was the major residue identified in Swiss chard, turnip root and top, and wheat forage and grain, at 44 to 93% of the TRRs (0.023-3.9 ppm) at all PBIs. Triazolylalanine accounted for a major portion of the radioactivity in wheat hay and straw, at 15 to 36% of the TRRs (0.197-0.85 ppm). THPA was a major residue in Swiss chard and

wheat forage, hay, and straw, at 18 to 39% of the TRRs (0.008-0.87 ppm). THPA was also found at ≤7% of the TRRs (≤ 0.047 ppm) in rotated turnip root and top and wheat grain from the 30- and 125-day PBIs. THPA was not found in these commodities from the 366-day PBI. Triazolylacetic acid accounted for significant radioactivity in wheat hay, straw, and grain (10-29% of the TRRs; 0.2-1.5 ppm). Triazolylacetic acid was found at ≤6% of the TRRs (≤0.034 ppm) in Swiss chard, turnip root and top, and wheat forage. Additional metabolites identified in rotational crops, each at ≤7% of the TRRs (≤0.063 ppm), were triazolyl-ethanol, triazolyl-ethanol glucoside, JAU6476-desthio, and JAU6476-α-hydroxy-desthio. Free triazole (1*H*-1,2,4-triazole) was not identified in any rotational crop commodity.

Based on the results of the study, it was concluded that the metabolism in rotational crops was qualitatively similar to that in the primary crops peanut, sugar beet and wheat, as the same major metabolites were detected. Additionally, the presence of minor unknown polar compounds indicated that composition of metabolites in rotational crops was influenced by the metabolism of prothioconazole in soil. In addition, it appeared that conjugation was more prevalent in rotational crop metabolism than in primary crop metabolism.

Conclusions: The submitted confined rotational crop data are adequate to satisfy data requirements. Based on the results of the phenyl-label study, the applicant concluded that metabolism in rotational crops was similar to that in the primary crops peanut and wheat, as the same major metabolites were detected. The presence of minor unknown polar compounds indicated that composition of metabolites in rotational crops was influenced by the metabolism of prothioconazole in soil. The applicant did not discuss prothioconazole metabolism in soil.

The applicant did not propose a metabolic pathway for [triazole-3,5-^{14}C]-prothioconazole in rotational crops. It appeared that conjugation was more prevalent in rotational crop metabolism than in primary crop metabolism, and that metabolism/degradation of the triazole ring to triazole conjugates was more extensive in rotational crops than in primary crops.

The submitted confined rotational crop studies indicate the potential for quantifiable prothioconazole and triazole conjugate residues in rotated crop commodities. No metabolites were identified in the confined rotational crop studies that were not identified in one or more of the primary crop metabolism studies. The residue definition in rotational crop commodities (for tolerance enforcement and for dietary intake assessment) is the same as for primary crop commodities, prothioconazole and the desthio metabolite.

860.1900 Field Accumulation in Rotational Crops

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Bayer CropScience has submitted a limited field rotational crop study on the representative crops mustard greens (leafy vegetable), turnip (root vegetable), and wheat (cereal grain). Three trial sites, in GA (Region 2), IN (Region 5), and KS (Region 5), were used for each crop. At each trial site, two spray applications of the 4 lb/gal (480 g/L) suspension concentrate formulation (flowable concentrate; FlC) were made to bare soil at ~0.36 lb a.i./A/application (~0.40 kg a.i./ha), for total application rates of ~0.72 lb a.i./A (0.81 kg a.i./ha); applications

were made with a ~14-day retreatment interval. Mustard, turnip, and wheat were planted at plantback intervals (PBIs) of 1, 4, 8, and 12 months, and samples of mustard greens, turnip roots and tops, and wheat forage, hay, grain, and straw were collected at crop maturity. For winter wheat, the 1-month and 4-month PBI trials were conducted in GA (Region 2), IN (Region 5), and KS (Region 5); the 8- and 12-month PBI trials for spring wheat were conducted in ID (Region 11), ND (Region 7), and OR (Region 12).

Samples were analyzed for the combined residue of prothioconazole and prothioconazole desthio (determined as prothioconazole sulfonic acid and prothioconazole desthio and designated "total prothioconazole-derived residues" in the method) using LC-MS/MS method RPA JA/03/01. The validated LOQs were 0.02 ppm for each analyte in wheat grain and 0.05 ppm for each analyte in mustard greens, turnip root and top, and wheat forage, hay, and straw. Samples were analyzed for residues of 1*H*-1,2,4-triazole and the triazole conjugates triazolylalanine and triazolylacetic acid using an LC-MS/MS method (Bayer Report No. G200598). The validated LOQs for 1*H*-1,2,4-triazole were 0.01 ppm (mustard greens, turnip top, and wheat forage, hay, grain, and straw) and 0.05 ppm (turnip root). The validated LOQs for triazolylalanine were 0.01 ppm (wheat forage, hay, grain, and straw), 0.05 ppm (turnip root and top), and 0.10 ppm (mustard greens). The validated LOQs for triazolylacetic acid were 0.01 ppm (mustard greens and wheat forage, hay, and grain), 0.025 ppm (wheat straw), and 0.05 ppm (turnip root and top). The methods are adequate for data collection based on acceptable concurrent method recovery data.

At the 1-month PBI, total prothioconazole-derived residues were below the LOQ (<0.02 ppm for wheat grain and <0.05 ppm for all other commodities) in mustard greens, turnip root and top, and wheat forage, hay grain, and straw. Because residues of prothioconazole were below the LOQ in all samples from the 1-month PBI, samples from the 4-, 8-, and 12-month PBIs were not analyzed for total prothioconazole-derived residues.

At the 1-month PBI, residues of 1*H*-1,2,4-triazole were below the LOQ (<0.01 ppm) in/on all rotational crop matrices except wheat straw and grain; quantifiable residues of 0.01 ppm were detected in/on two samples each of wheat straw and grain. At the 4-month PBI, residues of 1*H*-1,2,4-triazole were below the LOQ (<0.01 ppm) in/on all rotational crop matrices. Samples from the 8- and 12-month PBIs were not analyzed for 1*H*-1,2,4-triazole residues.

At the 1-month PBI, total residues of triazole conjugates (triazolylalanine and triazolylacetic acid) were 0.102-0.313 ppm (mustard greens), 0.301-0.567 ppm (turnip root), 0.263-0.484 ppm (turnip top), 0.080-1.174 ppm (wheat forage), 0.303-2.025 ppm (wheat hay), 0.710-3.465 ppm (wheat grain), and 0.074-0.719 ppm (wheat straw). At the 4-month PBI, total triazole conjugate residues were 0.081-0.392 ppm (mustard greens), 0.066-0.201 ppm (turnip root), 0.095-0.254 ppm (turnip top), 0.164-0.333 ppm (wheat forage), 0.406-0.763 ppm (wheat hay), 0.615-1.754 ppm (wheat grain), and 0.103-0.278 ppm (wheat straw). The average triazole conjugate residues in each commodity decreased from the 1-month PBI to the 4-month PBI. Samples from the 8- and 12-month PBIs were not analyzed for triazole conjugate residues.

The maximum storage intervals from harvest to analysis for the combined residue of prothioconazole and prothioconazole desthio (designated "total prothioconazole-derived

residues") were 1263 days (41.5 months) for mustard greens, 1243 days (40.8 months) for turnip tops and roots, 1002 days (32.9 months) for wheat forage, 943 days (31.0 months) for wheat hay, 919 days (30.2 months) for wheat grain, and 911 days (29.9 months) for wheat straw. The combined residues of prothioconazole and prothioconazole desthio ("prothioconazole-derived residues") are relatively stable up to 1 year (interim report) in wheat matrices, mustard greens, turnip tops and roots. Corrections due to apparent dissipation of the combined residue of prothioconazole and prothioconazole desthio (designated "prothioconazole-derived residues") in samples stored beyond a year are not necessary due to the low absolute (ppm) and % residue levels in plant matrices. Residues of prothioconazole desthio are stable for up to 1 year and the degree of loss is not expected to exceed 30% after 41.5 months after freezer storage.

Conclusions: The submitted field rotational crop residue data are adequate to satisfy data requirements. The applicant has proposed the following rotational crop restrictions: crops listed on the label may be planted as soon as practical after last application; all other crops may be planted 30 days following last application. The submitted field rotational crop data, which indicated no quantifiable total combined residues of prothioconazole and prothioconazole desthio ("prothioconazole-derived residues") in mustard greens, turnip root and top, and wheat forage, hay grain, and straw at the 1-month PBI, are adequate to support the proposed rotational crop restrictions. With these restrictions, tolerances will not be needed for prothioconazole rotated crops, based on a residue definition of the combined residues of prothioconazole and prothioconazole desthio, calculated as prothioconazole. Note that finite residues of triazole conjugates exist at the PBI of one month, and the issue of such residues in rotatational crops was noted in the aggregate exposure assessment of triazole derivatives for human-health risk assessment (D322215, 02/07/2006).

860.1550 Proposed Tolerances

Bayer CropScience has proposed the establishment of permanent tolerances for 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 the desthio metabolite in/on raw agricultural and processed commodities, and for residues of prothioconazole, the desthio and 4-hydroxy metabolites, and conjugates that can be converted to these three compounds by acid hydrolysis in/on cattle commodities.

The proposed tolerance expression for plant commodities should be revised to specify that residues of the desthio metabolite are calculated as parent. The tolerance expression for livestock commodities should be revised to residues of prothioconazole, prothioconazole desthio, and conjugates that are converted to these two compounds by acid hydrolysis, calculated as prothioconazole .

There are currently no established Codex or Mexican MRLs for prothioconazole. MRLs in Canada will be established as a result of this Joint Review project. An International Residue Limit Status sheet is attached to this review.

Pending receipt of the required storage stability data, the available crop field trial data will

support tolerances for residues of prothioconazole and the desthio metabolite in/on: barley, grain; barley, hay; barley, straw; barley, bran; grain, aspirated grain fractions; pea and bean, dried shelled, except soybean, subgroup 6C; peanut; peanut, hay; rapeseed, seed; rice, grain; rice, straw; rice, hulls; wheat, grain; wheat, forage; wheat, hay; and wheat, straw.

Tolerance values were determined by application of the NAFTA statistical calculation spreadsheet to the validated field trial data (See Appendix II). In those instances where a substantial quantity of censored data (values $\leq$ LOQ) existed, the recommended value based on a log normal distribution was replaced with the mean plus three standard deviation value. This occurred for pea and bean dried, rapeseed seed, rice grain, and wheat grain

The available data indicate that the proposed tolerance of 0.06 ppm for wheat grain is too low; a revised tolerance of 0.07 ppm should be proposed. The available crop field trial data indicate that the proposed tolerances of 13.0 ppm for aspirated grain fractions , of 7.0 ppm for wheat forage are too high. Revised tolerances of 11 ppm and 6.0 ppm, respectively, should be proposed. The available crop field trial data indicate that the proposed tolerances of 4.0 ppm for wheat hay and 2.3 ppm for wheat straw are too low. Revised tolerances of 4.5 ppm and 5.0 ppm, respectively, should be proposed.

The available crop field trial data indicate that proposed tolerances for barley grain of 0.2 ppm and for barley straw of 2.0 ppm are too low. Revised tolerances of 0.35 ppm and 4.0 ppm, respectively, should be proposed.

The available crop field trial data indicate that proposed tolerance for the pea and bean subgroup, dried, shelled, except soybean, of 0.8 ppm is too low. A revised tolerance of 0.9 ppm should be proposed.

The available crop field trial data indicate that proposed tolerance for peanut hay of 5.0 ppm is too low. A revised tolerance of 6.0 ppm should be proposed.

The available crop field trial data indicate that the proposed tolerance for rice grain of 0.25 ppm is too high. A revised tolerance of 0.20 ppm should be proposed. Likewise, the available field trial data indicate that the proposed tolerance for rice straw of 1.5 ppm is too high. A revised tolerance of 1.4 ppm should be proposed.

Additional crop field trial data are required to support the proposed tolerances for black mustard seed, borage seed, flax seed, and Indian mustard seed.

The proposed tolerances for canola seed, crambe seed, field mustard seed, and Indian rapeseed are not needed. According to 40 CFR §180.1(h), a tolerance for rapeseed will cover these commodities.

The ruminant feeding study will support tolerances for the combined residue of prothioconazole, prothioconazole desthio, and metabolites that are acid hydrolyzed to these two compounds, calculated as prothioconazole , in cattle, goat, hog, horse, and sheep commodities. The applicant must propose tolerances for the fat, meat, and meat byproducts of

goat, horse, and sheep, and must propose tolerances for the meat byproducts of hogs. The values proposed by the applicant are inappropriate as they included the 4-hydroxy prothioconazole metabolite and conjugates thereof that can be acid hydrolyzed to 4-hydroxy prothioconazole. The appropriate levels for the tolerances are listed in Table 9.

A poultry metabolism study will tentatively support tolerances for the combined residue of prothioconazole, prothioconazole desthio, and metabolites that are acid hydrolyzed to these two compounds, calculated as prothioconazole , at the LOQs of the analytical method, for poultry liver. The results of the poultry metabolism study indicate that tolerances are not needed for the remaining poultry commodities. A poultry feeding study must be conducted and the enforcement analytical method must be validated for poultry commodities to sustain these tentative conclusions.

The proposed tolerances should be revised to reflect the correct commodity definitions as specified in Table 9.

Table 9. Tolerance Summary for Prothioconazole.			
Commodity	Proposed Tolerance (ppm)	Recommended Tolerance (ppm)	Comments/ *Correct commodity definition*
Tolerances for the combined residues of prothioconazole [2-[2-(1-chlorocyclopropyl)-3-(2-chlorophenyl)-2-hydroxypropyl]-1,2-dihydro-3*H*-1,2,4-triazole-3-thione] and prothioconazole desthio [α-(1-chlorocyclopropyl)-α-[(2-chlorophenyl)methyl]-1H-1,2,4- triazole-1-ethanol], calculated as prothioconazole			
Barley, grain	0.2	0.35	The proposed tolerance is too low.
Barley, hay	7.0	7.0	
Barley, straw	2.0	4.0	The proposed tolerance is too low.
Barley, pearled barley	0.2	Delete, not needed	A separate tolerance is not needed for pearled barley.
Barley, bran	0.4	Delete, not needed	A separate tolerance is not need for barley, bran.
Black mustard, seed	0.1	Delete, not allowed at this time	Additional crop field trial data are needed to support this tolerance.
Borage, seed	0.1	Delete, not allowed at this time	Additional crop field trial data are needed to support this tolerance.
Canola, seed	0.1	Delete, not needed	As specified under 40 CFR §180.1(h), a tolerance for rapeseed applies to canola seed and crambe seed.
Crambe, seed	0.1	Delete, not needed	
Field mustard, seed	0.1	Delete, not needed	Covered under the tolerance for rapeseed.
Flax, seed	0.1	Delete, not allowed at this time	Additional crop field trial data are needed to support this tolerance.
Grain, aspirated fractions	13.	11	The proposed tolerance is too high; *Grain, aspirated grain fractions*
Indian mustard, seed	0.1	Delete, not allowed at this time	Additional crop field trial data are needed to support this tolerance.
Indian rapeseed	0.1	Delete, not needed	Covered under the tolerance for rapeseed.
Pea and bean, dried, shelled, except soybean, subgroup	0.8	0.90	The proposed tolerance is too low. *Pea and bean, dried shelled, except soybean, subgroup 6C*
Peanut, nutmeat	0.02	0.02	*Peanut*
Peanut, hay	5.0	6.0	The proposed tolerance is too low.
Peanut, meal	0.3	Delete, not needed	A separate tolerance is not needed for peanut meal.
Rapeseed, seed	0.1	0.15	The proposed tolerance is too low.
Rice, grain	0.25	0.20	The proposed tolerance is too high.
Rice, straw	1.5	1.4	The proposed tolerance is too high.
Rice, hulls	1.0	0.90	The proposed tolerance is too high.
Wheat, grain	0.06	0.07	The proposed tolerance is too low.

Table 9. Tolerance Summary for Prothioconazole.			
Commodity	Proposed Tolerance (ppm)	Recommended Tolerance (ppm)	Comments/ *Correct commodity definition*
Wheat, forage	7.0	6.0	The proposed tolerance is too high.
Wheat, hay	4.0	4.5	The proposed tolerance is too low.
Wheat, straw	2.3	5.0	The proposed tolerance is too low.
Wheat, bran	1.5	Delete, not needed.	Covered under the tolerance for wheat, grain
Wheat, germ	0.15	Delete, not needed.	Covered under the tolerance for wheat, grain.
Tolerances for the 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 prothioconazole desthio [α-(1-chlorocyclopropyl)-α -[(2-chlorophenyl)methyl]-1*H*-1,2,4-triazole-1-ethanol] and conjugates convertible to these two compounds by acid hydrolysis, calculated as prothioconazole			
Milk	0.006	0.02	The proposed tolerance is too low.
Cattle, fat	0.1	0.1	
Cattle, meat	0.01	0.02	The proposed tolerance is too low.
Cattle, meat byproducts	1.2	0.20	The proposed tolerance is too high.
Goat, fat	None	0.1	Extrapolated from cattle.
Goat, meat	None	0.02	Extrapolated from cattle.
Goat, meat byproducts	None	0.20	Extrapolated from cattle.
Hog, meat byproducts	None	0.05	Extrapolated from cattle.
Horse, fat	None	0.1	Extrapolated from cattle.
Horse, meat	None	0.02	Extrapolated from cattle.
Horse, meat byproducts	None	0.20	Extrapolated from cattle.
Sheep, fat	None	0.1	Extrapolated from cattle.
Sheep, meat	None	0.02	Extrapolated from cattle.
Sheep, meat byproducts	None	0.20	Extrapolated from cattle.
Poultry, liver	None	0.02	A tolerance is needed.

Attachments:
International Residue Limit Status sheet
Appendix I - Chemical Names and Structures of Prothioconazole and its Transformation Products

Appendix II - Statistical Calculation of Tolerances

INTERNATIONAL RESIDUE LIMIT STATUS

Chemical Name: [2-[2-(1-chlorocyclopropyl)-3-(2-chlorophenyl)-2-hydroxypropyl]-1,2-dihydro-3*H*-1,2,4-triazole-3-thione	Common Name: Prothioconazole	X Proposed tolerance Reevaluated tolerance Other	Date: 03/09/05

Codex Status (Maximum Residue Limits)	U. S. Tolerances
X No Codex proposal step 6 or above No Codex proposal step 6 or above for the crops requested	Petition Number: PP#4F6830 DP Barcode: D303508 and D314517 Other Identifier:
Residue definition (step 8/CXL): N/A	Reviewer/Branch: S.Funk/IO
	Residue definition: combined residues of prothioconazole [2-[2-(1-chlorocyclopropyl)-3- (2-chlorophenyl)-2-hydroxypropyl]-1,2-dihydro-3*H*-1,2,4-triazole-3-thione] and prothioconazole desthio [α-(1-chlorocyclopropyl)-α-[(2-chlorophenyl)methyl]-1H-1,2,4- triazole-1-ethanol], calculated as prothioconazole (plant commodities); or 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 prothioconazole desthio [α-(1-chlorocyclopropyl)-α -[(2-chlorophenyl)methyl]-1*H*-1,2,4-triazole-1-ethanol] and conjugates convertible to these two compounds by acid hydrolysis, calculated as prothioconazole (livestock commodities)

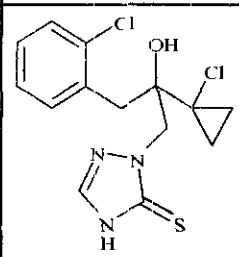
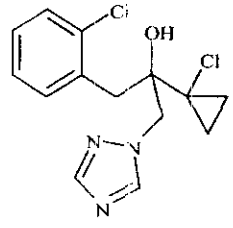
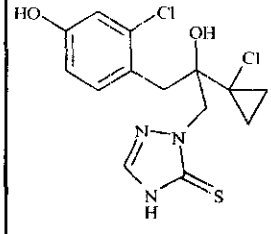
Crop (s)	MRL (mg/kg)	Crop(s)	Tolerance (ppm)
		Prothioconazole and the desthio metabolite:	
		Barley, grain	0.35
		Barley, hay	7.0
		Barley, straw	4.0
		Grain, aspirated fractions	11.
		Pea and bean, dried, shelled, except soybean, subgroup	0.90
		Peanut	0.02
		Peanut, hay	6.0
		Rapeseed, seed	0.15
		Rice, grain	0.20
		Rice, straw	1.4
		Rice, hulls	0.90
		Wheat, grain	0.07
		Wheat, forage	6.0
		Wheat, hay	4.5
		Wheat, straw	5.0
		Milk	0.02
		Cattle, fat	0.1

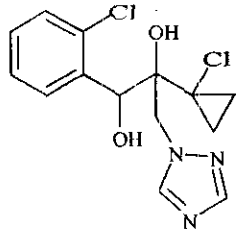
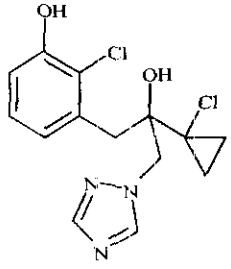
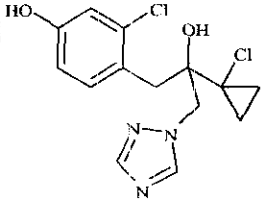
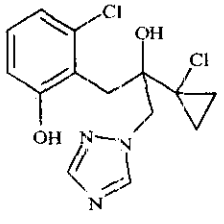
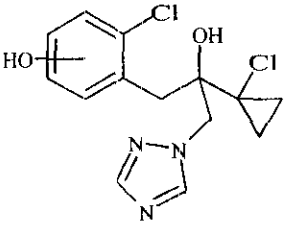
INTERNATIONAL RESIDUE LIMIT STATUS			
		Cattle, meat	0.02
		Cattle, meat byproducts	0.2
		Goat, fat	0.1
		Goat, meat	0.02
		Goat, meat byproducts	0.2
		Hog, meat byproducts	0.05
		Horse, fat	0.1
		Horse, meat	0.02
		Horse, meat byproducts	0.2
		Sheep, fat	0.1
		Sheep, meat	0.02
		Sheep, meat byproducts	0.2
		Poultry, liver	0.02

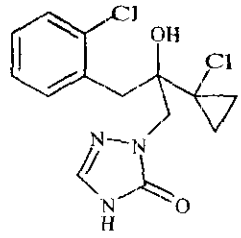
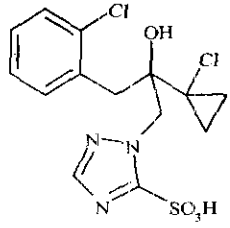
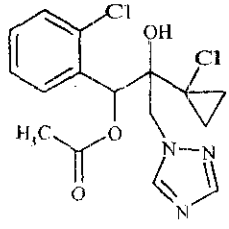
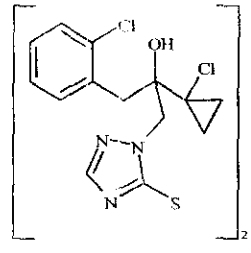
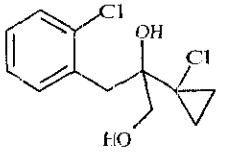
Limits for Canada	Limits for Mexico
☐ No Limits ☐ No Limits for the crops requested Limits to be established under this Joint Review project.	X No Limits ☐ No Limits for the crops requested
Residue definition: N/A	Residue definition: N/A

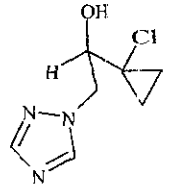
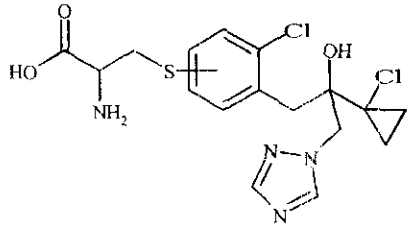
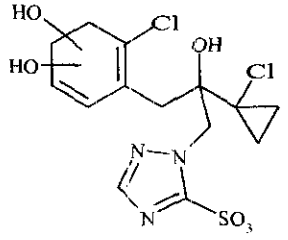
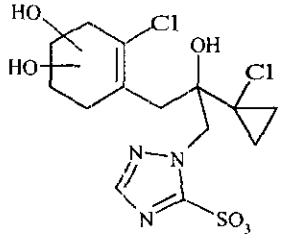
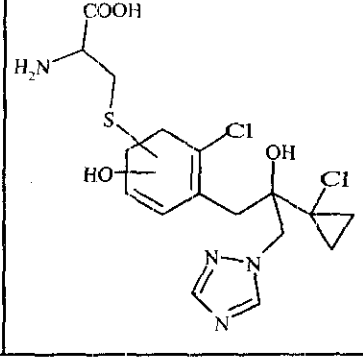
Crop(s)	MRL (mg/kg)	Crop(s)	MRL (mg/kg)

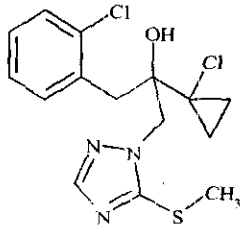
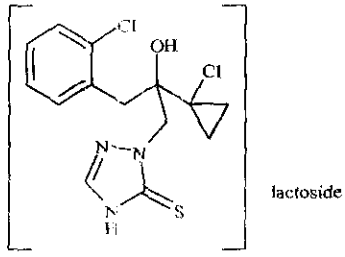
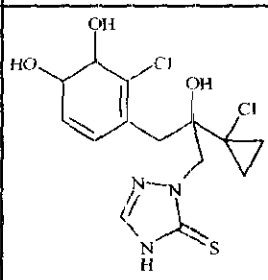
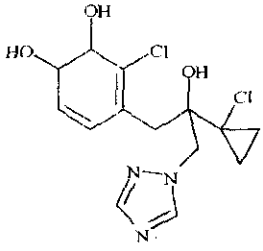
Notes/Special Instructions:S.Funk, 03/17/05

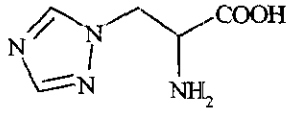
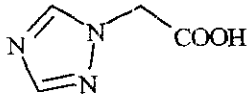
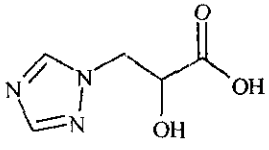
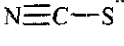
Appendix I. Chemical Names and Structures of Prothioconazole and its Transformation Products.		
Common Name/Code*Matrix*	Chemical Name	Structure
Prothioconazole; JAU6476 [Parent] *Wheat forage, hay, straw, and grain* *Peanut hay* *Sugar beet tops* *Rotated Swiss chard, turnip tops and root, and wheat straw* *Goat milk, liver, kidney, muscle, and fat* *Hen egg, liver, muscle, and fat*	2-[2-(1-chlorocyclopropyl)-3-(2-chlorophenyl)-2-hydroxypropyl]-1,2-dihydro-3H-1,2,4-triazole-3-thione	
Prothioconazole desthio; JAU6476-desthio [included in tolerance expression for plant and animal commodities] *Wheat forage, hay, straw, and grain* *Peanut hay and nutmeat* *Sugar beet tops and root* *Rotated Swiss chard, turnip tops and root, and wheat forage, hay, straw, and grain* *Goat milk, liver, kidney, muscle, and fat* *Hen egg, liver, muscle, and fat*	α-(1-chlorocyclopropyl)-α-[(2-chlorophenyl)methyl]-1H-1,2,4-triazole-1-ethanol	
Prothioconazole-4-hydroxy; JAU6476-4-hydroxy [included in tolerance expression for animal commodities] *Goat milk, liver, kidney, muscle, and fat* *Hen liver and muscle*	2-[2-(1-chlorocyclopropyl)-3-(2-chloro-4-hydroxyphenyl)-2-hydroxypropyl]-1,2-dihydro-3H-1,2,4-triazole-3-thione	

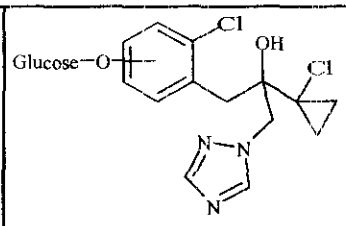
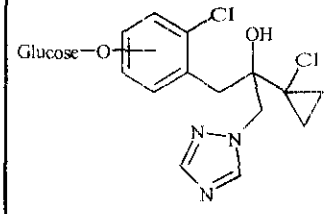
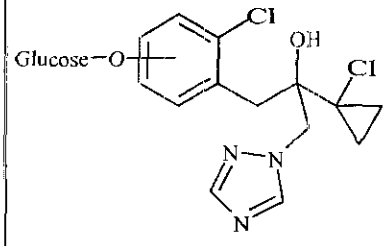
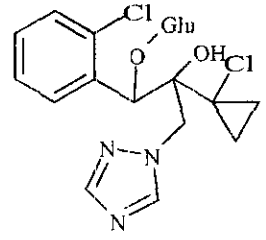
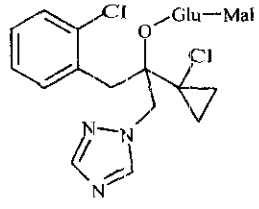
Appendix I. Chemical Names and Structures of Prothioconazole and its Transformation Products.		
Common Name/Code*Matrix*	Chemical Name	Structure
JAU6476-α-OH-desthio *Wheat forage, hay, straw, and grain* *Sugar beet tops* *Rotated Swiss chard, turnip tops and root, and wheat forage, hay, straw, and grain*	2-(1-chlorocyclopropyl)-1-(2-chlorophenyl)-3-(1H-1,2,4-triazol-1-yl)-1,2-propanediol	
JAU6476-3-OH-desthio *Wheat forage, hay, and straw* *Peanut hay* *Rotated Swiss chard, turnip tops and root, and wheat forage, hay, straw, and grain* *Goat milk, liver, kidney, muscle, and fat*	α-(1-chlorocyclopropyl)-α-[(2-chloro-3-hydroxyphenyl)-methyl]-1H-1,2,4-triazole-1-ethanol	
JAU6476-4-OH-desthio *Wheat forage, hay, and straw* *Peanut hay* *Rotated Swiss chard, turnip tops and root, and wheat forage, hay, straw, and grain* *Goat liver* *Hen egg and liver*	α-(1-chlorocyclopropyl)-α-[(2-chloro-4-hydroxyphenyl)-methyl]-1H-1,2,4-triazole-1-ethanol	
JAU6476-6-OH-desthio *Wheat forage, hay, and straw* *Rotated turnip tops and root and wheat forage and straw*	α-(1-chlorocyclopropyl)-α-[(2-chloro-6-hydroxyphenyl)-methyl]-1H-1,2,4-triazole-1-ethanol	
JAU6476-OH-desthio isomers *Wheat forage and straw* *Sugar beet tops*		

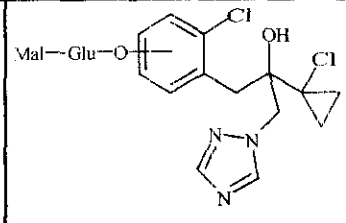
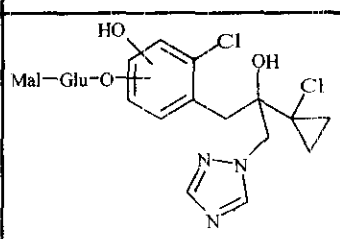
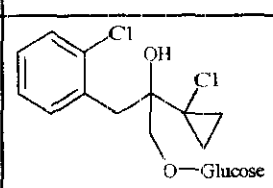
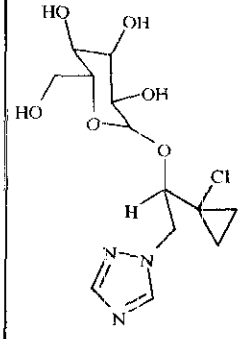
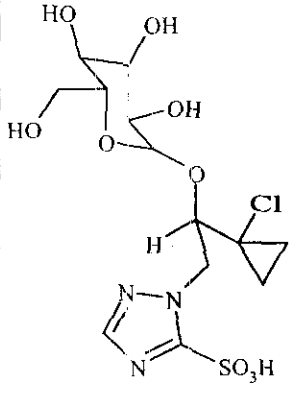
Appendix I. Chemical Names and Structures of Prothioconazole and its Transformation Products.		
Common Name/Code*Matrix*	Chemical Name	Structure
JAU6476-triazolinone *Wheat forage, hay, straw, and grain* *Peanut hay* *Sugar beet tops and root* *Rotated Swiss chard, turnip tops and root, and wheat forage, hay, and straw*	2-[2-(1-chlorocyclopropyl)-3-(2-chlorophenyl)-2-hydroxypropyl]-2,4-dihydro-3H-1,2,4-triazole-3-one	
JAU6476 sulfonic acid *Wheat forage, hay, and straw* *Peanut hay and nutmeat* *Sugar beet tops* *Rotated Swiss chard, turnip tops, and wheat forage, hay, and straw*	1-[2-(1-chlorocyclopropyl)-3-(2-chlorophenyl)-2-hydroxypropyl]-1H-1,2,4-triazole-5-sulfonic acid	
JAU6476-α-acetoxy-desthio *Wheat forage, hay, straw, and grain* *Rotated wheat forage, hay, and straw*	2-(1-chlorocyclopropyl)-1-(2-chlorophenyl)-2-hydroxy-3-(1H-1,2,4-triazol-1-yl)propyl acetate	
JAU6476-disulfide *Wheat forage, hay, straw, and grain* *Peanut hay* *Rotated Swiss chard, turnip tops and root, and wheat forage, hay, and straw*		
JAU6476-benzylpropyldiol *Wheat straw* *Rotated Swiss chard, turnip tops and root, and wheat straw*	2-(1-chlorocyclopropyl)-3-(2-chlorophenyl)propane-1,2-diol	

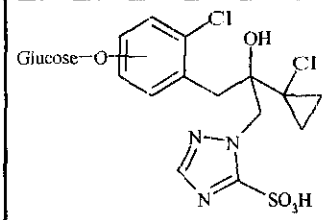
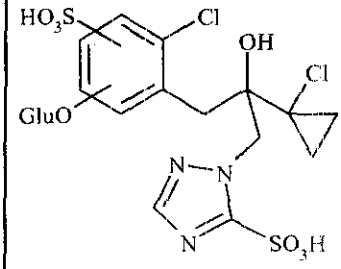
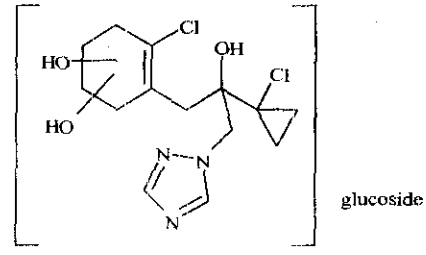
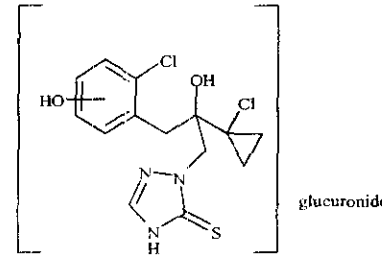
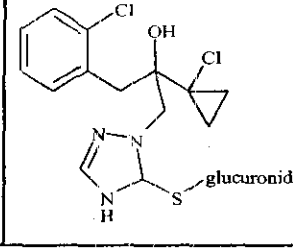
Appendix I. Chemical Names and Structures of Prothioconazole and its Transformation Products.		
Common Name/Code*Matrix*	Chemical Name	*Structure*
Triazolyl-ethanol *Wheat straw* *Peanut hay* *Sugar beet tops* *Rotated Swiss chard, turnip tops and root, and wheat forage, hay, and straw* *Hen egg, liver, muscle, and fat*	1-(1-chlorocyclopropyl)-2-(1*H*-1,2,4-triazol-1-yl)ethanol	
JAU6476-desthio-phenyl-cysteine *Wheat forage* *Peanut hay*		
JAU6476-dihydroxy-diene sulfonic acid *Peanut hay*		
JAU6476-dihydroxyolefin sulfonic acid *Peanut hay*		
JAU6476-desthio-hydroxy-dienyl-cysteine *Peanut hay and nutmeat* *Sugar beet tops and root*		

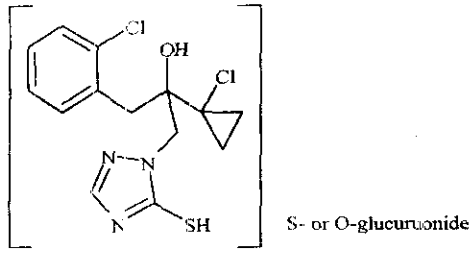
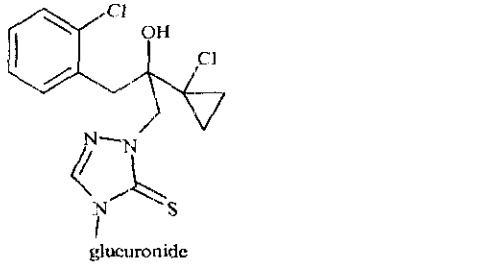
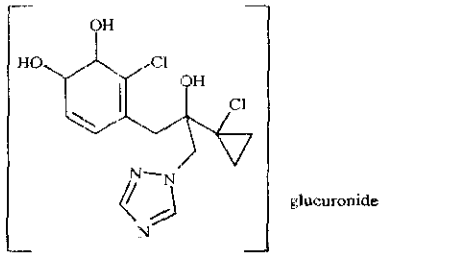
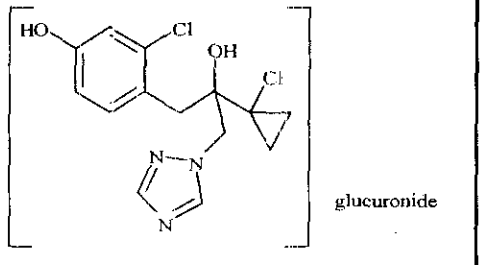
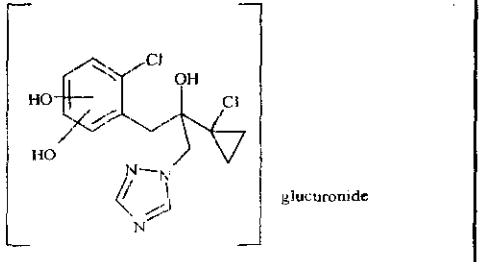
Appendix I. Chemical Names and Structures of Prothioconazole and its Transformation Products.		
Common Name/Code*Matrix*	Chemical Name	Structure
JAU6476-S-methyl *Goat liver* *Hen egg, liver, muscle, and fat*	α-(1-chlorocyclopropyl)-α-[(2-chlorophenyl)methyl]-3-(methylthio)-1H-1,2,4-triazole-1-ethanol	
JAU6476-lactoside *Goat milk*		 lactoside
JAU6476-dihydroxy-diene *Goat milk* *Hen liver and muscle*	2-[2-(1-chlorocyclopropyl)-3-(2-chloro-3,4-dihydroxycyclohexa-1,5-dien-1-yl)-2-hydroxypropyl]-2,4-dihydro-3H-1,2,4-triazole-3-thione [1]	
JAU6476-desthio-dihydroxy-diene *Goat milk and liver*	3-chloro-4-[2-(1-chlorocyclopropyl)-2-hydroxy-3-(1H-1,2,4-triazol-1-yl)propyl]cyclohexa-3,5-diene-1,2-diol [1]	

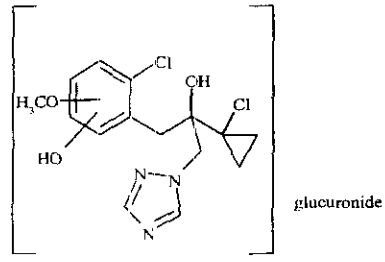
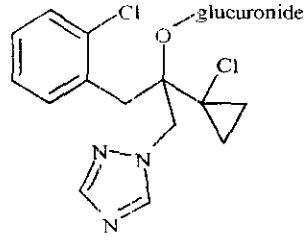
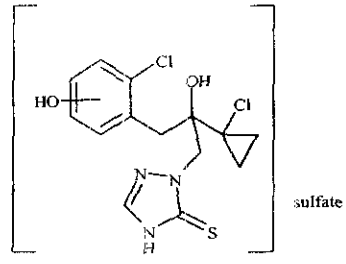
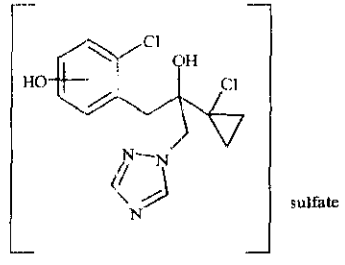
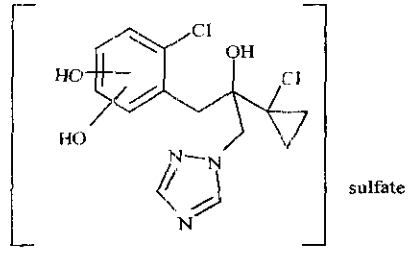
Appendix I. Chemical Names and Structures of Prothioconazole and its Transformation Products.		
Common Name/Code*Matrix*	Chemical Name	Structure
Triazole metabolites		
1,2,4-Triazole *Hen egg, liver, muscle, and fat*	1,2,4-triazole	
Triazolylalanine (TA) *Wheat forage, hay, straw, and grain* *Peanut hay and nutmeat* *Sugar beet tops and root* *Rotated Swiss chard, turnip tops and root, and wheat forage, hay, straw and grain*	α-amino-1H-1,2,4-triazole-1-propanoic acid	
Triazolylacetic acid (TAA) *Wheat forage, hay, straw, and grain* *Peanut hay and nutmeat* *Rotated Swiss chard, turnip tops and root, and wheat forage, hay, straw, and grain*	1H-1,2,4-triazole-1-acetic acid	
Triazolylhydroxypropionic acid (THPA) *Wheat forage, hay, straw, and grain* *Peanut hay and nutmeat* *Sugar beet tops* *Rotated Swiss chard, turnip tops and root, and wheat forage, hay, straw, and grain*	α-hydroxy-1H-1,2,4-triazole-1-propanoic acid	
Thiocyanate *Goat milk, liver, kidney, muscle, and fat* *Hen egg, liver, muscle, and fat*	thiocyanate ion	

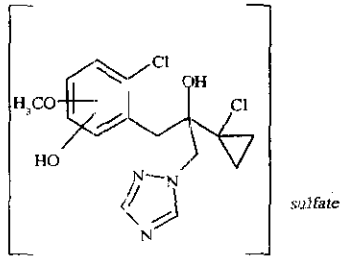
Appendix I. Chemical Names and Structures of Prothioconazole and its Transformation Products.		
Common Name/Code/Matrix	Chemical Name	Structure
Glucosides		
JAU6476-desthio-glucoside *Wheat hay and straw*		
JAU6476-OH-desthio glucoside isomers [2] *Wheat forage, hay, straw, and grain* *Peanut nutmeat and hay* *Rotated Swiss chard, turnip tops and root, and wheat forage, hay, and straw*		
JAU6476-OH-desthio glucoside isomers *Sugar beet tops*		 or 
JAU6476-desthio-malonyl-glucoside *Wheat forage and hay*		

Appendix I. Chemical Names and Structures of Prothioconazole and its Transformation Products.		
Common Name/Code*Matrix*	Chemical Name	Structure
JAU6476-OH-desthio-malonyl-glucoside *Wheat forage, hay, and straw* *Peanut hay*		
JAU6476-dihydroxy-desthio-malonyl-glucoside *Wheat forage*		
JAU6476-benzylpropyldiol glucoside *Wheat hay, straw, and grain* *Rotated turnip tops and root and wheat forage and straw*	2-(1-chlorocyclopropyl)-3-(2-chlorophenyl)propane-1,2-diol glucoside	
Triazolyl-ethanol-glucoside *Wheat forage, hay, and straw* *Peanut hay* *Sugar beet tops* *Rotated turnip tops and root and wheat forage, hay, and straw*		
Triazolyl-sulfonic acid-ethanol-glucoside *Sugar beet tops*		

Appendix I. Chemical Names and Structures of Prothioconazole and its Transformation Products.		
Common Name/Code*Matrix*	Chemical Name	Structure
JAU6476-OH-sulfonic acid-glucoside isomers *Wheat forage and hay* *Sugar beet tops*		
JAU6476-hydroxy-di-sulfonic acid glucoside *Sugar beet tops*		
JAU6476-desthio-dihydroxy-olefin glucosides *Peanut hay and nutmeat* *Rotated Swiss chard, turnip tops and root, and wheat forage, hay, and straw*		
Glucuronides		
JAU6476-hydroxy-glucuronide[2] *Goat milk, liver, kidney, muscle, and fat* *Hen liver*		
JAU6476-S-glucuronide *Goat milk, liver, kidney, muscle, and fat* *Hen egg, liver, muscle, and fat*		

Appendix I. Chemical Names and Structures of Prothioconazole and its Transformation Products.		
Common Name/Code*Matrix*	Chemical Name	Structure
JAU6476-O- or S-glucuronide *Goat milk, liver, kidney, muscle, and fat* *Hen egg, liver, muscle, and fat*		
JAU6476-N-glucuronide *Goat milk, liver, kidney, muscle, and fat* *Hen liver and muscle*		
JAU6476-desthio-3,4-dihydroxy-dienyl-glucuronide *Goat liver* *Hen liver and muscle*		
JAU6476-4-hydroxy-desthio-glucuronide *Goat milk*		
JAU6476-dihydroxy-desthio-glucuronide *Goat milk*		

Appendix I. Chemical Names and Structures of Prothioconazole and its Transformation Products.		
Common Name/Code*Matrix*	Chemical Name	Structure
JAU6476-hydroxy-methoxy-desthio-glucuronide *Goat milk*		
JAU6476-desthio-glucuronide *Goat milk*		
Sulfate conjugates		
Sulfate conjugate of JAU6476-hydroxy *Goat liver*		
Sulfate conjugate of JAU6476-hydroxy-desthio *Hen liver and fat*		
Sulfate conjugate of JAU6476-dihydroxy-desthio *Hen liver, muscle, and fat*		

Appendix I. Chemical Names and Structures of Prothioconazole and its Transformation Products.		
Common Name/Code*Matrix*	Chemical Name	Structure
Sulfate conjugate of JAU6476-hydroxy-methoxy-desthio *Hen liver, muscle, and fat*		

[1] When chemical names were not provided by the petitioner, the chemical naming feature of ISIS/Draw was used to generate the name.

[2] Including 3-hydroxy and/or 4-hydroxy isomers.

Appendix II – Statistical Calculation of Tolerances

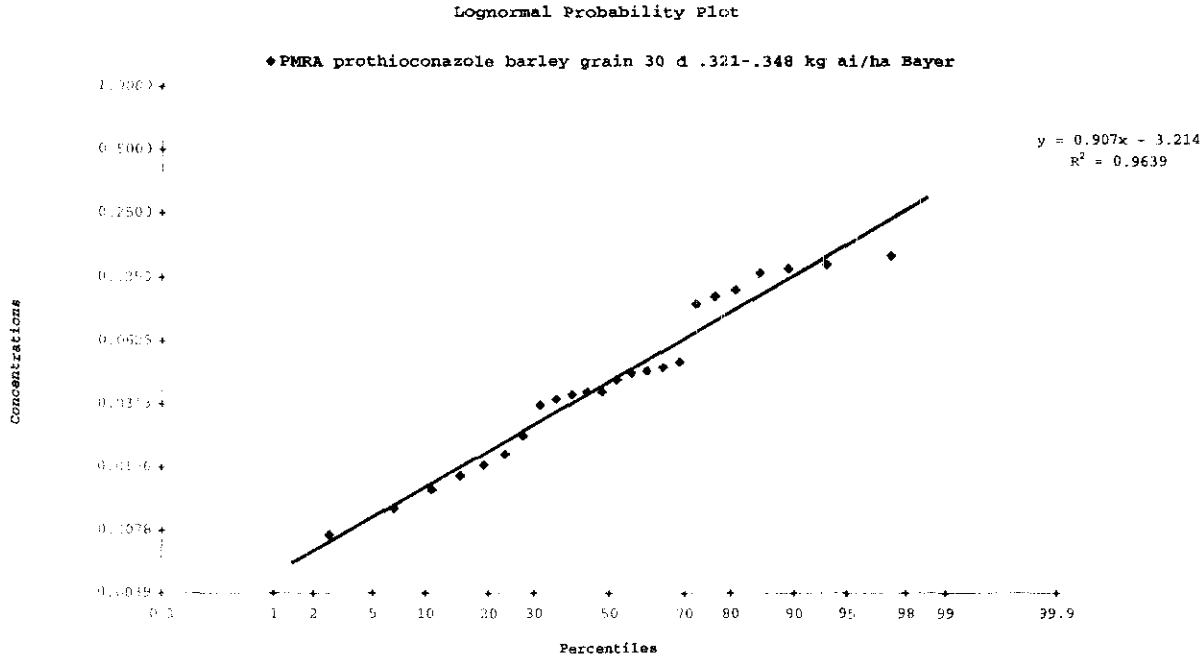
Barley Grain

Regulator:	EPA
Chemical:	prothioconazole
Crop:	barley grain
PHI:	32 d (30 d Canada)
App. Rate:	.321-.348 kg ai/ha
Submitter:	Bayer
n:	24
min:	0.01
max:	0.16
median:	0.04
average:	0.06

	95th Percentile	99th Percentile	99.9th Percentile
EU Method I Normal	0.15 (0.20)	0.20 (0.25)	0.25 (--)
EU Method I Log Normal	0.20 (0.35)	0.35 (0.70)	0.70 (--)
EU Method II Distribution-Free		0.20	
California Method $\mu + 3\sigma$		**0.25**	
UPLMedian95th		**0.25**	
Approximate Shapiro-Francia Normality Test		0.9639 p-value > 0.05 : Do not reject lognormality assumption	

Residues (LOQ = 0.02)	LN(Residues)	Z-scores
0.05	-3.00	0.48
0.036	-3.32	-0.16
0.144	-1.94	1.50
0.007419992	-4.90	-1.95
0.010009059	-4.60	-1.50
0.012189445	-4.41	-1.24
0.031	-3.47	-0.48
0.01420563	-4.25	-1.04
0.132	-2.02	1.04
0.102	-2.28	0.73
0.041	-3.19	0.05
0.036	-3.32	-0.05
0.094	-2.36	0.60
0.044	-3.12	0.16
0.158	-1.85	1.95
0.016149969	-4.13	-0.88
0.022	-3.82	-0.60
0.01807106	-4.01	-0.73

0.035	-3.35	-0.26
0.033	-3.41	-0.37
0.138	-1.98	1.24
0.109	-2.22	0.88
0.047	-3.06	0.37
0.045	-3.10	0.26

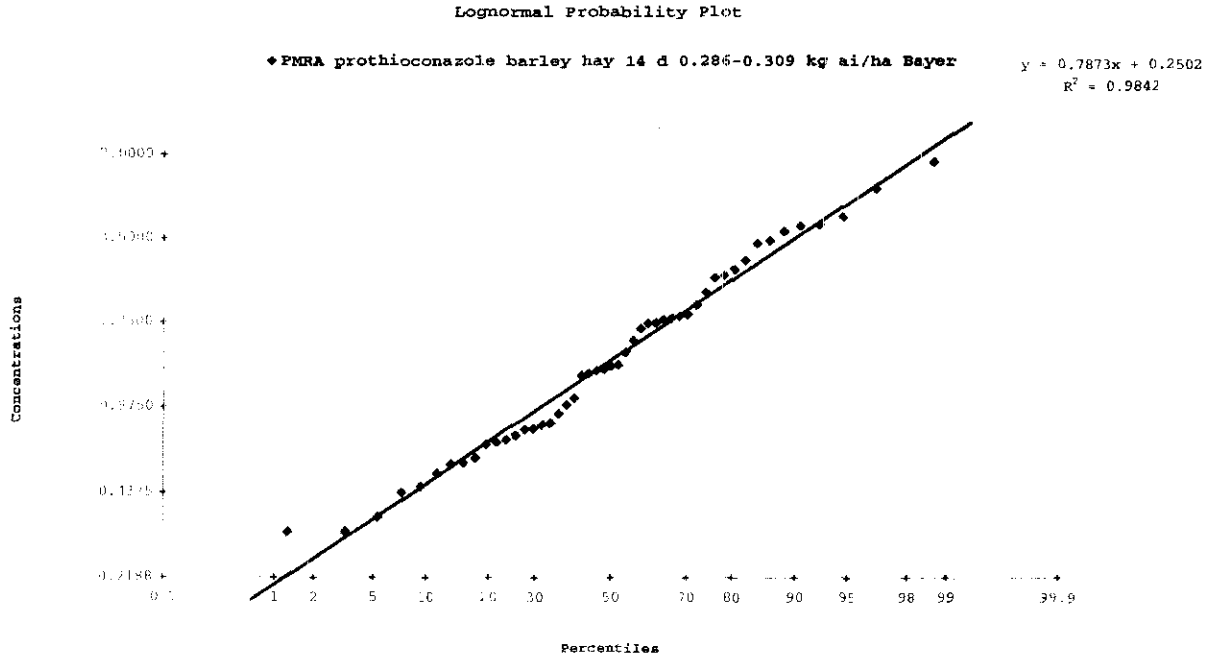


Barley Hay

	Regulator: EPA **Chemical:** prothioconazole **Crop:** barley hay **PHI:** 14 d **App. Rate:** 0.286-0.309 kg ai/ha **Submitter:** Bayer **n:** 49 **min:** 0.32 **max:** 6.59 **median:** 1.22 **average:** 1.72		
	95th Percentile	**99th Percentile**	**99.9th Percentile**
EU Method I Normal	4.5 (5.0)	5.0 (6.0)	7.0 (--)
EU Method I Log Normal	5.0 (7.0)	**8.0** (13)	15 (--)
EU Method II *Distribution-Free*	5.0		
California Method $\mu + 3\sigma$	**6.0**		
UPLMedian95th	**7.0**		
Approximate Shapiro-Francia Normality Test	0.9842 p-value > 0.05 : Do not reject lognormality assumption		

Residues	LN(Residues)	Z-scores
0.438	-0.83	-1.45
1.133	0.12	-0.21
2.246	0.81	0.65
0.647	-0.44	-0.86
0.318	-1.15	-1.84
0.668	-0.40	-0.72
1.191	0.17	-0.05
5.305	1.67	1.84
1.178	0.16	-0.10
1.151	0.14	-0.15
1.865	0.62	0.53
1.741	0.55	0.26
0.656	-0.42	-0.79
0.551	-0.60	-1.11
0.317	-1.15	-2.24
0.556	-0.59	-1.02
2.933	1.08	0.93
2.718	1.00	0.86
0.512	-0.67	-1.20
1.669	0.51	0.21

3.366	1.21	1.02
0.890	-0.12	-0.31
1.747	0.56	0.31
3.931	1.37	1.45
1.796	0.59	0.36
0.462	-0.77	-1.32
1.220	0.20	0.00
2.534	0.93	0.72
0.731	-0.31	-0.59
0.358	-1.03	-1.61
0.758	-0.28	-0.48
1.235	0.21	0.05
6.590	1.89	2.24
1.375	0.32	0.10
1.514	0.41	0.15
2.021	0.70	0.59
1.814	0.60	0.42
0.829	-0.19	-0.36
0.578	-0.55	-0.93
0.734	-0.31	-0.53
0.767	-0.27	-0.42
3.445	1.24	1.11
0.697	-0.36	-0.65
2.577	0.95	0.79
3.715	1.31	1.20
0.941	-0.06	-0.26
1.843	0.61	0.48
4.197	1.43	1.61
3.895	1.36	1.32


Lognormal Probability Plot
◆PMRA prothioconazole barley hay 14 d 0.286-0.309 kg ai/ha Bayer
y = 0.7873x + 0.2502
R² = 0.9842
Concentrations
Percentiles
1
2
5
10
20
30
50
70
80
90
95
98
99
99.9

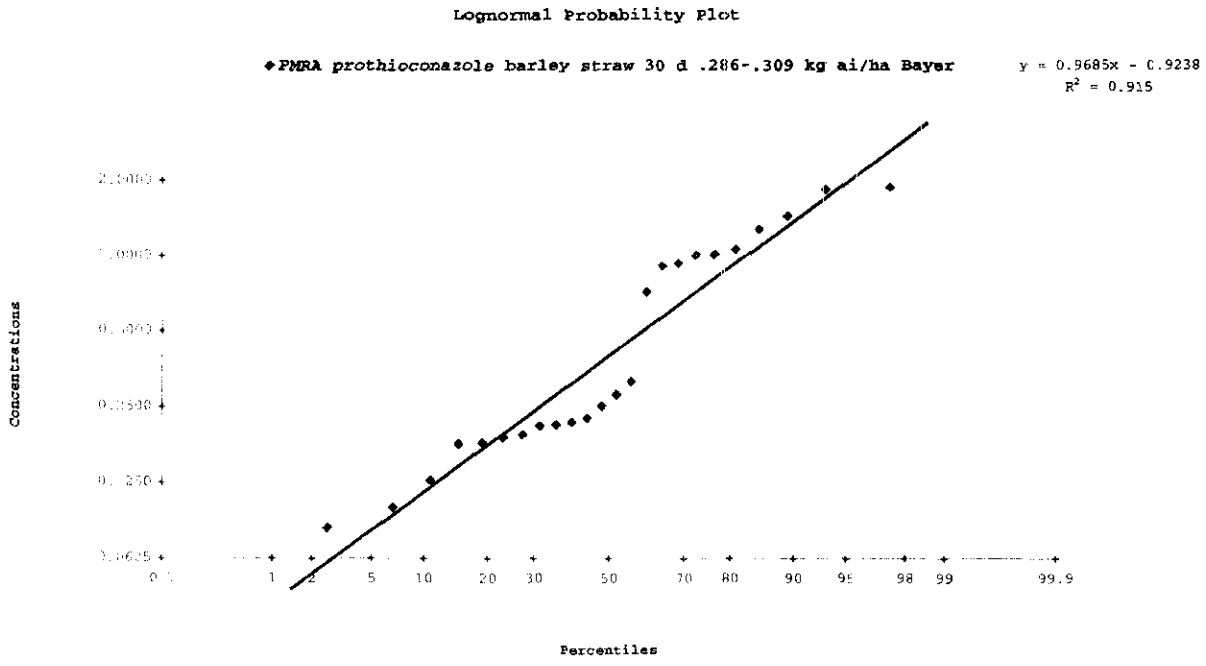
Barley Straw

Regulator:	EPA
Chemical:	prothioconazole
Crop:	barley straw
PHI:	30 d
App. Rate:	.286-.309 kg ai/ha
Submitter:	Bayer
n:	24
min:	0.08
max:	1.87
median:	0.26
average:	0.62

	95th Percentile	99th Percentile	99.9th Percentile
EU Method I Normal	1.6 (2.0)	2.0 (2.5)	2.5 (--)
EU Method I Log Normal	2.0 (4.0)	4.0 (9.0)	9.0 (--)
EU Method II Distribution-Free	2.5		
California Method $\mu + 3\sigma$	2.5		
UPLMedian95th	1.6		
Approximate Shapiro-Francia Normality Test	0.9150 p-value > 0.05 : Do not reject lognormality assumption		

Residues	LN(Residues)	Z-scores
1.003	0.00	0.60
0.180	-1.71	-0.88
1.282	0.25	1.04
0.083	-2.49	-1.95
0.251	-1.38	-0.05
0.911	-0.09	0.37
0.209	-1.57	-0.48
0.128	-2.06	-1.24
0.711	-0.34	0.26
1.439	0.36	1.24
0.188	-1.67	-0.73
0.177	-1.73	-1.04
1.056	0.05	0.88
0.193	-1.65	-0.60
1.828	0.60	1.50
0.100	-2.30	-1.50
0.278	-1.28	0.05
1.008	0.01	0.73
0.313	-1.16	0.16
0.211	-1.56	-0.37
0.932	-0.07	0.48
1.871	0.63	1.95

0.225	-1.49	-0.16
0.217	-1.53	-0.26



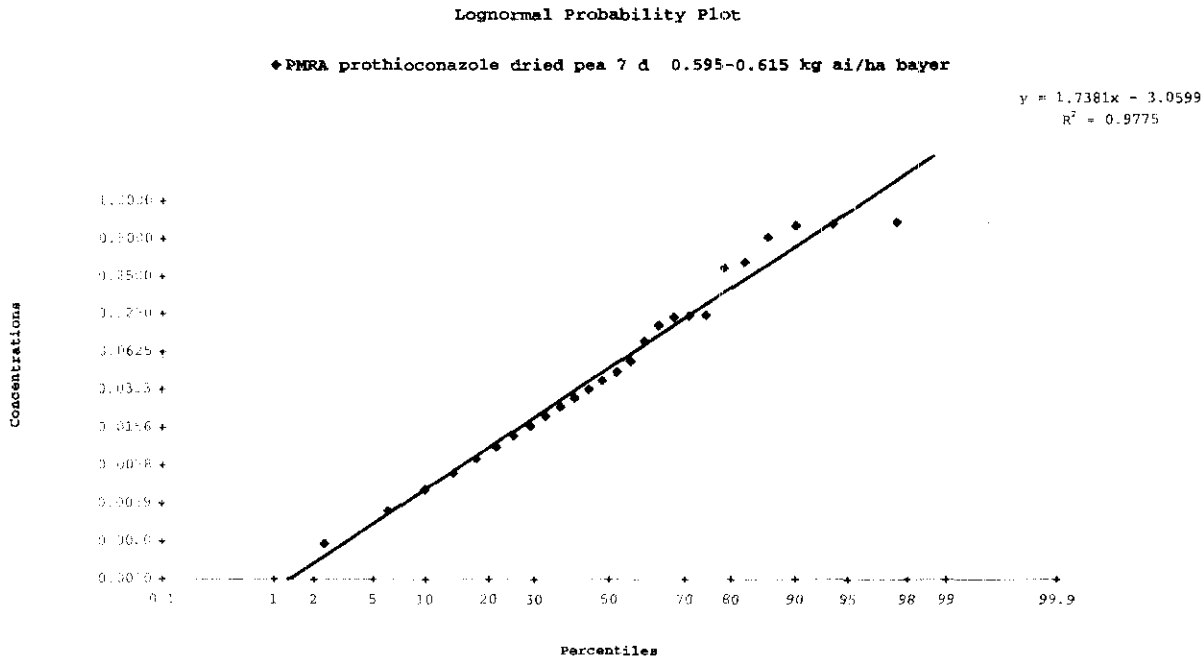
Pea and Bean (dry)

Pea

	PHI: 7 d App. Rate: 0.595-0.615 kg ai/ha Submitter: bayer n: 26 min: 0.00 max: 0.68 median: 0.04 average: 0.15		
	95th Percentile	**99th Percentile**	**99.9th Percentile**
EU Method I Normal	0.60 (0.70)	0.70 (0.90)	0.90 (--)
EU Method I *Log Normal*	0.80 (2.5)	2.5 (10)	10 (--)
EU Method II Distribution-Free	0.35		
California Method $\mu + 3\sigma$	**0.90**		
UPLMedian95th	**0.25**		
Approximate Shapiro-Francia Normality Test	0.9775 p-value > 0.05 : Do not reject lognormality assumption		

Residues (LOQ = 0.05 ppm)	LN(Residues)	Z-scores
0.12	-2.12	0.55
0.102	-2.28	0.34
0.001895462	-6.27	-1.98
0.003411318	-5.68	-1.54
0.292	-1.23	0.79
0.005030396	-5.29	-1.28
0.006808825	-4.99	-1.09
0.008782788	-4.73	-0.93
0.010986121	-4.51	-0.79
0.013455115	-4.31	-0.67
0.519	-0.66	1.09
0.016231262	-4.12	-0.55
0.639	-0.45	1.28
0.122	-2.10	0.67
0.118	-2.14	0.44
0.019363226	-3.94	-0.44
0.022909197	-3.78	-0.34
0.328	-1.11	0.93
0.026939787	-3.61	-0.24
0.031541633	-3.46	-0.14
0.076	-2.58	0.24

0.036822359	-3.30	-0.05
0.042917445	-3.15	0.05
0.655	-0.42	1.54
0.053	-2.94	0.14
0.684	-0.38	1.98

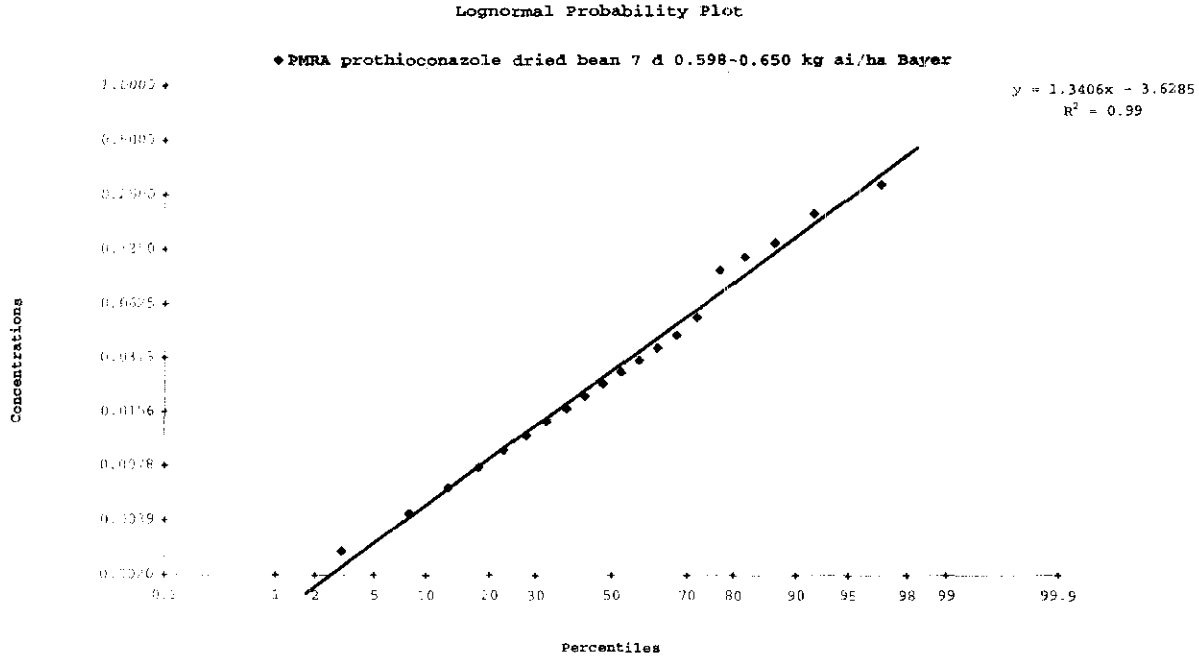


Bean

Regulator:	EPA
Chemical:	prothioconazole
Crop:	dried bean
PHI:	7 d
App. Rate:	0.598-0.650 kg ai/ha
Submitter:	Bayer
n:	20
min:	0.00
max:	0.29
median:	0.02
average:	0.06

	95th Percentile	99th Percentile	99.9th Percentile
EU Method I Normal	0.20 (0.25)	0.25 (0.35)	0.30 (--)
EU Method I Log Normal	**(0.60)**	**0.60** (2.0)	1.5 (--)
EU Method II Distribution-Free		0.20	
California Method μ + 3σ		**0.30**	
UPLMedian95th		**0.20**	
Approximate Shapiro-Francia Normality Test		0.9900 p-value > 0.05 : Do not reject lognormality assumption	

Residues (LOQ = 0.05 ppm)	LN(Residues)	Z-scores
0.053	-2.94	0.59
0.002656732	-5.93	-1.87
0.004293274	-5.45	-1.40
0.115	-2.16	0.92
0.005924704	-5.13	-1.13
0.199	-1.61	1.40
0.007642865	-4.87	-0.92
0.009497614	-4.66	-0.74
0.011530548	-4.46	-0.59
0.013784693	-4.28	-0.45
0.096	-2.34	0.74
0.016309357	-4.12	-0.31
0.019164827	-3.95	-0.19
0.137	-1.99	1.13
0.022427309	-3.80	-0.06
0.288	-1.24	1.87
0.026196504	-3.64	0.06
0.030606471	-3.49	0.19
0.0358432	-3.33	0.31
0.042174067	-3.17	0.45


Lognormal Probability Plot
◆PMRA prothioconazole dried bean 7 d 0.598-0.650 kg ai/ha Bayer
y = 1.3406x - 3.6285
R² = 0.99
Concentrations
0.6625
0.0156
0.0078
0.0039
0.1
1
2
5
10
20
30
50
70
80
90
95
98
99
99.9
Percentiles

Peanut

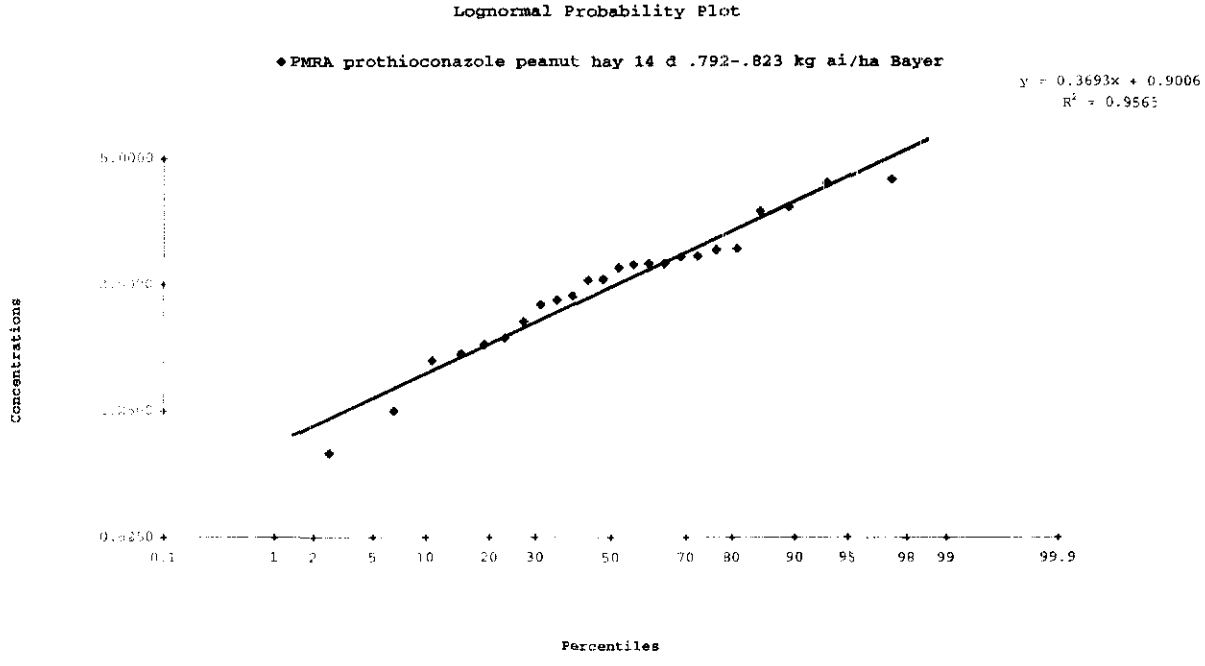
All values were below the limit of quantitation, 0.02 ppm.

Peanut Hay

	Regulator: EPA **Chemical:** prothioconazole **Crop:** peanut hay **PHI:** 14 d **App. Rate:** .792-.823 kg ai/ha **Submitter:** Bayer **n:** 24 **min:** 0.99 **max:** 4.46 **median;** 2.66 **average:** 2.61		
	95th Percentile	**99th Percentile**	**99.9th Percentile**
EU Method I Normal	4.5 (5.0)	5.0 (6.0)	6.0 (--)
EU Method I Log Normal	4.5 **(6.0)**	**6.0** (8.0)	8.0 (--)
EU Method II Distribution-Free	6.0		
California Method $\mu + 3\sigma$	**6.0**		
UPLMedian95th	**16**		
Approximate Shapiro-Francia Normality Test	0.9565 p-value > 0.05 : Do not reject lognormality assumption		

Residues	LN(Residues)	Z-scores
1.645	0.50	-1.24
2.289	0.83	-0.37
2.908	1.07	0.48
2.787	1.02	0.16
2.036	0.71	-0.60
1.709	0.54	-1.04
2.801	1.03	0.37
2.564	0.94	-0.16
0.989	-0.01	-1.95
2.574	0.95	-0.05
1.863	0.62	-0.73
1.797	0.59	-0.88
2.921	1.07	0.60
3.831	1.34	1.24
4.35	1.47	1.50
3.741	1.32	1.04
2.74	1.01	0.05
2.235	0.80	-0.48

4.458	1.49	1.95
3.025	1.11	0.73
1.249	0.22	-1.50
3.044	1.11	0.88
2.342	0.85	-0.26
2.799	1.03	0.26

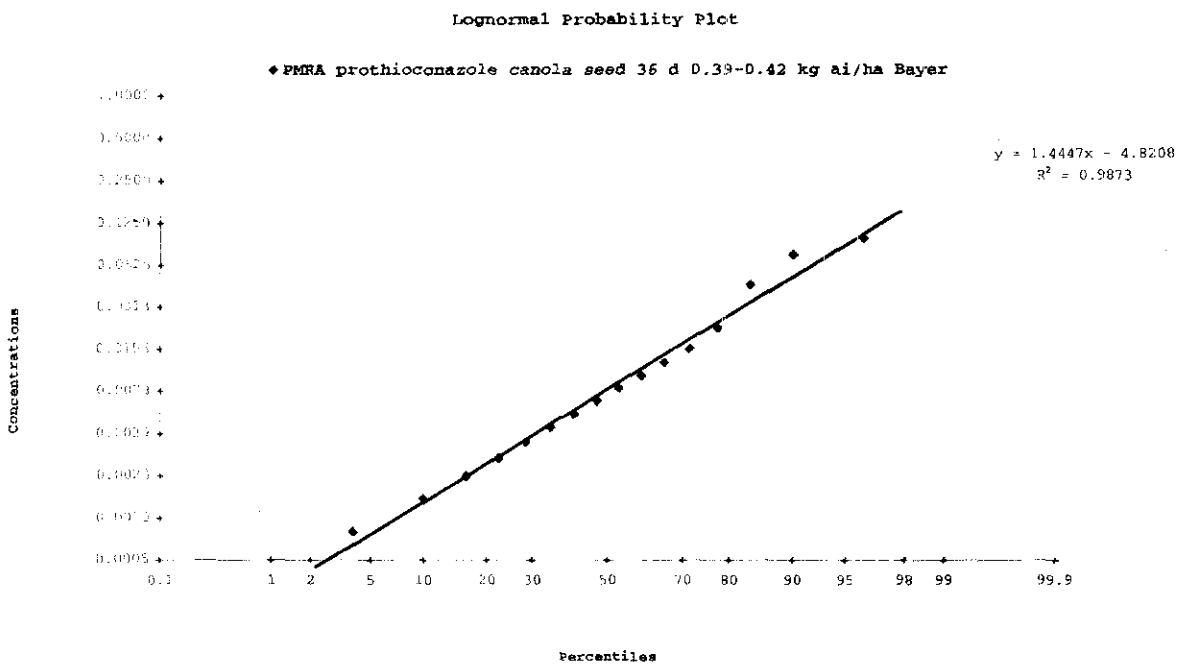


Rapeseed (Canola)

Regulator:	EPA
Chemical:	prothioconazole
Crop:	canola seed
PHI:	36 d
App. Rate:	0.39-0.42 kg ai/ha
Submitter:	Bayer
n:	16
min:	0.00
max:	0.10
median:	0.01
average:	0.02

	95th Percentile	99th Percentile	99.9th Percentile
EU Method I Normal	0.07 (0.10)	0.09 (0.15)	0.15 (--)
EU Method I Log Normal	0.08 **(0.30)**	**0.25** (1.0)	0.60 (--)
EU Method II Distribution-Free		0.05	
California Method μ + 3σ		**0.15**	
UPLMedian95th		**0.05**	
Approximate Shapiro-Francia Normality Test		0.9873 p-value > 0.05 : Do not reject lognormality assumption	

Residues (LOQ = 0.02 ppm)	LN(Residues)	Z-scores
0.022	-3.82	0.76
0.000778668	-7.16	-1.77
0.074	-2.60	1.28
0.001344005	-6.61	-1.28
0.001949138	-6.24	-0.99
0.002626332	-5.94	-0.76
0.003400676	-5.68	-0.57
0.004299853	-5.45	-0.40
0.045	-3.10	0.99
0.005358714	-5.23	-0.23
0.097	-2.33	1.77
0.00662391	-5.02	-0.08
0.008160845	-4.81	0.08
0.010065174	-4.60	0.23
0.012483685	-4.38	0.40
0.015655229	-4.16	0.57


Lognormal Probability Plot
PMRA prothioconazole canola seed 35 d 0.39-0.42 kg ai/ha Bayer
y = 1.4447x - 4.8208
R² = 0.9873
Concentrations
0.1
1
2
5
10
20
30
50
70
80
90
95
98
99
99.9
Percentiles

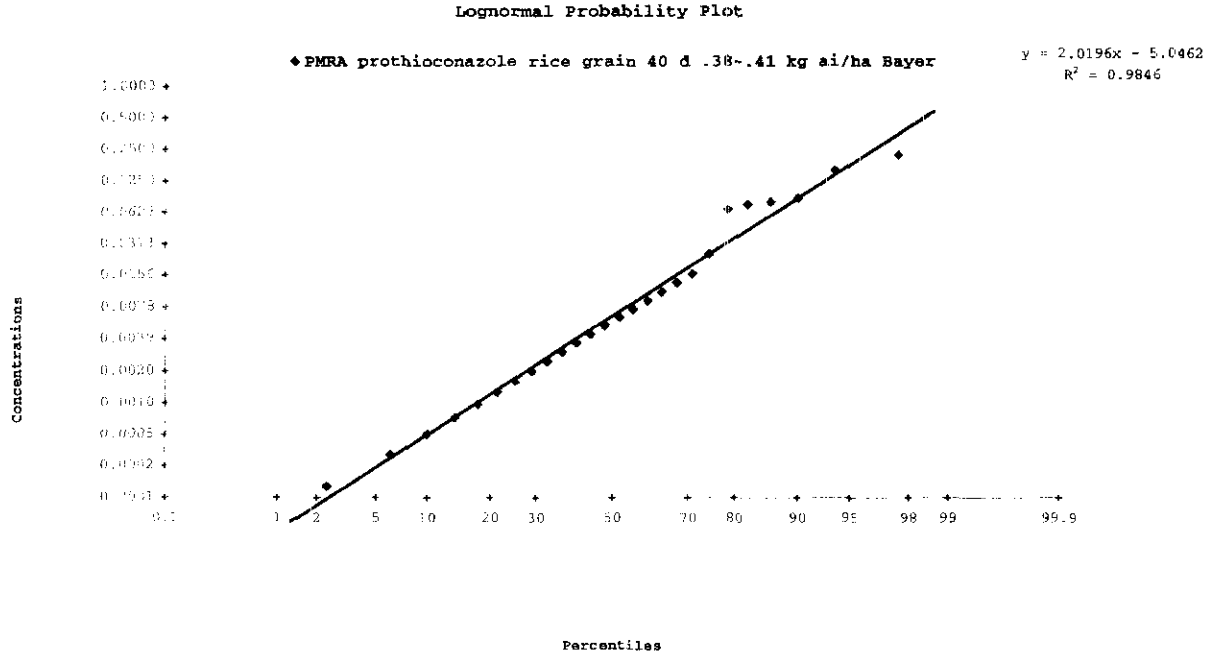
Rice Grain

Regulator:	EPA
Chemical:	prothioconazole
Crop:	rice grain
PHI:	40 d
App. Rate:	.38-.41 kg ai/ha
Submitter:	Bayer
n:	26
min:	0.00
max:	0.22
median:	0.01
average:	0.03

	95th Percentile	99th Percentile	99.9th Percentile
EU Method I Normal	0.15 (0.20)	0.20 (0.25)	0.25 (--)
EU Method I Log Normal	0.20 **(0.60)**	**0.70** (3.5)	3.0 (--)
EU Method II Distribution-Free		0.08	
California Method $\mu + 3\sigma$		**0.20**	
UPLMedian95th		**0.04**	
Approximate Shapiro-Francia Normality Test		0.9846 p-value > 0.05 : Do not reject lognormality assumption	

Residues (LOQ = 0.02 ppm)	LN(Residues)	Z-scores
0.159	-1.84	1.54
0.079	-2.54	1.09
0.00015637	-8.76	-1.98
0.00031169	-8.07	-1.54
0.00049206	-7.62	-1.28
0.00070271	-7.26	-1.09
0.00094866	-6.96	-0.93
0.00123556	-6.70	-0.79
0.00157018	-6.46	-0.67
0.00196068	-6.23	-0.55
0.00241708	-6.03	-0.44
0.00295171	-5.83	-0.34
0.067	-2.70	0.79
0.222	-1.51	1.98
0.086	-2.45	1.28
0.00357997	-5.63	-0.24
0.00432132	-5.44	-0.14
0.00520058	-5.26	-0.05
0.00624988	-5.08	0.05
0.00751159	-4.89	0.14
0.025	-3.69	0.67

0.00904243	-4.71	0.24
0.01092018	-4.52	0.34
0.01325397	-4.32	0.44
0.01620166	-4.12	0.55
0.075	-2.59	0.93

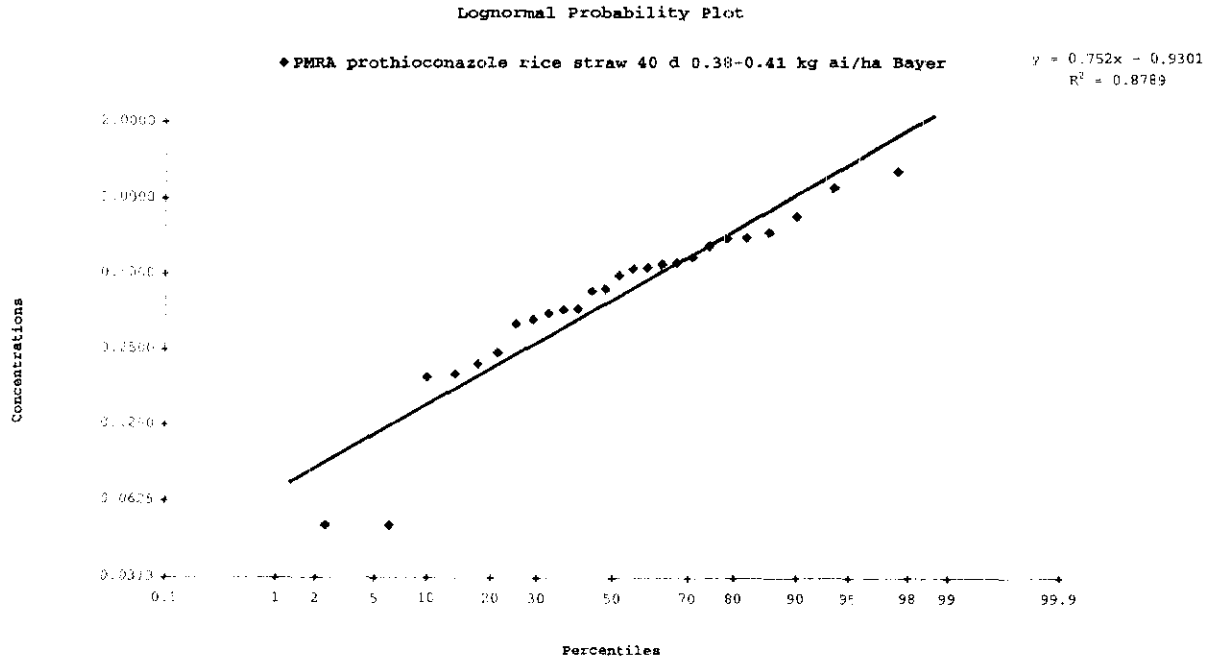


Rice Straw

	Regulator: EPA **Chemical:** prothioconazole **Crop:** rice straw **PHI:** 40 d **App. Rate:** 0.38-0.41 kg ai/ha **Submitter:** Bayer **n:** 26 **min:** 0.05 **max:** 1.28 **median;** 0.47 **average:** 0.49		
	95th Percentile	**99th Percentile**	**99.9th Percentile**
EU Method I Normal	1.0 (1.2)	1.2 (1.5)	1.4 (--)
EU Method I *Log Normal*	1.5 **(2.5)**	**2.5** (5.0)	4.5 (--)
EU Method II Distribution-Free		1.4	
California Method $\mu + 3\sigma$		**1.4**	
UPLMedian95th		**3.0**	
Approximate Shapiro-Francia Normality Test	0.8789 p-value <= 0.01: Reject lognormality assumption		

Residues	LN(Residues)	Z-scores
0.694	-0.37	0.79
0.363	-1.01	-0.24
0.05	-3.00	-1.98
0.526	-0.64	0.14
0.7	-0.36	0.93
0.315	-1.16	-0.67
0.427	-0.85	-0.14
1.101	0.10	1.54
0.194	-1.64	-1.28
0.243	-1.41	-0.79
0.2	-1.61	-1.09
0.497	-0.70	0.05
0.531	-0.63	0.24
0.85	-0.16	1.28
0.548	-0.60	0.34
0.05	-3.00	-1.54
0.556	-0.59	0.44
0.736	-0.31	1.09
0.36	-1.02	-0.34
0.437	-0.83	-0.05
1.277	0.24	1.98

0.33	-1.11	-0.55
0.349	-1.05	-0.44
0.219	-1.52	-0.93
0.587	-0.53	0.55
0.65	-0.43	0.67

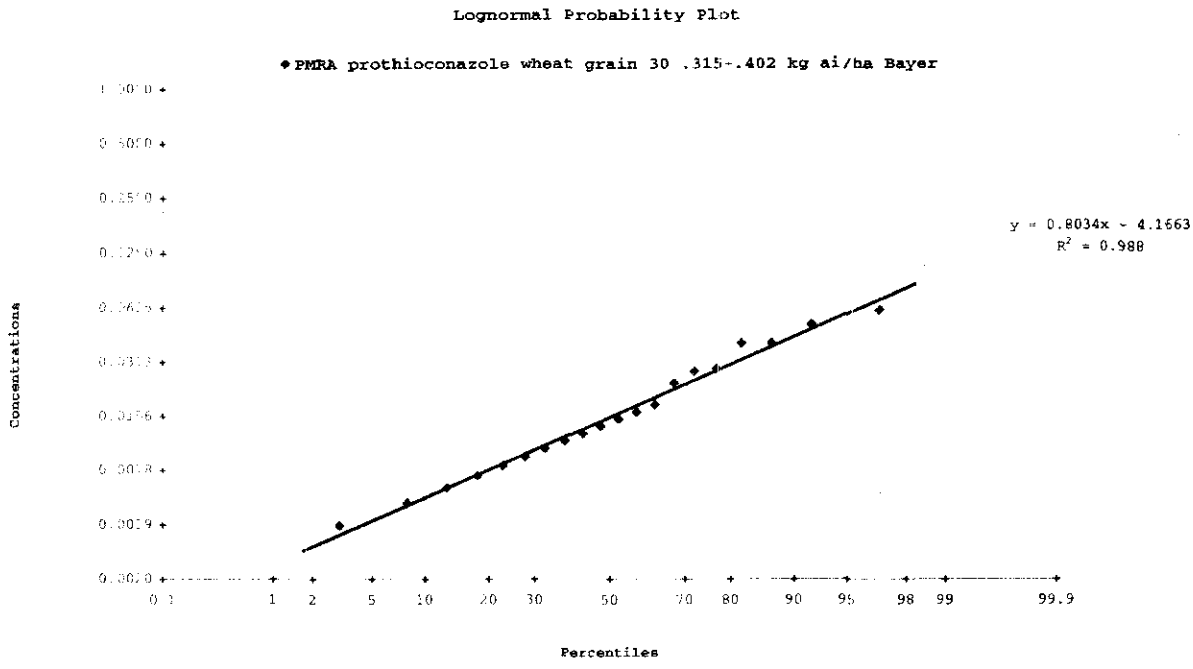


Wheat Grain

Regulator:	EPA
Chemical:	prothioconazole
Crop:	wheat grain
PHI:	30
App. Rate:	.315-.402 kg ai/ha
Submitter:	Bayer
n:	20
min:	0.00
max:	0.06
median;	0.01
average:	0.02

	95th Percentile	99th Percentile	99.9th Percentile
EU Method I Normal	0.05 (0.06)	0.06 (0.08)	0.08 (--)
EU Method I Log Normal	0.06 **(0.15)**	**0.10** (0.25)	0.20 (--)
EU Method II Distribution-Free		0.06	
California Method $\mu + 3\sigma$		**0.07**	
UPLMedian95th		**0.09**	
Approximate Shapiro-Francia Normality Test		0.9880 p-value > 0.05 : Do not reject lognormality assumption	

Residues (LOQ = 0.02 ppm)	LN(Residues)	Z-scores
0.040	-3.22	0.92
0.028	-3.58	0.59
0.029	-3.54	0.74
0.004	-5.55	-1.87
0.005	-5.26	-1.40
0.006	-5.07	-1.13
0.007	-4.92	-0.92
0.008	-4.79	-0.74
0.009	-4.68	-0.59
0.010	-4.57	-0.45
0.051	-2.98	1.40
0.061	-2.80	1.87
0.040	-3.22	1.13
0.011	-4.47	-0.31
0.013	-4.38	-0.19
0.014	-4.28	-0.06
0.015	-4.19	0.06
0.024	-3.73	0.45
0.017	-4.10	0.19
0.018	-4.01	0.31


Lognormal Probability Plot
PMRA prothioconazole wheat grain 30 .315-.402 kg ai/ha Bayer
y = 0.8034x - 4.1663
R² = 0.988
Concentrations
0.1
1
2
5
10
20
30
50
70
80
90
95
98
99
99.9
Percentiles

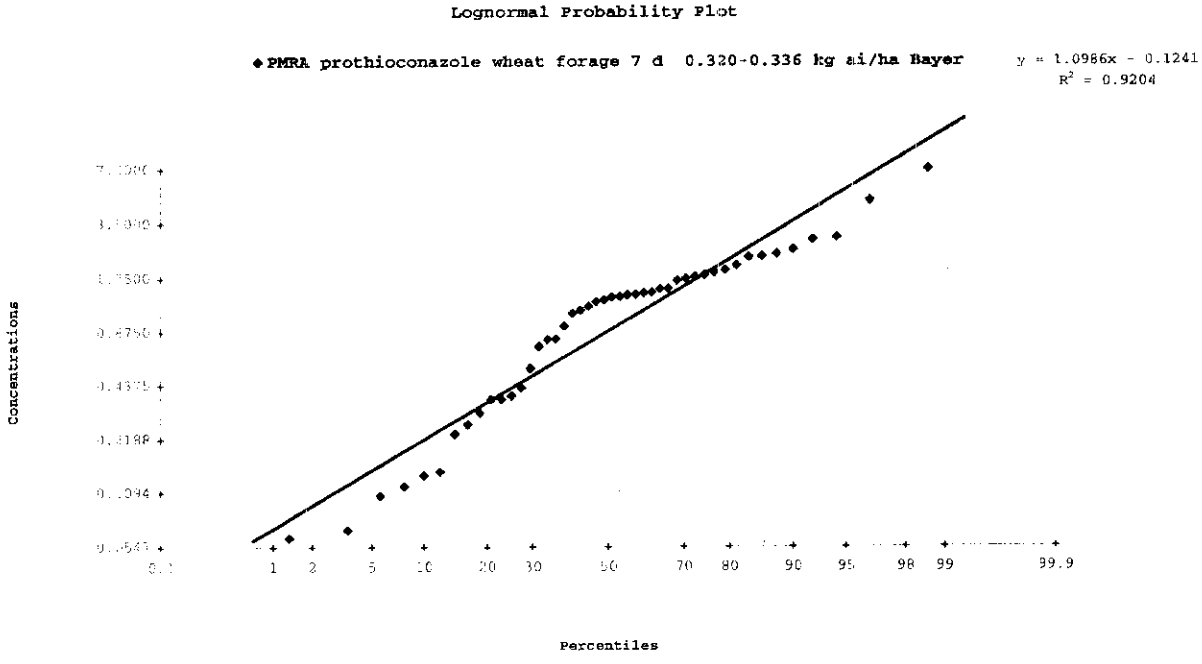
Wheat Forage

Regulator:	EPA
Chemical:	prothioconazole
Crop:	wheat forage
PHI:	7 d
App. Rate:	0.320-0.336 kg ai/ha
Submitter:	Bayer
n:	46
min:	0.06
max:	6.99
median:	1.35
average:	1.40

	95th Percentile	99th Percentile	99.9th Percentile
EU Method I Normal	3.5 (4.5)	4.5 (6.0)	6.0 (--)
EU Method I Log Normal	6.0 **(10)**	**13** (25)	30 (--)
EU Method II Distribution-Free		4.0	
California Method μ + 3σ		**6.0**	
UPLMedian95th		**8.0**	
Approximate Shapiro-Francia Normality Test		0.9204 p-value <= 0.01: Reject lognormality assumption	

Residues	LN(Residues)	Z-scores
1.383	0.32	0.08
0.233	-1.46	-1.07
0.383	-0.96	-0.67
2.528	0.93	1.28
0.307	-1.18	-0.89
1.112	0.11	-0.25
4.696	1.55	1.81
0.365	-1.01	-0.81
0.105	-2.25	-1.58
0.061	-2.80	-2.21
2.388	0.87	1.17
1.413	0.35	0.14
1.461	0.38	0.30
1.325	0.28	-0.03
2.294	0.83	0.97
1.296	0.26	-0.08
0.794	-0.23	-0.42
0.136	-2.00	-1.28
0.727	-0.32	-0.48
1.222	0.20	-0.14
1.378	0.32	0.03
1.532	0.43	0.42
1.703	0.53	0.48

1.827	0.60	0.67
0.263	-1.34	-0.97
0.547	-0.60	-0.54
2.864	1.05	1.42
0.366	-1.01	-0.74
1.161	0.15	-0.19
6.987	1.94	2.21
0.425	-0.86	-0.60
0.119	-2.13	-1.42
0.068	-2.69	-1.81
2.941	1.08	1.58
1.792	0.58	0.60
1.749	0.56	0.54
1.883	0.63	0.74
2.321	0.84	1.07
1.448	0.37	0.25
0.948	-0.05	-0.30
0.143	-1.94	-1.17
0.802	-0.22	-0.36
1.529	0.42	0.36
1.42	0.35	0.19
2.061	0.72	0.89
1.944	0.66	0.81



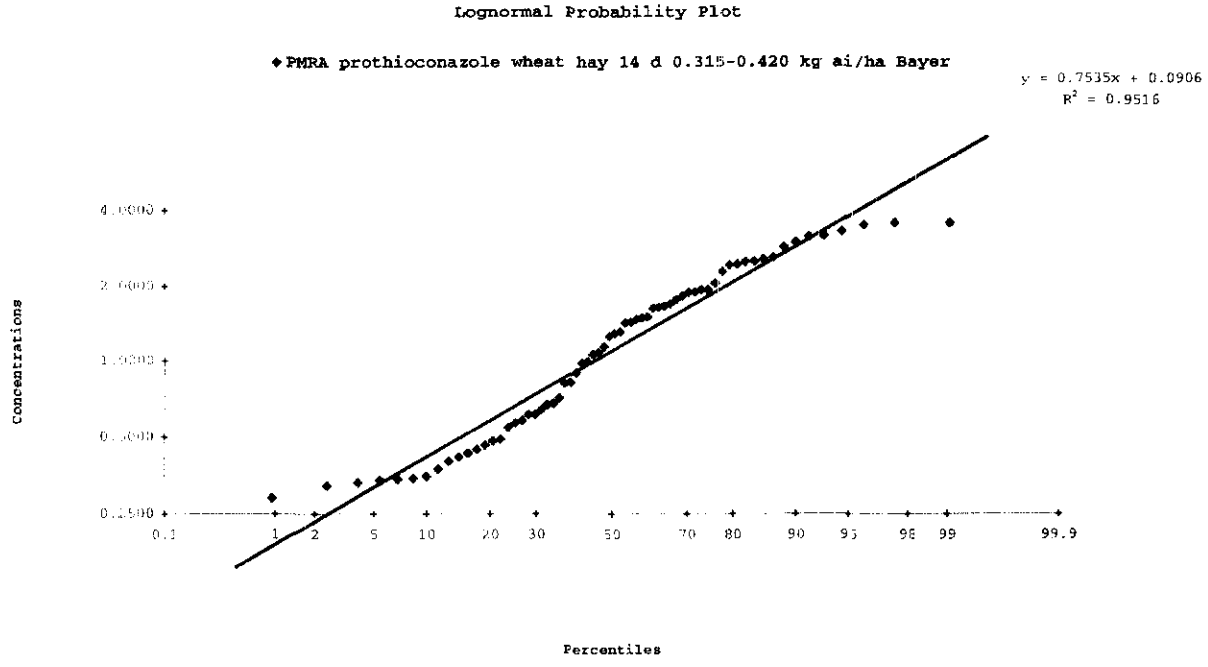
Wheat Hay

	Regulator: EPA **Chemical:** prothioconazole **Crop:** wheat hay **PHI:** 14 d **App. Rate:** 0.315-0.420 kg ai/ha **Submitter:** Bayer **n:** 66 **min:** 0.29 **max:** 3.57 **median;** 1.27 **average:** 1.42		
	95th Percentile	**99th Percentile**	**99.9th Percentile**
EU Method I Normal	3.5 (3.5)	4.0 (4.5)	4.5 (--)
EU Method I Log Normal	4.0 **(6.0)**	**7.0** (10)	12 (--)
EU Method II Distribution-Free	4.0		
California Method $\mu + 3\sigma$	4.5		
UPLMedian95th	**7.0**		
Approximate Shapiro-Francia Normality Test	0.9516 0.05 >= p-value > 0.01 : Reject lognormality assumption		

Residues	LN(Residues)	Z-scores
0.545	-0.61	-0.72
0.71	-0.34	-0.37
2.866	1.05	1.20
1.42	0.35	0.09
0.339	-1.08	-1.60
1.889	0.64	0.58
0.322	-1.13	-1.97
2.055	0.72	0.72
2.442	0.89	0.88
1.47	0.39	0.17
0.417	-0.87	-1.06
0.675	-0.39	-0.41
2.998	1.10	1.28
0.612	-0.49	-0.58
1.252	0.22	-0.02
0.432	-0.84	-0.99
0.567	-0.57	-0.67
0.341	-1.08	-1.48
1.822	0.60	0.49
1.928	0.66	0.62
1.489	0.40	0.21
3.515	1.26	1.76

0.809	-0.21	-0.33
2.601	0.96	1.13
1.08	0.08	-0.09
1.308	0.27	0.06
1.286	0.25	0.02
1.641	0.50	0.33
0.821	-0.20	-0.29
1.431	0.36	0.13
0.344	-1.07	-1.37
0.288	-1.24	-2.35
0.447	-0.81	-0.93
0.58	-0.54	-0.62
1.063	0.06	-0.13
3.305	1.20	1.60
1.632	0.49	0.29
0.374	-0.98	-1.20
1.928	0.66	0.67
0.401	-0.91	-1.13
2.568	0.94	1.06
3.569	1.27	1.97
1.761	0.57	0.45
0.482	-0.73	-0.82
0.898	-0.11	-0.25
3.182	1.16	1.48
0.668	-0.40	-0.45
1.66	0.51	0.37
0.49	-0.71	-0.77
0.638	-0.45	-0.49
0.35	-1.05	-1.28
2.509	0.92	0.99
2.501	0.92	0.93
1.5	0.41	0.25
3.571	1.27	2.35
0.981	-0.02	-0.21
3.149	1.15	1.37
1.142	0.13	-0.06
2.287	0.83	0.77
1.886	0.63	0.54
2.428	0.89	0.82
0.995	-0.01	-0.17
1.693	0.53	0.41
0.612	-0.49	-0.54
0.332	-1.10	-1.76

0.463	-0.77	-0.88

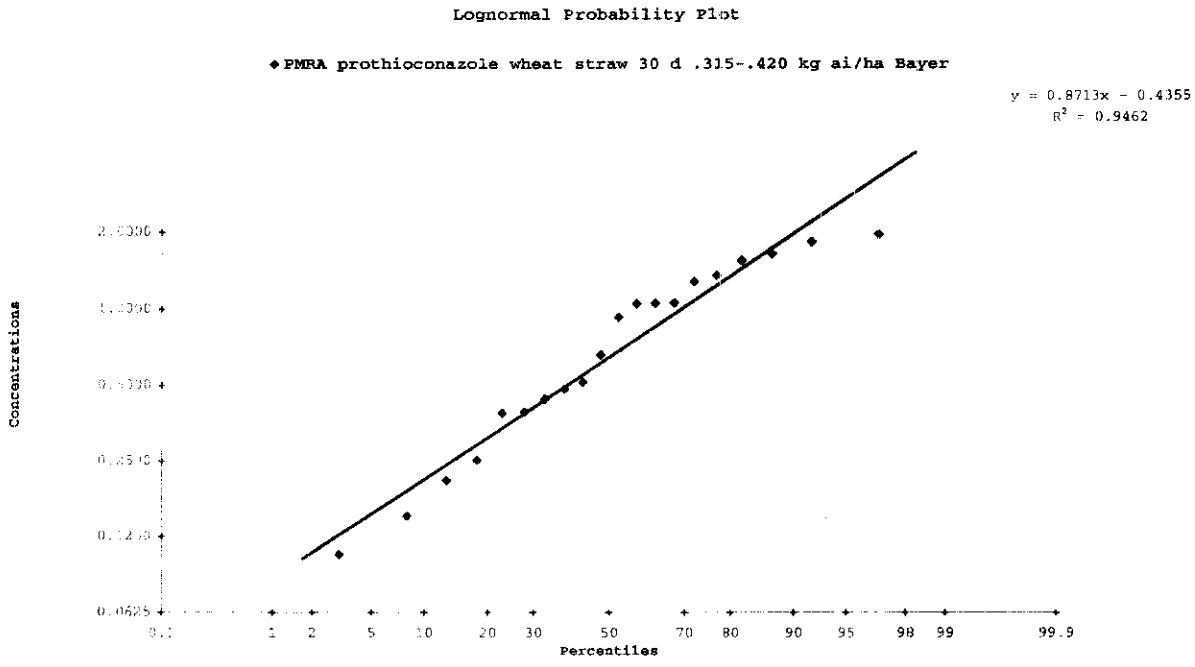

Lognormal Probability Plot
PMRA prothioconazole wheat hay 14 d 0.315-0.420 kg ai/ha Bayer
y = 0.7535x + 0.0906
R² = 0.9516
Concentrations
4.0000
2.0000
1.0000
0.5000
0.2500
0.1
1
2
5
10
20
30
50
70
80
90
95
98
99
99.9
Percentiles

Wheat Straw

Regulator:	EPA
Chemical:	prothioconazole
Crop:	wheat straw
PHI:	30 d
App. Rate:	.315-.420 kg ai/ha
Submitter:	Bayer
n:	20
min:	0.11
max:	1.96
median;	0.80
average:	0.87

	95th Percentile	99th Percentile	99.9th Percentile
EU Method I Normal	1.9 (2.5)	2.5 (3.0)	3.0 (--)
EU Method I Log Normal	3.0 **(6.0)**	**5.0** (12)	10 (--)
EU Method II Distribution-Free		3.0	
California Method $\mu + 3\sigma$		**3.0**	
UPLMedian95th		**5.0**	
Approximate Shapiro-Francia Normality Test	0.9462 p-value > 0.05 : Do not reject lognormality assumption		

Residues	LN(Residues)	Z-scores
0.393	-0.93	-0.59
0.443	-0.81	-0.45
1.838	0.61	1.40
0.93	-0.07	0.06
0.106	-2.24	-1.87
0.21	-1.56	-1.13
1.359	0.31	0.74
1.052	0.05	0.19
0.389	-0.94	-0.74
1.284	0.25	0.59
0.485	-0.72	-0.31
0.515	-0.66	-0.19
1.96	0.67	1.87
1.053	0.05	0.31
0.151	-1.89	-1.40
0.252	-1.38	-0.92
1.643	0.50	1.13
1.058	0.06	0.45
0.661	-0.41	-0.06
1.548	0.44	0.92


Lognormal Probability Plot
PMRA prothioconazole wheat straw 30 d .315-.420 kg ai/ha Bayer
y = 0.8713x - 0.4355
R² = 0.9462
Concentrations
0.0625
0.1
1
2
5
10
20
30
50
70
80
90
95
98
99
99.9
Percentiles

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON D.C., 20460
Analytical Chemistry Branch
Environmental Science Center
Fort George G. Meade, Maryland 20755-5350

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

November 6, 2006

MEMORANDUM

SUBJECT: PP#4F6830. New Chemical – Prothioconazole in/on Plant and Livestock Commodities. Tolerance Method Validation Report.
(MRID #'s 462462-04, 462462-06, 462462-07, and 462462-09)
Chemical # 113961. Decision Number: 318440. ACB # B05-39.
DP Barcode 318440.

FROM: Patricia G. Schermerhorn, Chemist
Paul E. Golden, Chemist
Analytical Chemistry Branch
Biological and Economic Analysis Division (7503P)

THRU: Frederic L. Siegelman, Chief
Analytical Chemistry Branch
Biological and Economic Analysis Division (7503P)

TO: Paula Deschamp, Chief
Registration Action Branch III
Health Effects Division (7509P)

AND

Cynthia Giles-Parker, Chief
Fungicide Branch
Registration Division (7505C)

INTRODUCTION

The Analytical Chemistry Branch (ACB) was requested by the Registration Action Branch III (RAB-3) to conduct a tolerance method validation for the new fungicide, prothioconazole, [2-[2-(1-chlorocyclopropyl)-3-(2-chlorophenyl)-2-hydroxyproply]-1,2-dihydro-3*H*-1,2,4-triazole-3-thione] and its desthio metabolite for agricultural commodities. The petitioner, Bayer CropScience, has submitted a petition (PP#4F6830) for the establishment of permanent tolerances for the combined residues

of the fungicide, prothioconazole (JAU 6476) , and its desthio metabolite (JAU 6476- desthio) on the following agricultural commodities at the following part per million (ppm) levels shown in parenthesis: 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); and wheat, germ (0.15 ppm).

Bayer is also proposing the establishment of permanent tolerances for residues of prothioconazole (JAU 6476), its desthio (JAU 6476-desthio) and 4-hydroxy (JAU 6476-4-hydroxy) metabolites, and conjugates that can be converted to these three compounds by acid hydrolysis in/on the following livestock commodities at the following ppm levels, shown in parenthesis: milk (0.006 ppm); cattle, fat (0.1 ppm); cattle, meat (0.01 ppm); and cattle, meat byproducts (1.2 ppm).

RAB-3 requested the ACB to fortify control peanut nutmeat for prothioconazole at the 0.02 ppm Level of Quantitation (LOQ) and the prothioconazole-desthio metabolite at 0.02 ppm (LOQ). Control wheat forage was requested to be fortified at two levels (0.05 ppm (LOQ) and 7 ppm) for both the parent, prothioconazole, and its desthio metabolite using the plant method found in MRID # 462462-06. In addition, RAB-3 requested ACB to fortify control milk at two levels (0.005 ppm (LOQ) and 0.01 ppm) for the parent, prothioconazole, and its desthio and 4-hydroxy metabolites. Control beef liver was requested at the following levels: prothioconazole, 0.01 ppm and 0.5 ppm; desthio metabolite, 0.01 ppm and 1.2 ppm; and 4-hydroxy metabolite, 0.01 ppm and 0.5 ppm; using the livestock method found in MRID # 462462-04.

METHOD SUMMARY – PLANT

The analytical method submitted for trial was titled: "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," written by T.J. Gould, B.C. Timberlake, M.E. Krolski, et al. Dated March 15, 2004. Bayer Study No. J6111401. Bayer Report No. 200799. Unpublished study prepared by Bayer Corp. **MRID # 462462-06**.

A 2 gram sample of homogenized matrix is extracted with a mixture of methanol (MeOH), 30% hydrogen peroxide (H_2O_2), and aqueous sodium bicarbonate ($NaHCO_3$) at 65 °C for 2 hours. This extraction procedure converts JAU 6476 to a mixture of JAU 6476 sulfonic acid and JAU 6476-desthio. Residues of JAU 6476-desthio are extracted without change. Following the addition of a mixture of isotopically labeled JAU 6476 sulfonic acid and JAU 6476-desthio internal standard, the sample extracts are purified by octadecyl solid phase extraction (C-18 SPE) and then mixed with aqueous acetic

acid. An aliquot of the sample is transferred to an autosampler vial for LC-MS/MS analysis.

METHOD SUMMARY – LIVESTOCK

The analytical method submitted for trial was titled: "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," written by S. M. Moore and A. M. Harbin. Dated October 31, 2003. Bayer Report No. 200537. Unpublished study prepared by Bayer Corp. **MRID # 462462-04.**

The method for analyzing liver, kidney, and muscle tissues consisted of an initial extraction using acetonitrile/water containing 250 mg/mL L-cysteine HCl (4:1), followed by the addition of an internal standards solution (labeled analogs of each analyte of interest). A portion of the extract was hydrolyzed in aqueous hydrochloric acid, and the analytes of interest transferred to the organic phase by partitioning with methylene chloride/acetone (3:2). An aliquot of this organic extract was concentrated to an aqueous remainder and diluted using acetonitrile and water prior to LC-MS/MS analysis.

The method for analyzing milk, skim milk, and cream omitted the initial extraction step. An aliquot of the labeled internal standards of JAU 6476, JAU 6476-4-hydroxy, and JAU 6476-desthio was added to the milk sample, which was then hydrolyzed, partitioned, and prepared for LC-MS/MS analysis as described above.

RECOMMENDATIONS

1. ACB finds that both methods (plant and livestock) which use liquid chromatography with tandem mass spectrometry using electrospray ionization in both the positive and negative modes meets the requirements of the Residue Chemistry Test Guidelines, OPPTS 860.1340, for acceptable tolerance enforcement methods.

2. ACB recommends that the petitioner include a confirmatory procedure for each method submitted. The OPP guidelines require either a confirmatory method or an interference study to eliminate the possibility of false positives while using the primary enforcement method. When mass spectrometry is used for detection in the primary enforcement method a confirmatory method is waived as long as the detector provides enough selectivity to eliminate false positives. While we fully agree that MS/MS provides excellent selectivity, expert opinion has changed over the past several years as mass spectrometrists have gained more experience with the instrumentation. A single MS/MS ion transition used to be considered sufficient for positive confirmation of analyte residue. However, instances of false positive interferences have led towards consensus that two ion transitions are needed to provide "confirmation" of residues. The ACB recommends that future revisions of these methods include at least two multiple reaction monitoring (MRM) transitions. References include the following:

a. Commission Decision 2002/657/EC, *Official Journal of the European Communities*, August 12, 2002.

b. Guidance for Industry: Mass Spectrometry for Confirmation of the Identity of Animal Drug Residues, US FDA Center for Veterinary Medicine, May 1, 2003.

c. Bethem, et al, Establishing the Fitness for Purpose of Mass Spectrometric Methods, *J Am Soc Mass Spectrom* 2003, 14, 523-541.

3. ACB reviewed the Independent Laboratory Validation (ILV) reports for both the plant and livestock methods (MRID #'s 462462-09 and 462462-07, respectively). ACB recommends that there were successful ILV's.

a. However, the first ILV trial of the plant method failed due to incomplete mixing following the addition of internal standard and prior to the removal of an aliquot for the C-18 SPE cartridge cleanup. This step may require a precautionary note in any future revisions of the method.

b. The ILV performing the livestock method found that a quadratic regression calibration curve with $1/x^2$ weighting gave the best fit for the milk. ACB was able to use a linear regression of $y = mx + b$ for the milk. Both the ILV and ACB used a 1/x weighted least square regression fit for the liver. A 1/x weighted linear regression was recommended and used by the petitioner.

COMMENTS

1. ACB's results are tabulated on pages 6 and 7. ACB concludes that there has been a successful method validation of both the plant and animal methods.

a. ACB observed trace amount of JAU 6476-Desthio in the control milk samples but these were below the estimated LOD.

b. ACB observed approximately 2-5 ppb levels of JAU 6476-Desthio in the initial control liver samples. ACB assayed another set of control liver and found that there were no detectable levels of JAU 6476-Desthio. There may have been some contamination during the extraction procedure that accounted for these levels in ACB's first set of samples.

2. ACB's chromatograms were similar to those of the ILV's and the petitioner's. The ILV's and the petitioner used the smoothing function to improve the appearance of their chromatograms. ACB chose not to use the smoothing function.

3. Control wheat forage was sent to ACB from Bayer CropScience. The peanuts nutmeats were from a previous project that had been stored in the -80 °C freezer. Permission from Bayer was given, during a telephone conversation, to use them in this TMV. The milk (4% whole shelf milk) and liver were purchased from local grocery stores.

4. The prothioconazole and its desthio and 4-hydroxy metabolites plus JAU 6476 sulfonic acid were obtained from the EPA National Pesticide Standard Repository located at Fort Meade, Maryland. The isotopically labeled internal standards: JAU 6476, JAU 6476-Desthio, JAU 6476-4-Hydroxy and JAU 6476 sulfonic acid were also obtained from the Repository.

5. The ACB's Limits of Detection (LOD) and Limits of Quantitation (LOQ) were estimated to be similar to those of the petitioner. The petitioner determined the LOQ as the lowest fortification level with adequate recovery.

a. For the livestock method: ACB concurs with the petitioner's LOQ for JAU 6476, JAU 6476-desthio, and JAU 6476-4-hydroxy at 5 ppb in milk, and 10 ppb in liver. ACB also concurs with the petitioner's LOD for JAU 6476 ranged from 0.7-11.5 ppb; for JAU 6476-desthio, ranged from 0.7-2.9 ppb; and for JAU 6476-4-hydroxy, ranged from 0.6-7.7 ppb. ACB estimated the LOD for milk and liver to be approximately 1 ppb for each analyte.

b. For the plant method: ACB concurs with the petitioner's LOQ for JAU 6476 and JAU 6476-desthio at 20 ppb for peanut nutmeat and 50 ppb for wheat forage. ACB estimated the LOD for JAU 6476 and JAU 6476-desthio to be 2 ppb each for peanut nutmeat and 10 ppb each for wheat forage.

6. The time to prepare a set of six samples varied depending on the commodity and the method.

a. A set of 6 samples and up to 12 samples could be prepared in one 8-hour day and set up for overnight unattended instrumental analysis using the plant method.

b. A set of 6 milk samples could be prepared in one 8-hour day and set up for overnight unattended instrumental analysis using the livestock method.

c. A set of 6 liver samples required more than one 8-hour day by a single analyst, with a stopping point prior to a two hour acid hydrolysis step in the livestock method. There is the possibility that a set of 6 liver samples could be completed in one day by a team of analysts working a staggered work schedule. Overnight unattended analysis followed by data reduction requires another 6 hours, regardless of the method used.

Analytical Chemistry Branch

Method Validation Results - Plant
PP#4F6830

Commodity	Chemical Added	ppb Added	ppb Found [b]	Percent Recovery
Peanut nutmeat	Prothioconazole	0 (control-1)	ND [a]	
		0 (control-2)	ND	
		20 (set-1) LOQ	18.2	91
		20 (set-2) LOQ	20.3	102
	prothioconazole-desthio	0 (control-1)	ND	
		0 (control-2)	ND	
		20 (set-1) LOQ	20.2	101
		20 (set-2) LOQ	19.2	96
Wheat Forage	Prothioconazole	0 (control-1)	ND	
		0 (control-2)	ND	
		50 (set-1) LOQ	41.4	83
		50 (set-2) LOQ	38.8	78
		7000 (set-1)	5685.3	81
		7000 (set-2)	5678.8	81
	prothioconazole-desthio	0 (control-1)	ND	
		0 (control-2)	ND	
		50 (set-1) LOQ	44.7	89
		50 (set-2) LOQ	49.8	100
		7000 (set-1)	6025.0	86
		7000 (set-2)	6290.3	90

[a] ND = none detected
[b] ppb found is the average of duplicate injections

Method Validation Results - Livestock
PP#4F6830

Commodity	Chemical Added	ppb Added	ppb Found [b]	Percent Recovery
Cattle liver	Prothioconazole	0 (control-1)	ND [a]	
		0 (control-2)	ND	
		10 (set-1) LOQ	9.2	92
		10 (set-2) LOQ	9.7	97
		500 (set-1)	457.6	92
		500 (set-2)	465.3	93
	Prothioconazole-desthio	0 (control-1)	< LOD [c]	
		0 (control-2)	< LOD [c]	
		10 (set-1) LOQ	10.0	100
		10 (set-2) LOQ	10.8	108
		1200 (set-1)	1152.2	96
		1200 (set-2)	1087.5	91
	Prothioconazole-4-hydroxy	0 (control-1)	ND	
		0 (control-2)	ND	
		10 (set-1) LOQ	9.1	91
		10 (set-2) LOQ	9.7	97
		500 (set-1)	451.9	90
		500 (set-2)	466.1	93
Milk	Prothioconazole	0 (control-1)	ND	
		0 (control-2)	ND	
		5 (set-1) LOQ	5.2	104
		5 (set-2) LOQ	5.4	108
		10 (set-1)	10.5	105
		10 (set-2)	10.9	109
	Prothioconazole-desthio	0 (control-1)	< LOD [c]	
		0 (control-2)	< LOD [c]	
		5 (set-1) LOQ	4.9	98
		5 (set-2) LOQ	4.8	96
		10 (set-1)	9.9	99
		10 (set-2)	10.2	102
	Prothioconazole-4-hydroxy	0 (control-1)	ND	
		0 (control-2)	ND	
		5 (set-1) LOQ	5.2	104
		5 (set-2) LOQ	4.9	98
		10 (set-1)	9.9	99
		10 (set-2)	10.1	101

[a] ND = none detected
[b] ppb found is the average of duplicate injections
[c] A trace amount detected but less than the estimated LOD

Primary Evaluators	Stephen Funk, Ph.D., Senior Chemist Immediate Office	Date: Mon 13 2006
	Louise G. Croteau, Senior Evaluation Officer FREAS, HED	Date: 23/01/06
	Suzan Mathew, Evaluation Officer FREAS, HED	Date: January 23/06
Approved by	Leung Cheng, Ph. D., Team Leader HED/RAB3	Date:
	Henri P. Bietlot, Acting Section Head FREAS, HED	Date: JAN 24/06

In the absence of signatures, this document is considered to be a draft with deliberative material for internal use only.

This DER was originally prepared under contract by Dynamac Corporation (20440 Century Boulevard, Suite 100; Germantown, MD 20874; submitted 04/13/2005). The DER has been rewritten by the Health Effects Division (HED) and Health Canada's Pest Management Regulatory Agency (PMRA). The DER has been revised to reflect current Office of Pesticide Programs (OPP) policies and PMRA Directive 98-02.

STUDY REPORT:

46246150 Weber, H.; Spiegel, K. (2001) [Phenyl-UL-(Carbon 14)]JAU6476: Absorption, Distribution, Excretion, and Metabolism in the Lactating Goat. Project Number: M/91819082, MR/092/01. Unpublished study prepared by Bayer Ag Institut fuer Ruckstands-Analytik. 205 p.

46246149 Weber, E.; Weber, H.; Spiegel, K. (2003) [Triazole-UL-(Carbon 14)]JAU6476: Absorption, Distribution, Excretion, and Metabolism in the Lactating Goat. Project Number: M51819114. MR/448/02. Unpublished study prepared by Bayer Ag, Institute of Product Info. 308 p.

EXECUTIVE SUMMARY:

Bayer CropScience has submitted studies investigating the metabolism of [phenyl-UL-^{14}C]-prothioconazole (specific activity 0.381 MBq/mg), and [triazole-UL-^{14}C]-prothioconazole (specific activity 369.6 kBq/mg) in lactating goats. The test substances were administered orally to a single goat for each study at 246 ppm (phenyl-label study), and 195 ppm (triazole-label study) in the diet. The goats were dosed once per day for 3 consecutive days. Milk was collected twice daily throughout the studies, and tissues (muscle, fat, liver, and kidney) were collected at sacrifice. Residues were characterized primarily by HPLC analysis, using TLC analyses for confirmation and LC-MS/MS analyses for identification of metabolites. These methods successfully identified the predominant residues in the major extract of each goat matrix. Additionally, adequate storage stability data were submitted demonstrating the stability of the metabolite profile in goat samples and extracts for the duration of the studies.

Phenyl-label study:
The recovered radioactivity accounted for 67.57% of the administered dose. A total of 0.02% of the radioactivity was recovered in milk (0.020-0.071 ppm), while 0.96% was present in the tissues and organs (6.092 ppm in liver, 6.762 ppm in kidney, 0.084-0.106 ppm in muscle, and 0.149-0.172 ppm in fat). Approximately 66% of the total administered dose was excreted in urine and feces. The remaining administered dose was assumed to be absorbed from the intestinal tract prior to excretion. Prothioconazole was monophasically eliminated from plasma with a half-life of 5.3 hours, and the mean residence time (MRT) in plasma was 8.2 hours.

Approximately 29-71% of the TRRs were identified in goat matrices. Prothioconazole was found to be a major residue in liver, kidney, muscle, and fat at 12.94-17.97% of the TRRs (0.012-1.215 ppm), and at 0.89% of the TRRs (<0.001 ppm) in milk. JAU6476-*O*- or *S*-glucuronide and JAU6476-3-hydroxy-desthio were major metabolites in milk and tissues at 34.32% of the TRRs (2.321 ppm) in kidney, and 10.02-14.80% of the TRRs (0.004-0.610 ppm) in the other matrices. JAU6476-desthio was a major metabolite in fat at 18.98% of the TRRs (0.032 ppm), but <3% of the TRRs (<0.087 ppm) in the other matrices. JAU6476-4-hydroxy was identified at 11.21% of the TRRs (0.683 ppm) in liver, and at <5% of the TRRs (≤0.210 ppm) in other goat matrices. Minor metabolites, each at <8% of the TRRs (<0.51 ppm included): JAU6476-4-hydroxy-glucuronide, JAU6476-hydroxy-glucuronide, JAU6476-4-hydroxy-desthio, and JAU6476-*N*-glucuronide. This also included glucuronic acid conjugates of JAU6476-desthio, JAU6476-hydroxy-desthio, JAU6476-dihydroxy-desthio, and JAU6476-hydroxymethoxy-desthio, JAU6476-dihydroxy-diene and JAU6476-desthio-dihydroxy-diene. Unknowns accounted for <8% of the TRRs (<0.182 ppm) in milk, liver, and fat. Accountabilities were normalized to 100%. Non-extractable residues accounted for 16-23% of the TRRs (≤0.04 ppm) in milk, muscle, and fat; <3% of the TRRs (0.166 ppm) in kidney; and 16.7% of the TRRs (1.018 ppm) in liver. While the applicant did not attempt to release the non-extractable residues in liver, a significant portion of the residues were expected to be the same non-label specific metabolites as found in the solvent extracts, so no further extraction was conducted.

Based on the results of the phenyl-label study, the applicant concluded that prothioconazole is metabolized in goats via several steps: conjugation of the unchanged parent compound with glucuronic acid resulting in an *S*- or *O*-glucuronide; additional glucuronidation of the triazole-thione nitrogen atom of the parent compound to form JAU6476-*N*-glucuronide; hydroxylation of the parent compound to form JAU6476-4-hydroxy and a further hydroxy isomer, followed by conjugation with glucuronic acid; oxidation of the phenyl ring of the parent compound to form JAU6476-dihydroxy-diene; elimination of sulfur to form JAU6476-desthio; further hydroxylation of the chlorophenyl moiety to form JAU6476-3-hydroxy-desthio and JAU6476-4-hydroxy-desthio, followed by conjugation with glucuronic acid; and oxidation of the chlorophenyl moiety of JAU6476-desthio to form JAU6476-desthio-dihydroxy-diene. The presence of JAU6476-dihydroxy-desthio-glucuronides indicated that isomers of JAU6476-dihydroxy-desthio were formed as intermediates. Methylation of JAU6476-hydroxy-desthio-glucuronides to form JAU6476-hydroxymethoxy-desthio-glucuronides occurred to a small extent, as did the glucuronidation of JAU6476-desthio.

Triazole-label study:
The recovered radioactivity accounted for 59.52% of the administered dose. A total of 0.03% of the radioactivity was recovered in milk (0.080-0.249 ppm), while 0.74% was present in the tissues and organs (6.248 ppm in liver, 4.507 ppm in kidney, 0.115-0.142 ppm in muscle, and 0.109-0.213 ppm in fat). Approximately 58% of the total administered dose was excreted in urine and feces. The remaining administered dose was assumed to be absorbed from the intestinal tract prior to excretion. Prothioconazole was monophasically eliminated from plasma with a half-life of 7 hours, and the mean residence time (MRT) in plasma was 10.6 hours.

Approximately 61-84% of the TRRs were identified in goat matrices. Prothioconazole was found to be a major residue in liver, kidney, and fat at 16.12-19.50% of the TRRs (0.028-1.047 ppm), at 3.18% of the TRRs (0.005 ppm) in milk, and at 7.17% of the TRRs (0.008 ppm) in muscle. Thiocyanate accounted for 9.01-41.12% of the TRRs (0.022-0.406 ppm) in milk, kidney, muscle and fat; and at 2.04% of the TRRs (0.128 ppm) in liver. Radioactivity corresponding to 10.76% of the TRRs (0.016 ppm) was attributed to lactose in milk. JAU6476-*S*-glucuronide and JAU6476-3-hydroxy-desthio were major metabolites in kidney, muscle and fat at 11.92-33.85% of the TRRs (0.016-1.526 ppm), and in milk and liver at <6.1% of the TRRs (<0.38 ppm). JAU6476-desthio was a major metabolite in fat at 15.11% of the TRRs (0.026 ppm), and <5% of the TRRs (<0.31 ppm) in other goat matrices. Other metabolites included JAU6476-4-hydroxy (10.97% of the TRRs; 0.686 ppm) in liver, and <8.5% of the TRRs (<0.164 ppm) in other goat matrices; and JAU6476-hydroxy-glucuronide at 11.15% of the TRRs (0.019 ppm) in fat, but <7% of the TRRs (<0.32 ppm) in other goat matrices. Minor metabolites, each at <7% of the TRRs (<0.41 ppm) included: JAU6476-4-hydroxy-glucuronide, JAU6476-hydroxy sulfate and sulfate conjugate, JAU6476-lactoside, JAU6476-4-hydroxy-desthio, JAU6476-*N*-glucuronide, and JAU6476-*S*-methyl. Unknown metabolites accounted for 5.1-11.0% of the TRRs (<0.32 ppm) in liver and kidney. Accountabilities were normalized to 100%. Non-extractable residues accounted for 16-23% of the TRRs (≤0.04 ppm) in milk, muscle, and fat; <6% of the TRRs (0.237 ppm) in kidney; and ~10% of the TRRs (0.662 ppm) in liver. The

non-extractable residues of liver were subjected to microwave extraction which released an additional 4.1% of the TRRs (0.256 ppm).

Based on the results of the submitted goat metabolism studies with prothioconazole, the applicant concluded that prothioconazole is metabolized in goats via several steps: conjugation of the triazolinethione moiety of the parent compound with glucuronic acid to form the *S*-glucuronide and *N*-glucuronide of the parent; elimination of sulfur to form the metabolite JAU6476-desthio; oxidative hydroxylation of the phenyl moiety in prothioconazole and JAU6476-desthio to monohydroxy, dihydroxy, and dihydroxy-diene compounds, partly followed by conjugation with glucuronic acid; conjugation of the triazolinethione moiety of the parent compound with lactose; conjugation of hydroxylated metabolites of prothioconazole with sulfate; methylation of the triazolinethione moiety of prothioconazole to form JAU6476-*S*-methyl; and cleavage of the parent compound to form thiocyanate.

STUDY/WAIVER ACCEPTABILITY/DEFICIENCIES/CLARIFICATIONS:

The acceptability of these studies for regulatory purposes is addressed in the forthcoming U.S. EPA Residue Chemistry Summary Document, DP Barcode D303508, and in Canada's Regulatory Decision Document.

COMPLIANCE:

Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided. No deviations from regulatory requirements were reported which would have an impact on the validity of the studies.

A. BACKGROUND INFORMATION

Prothioconazole is a broad-spectrum systemic fungicide intended for the control of many diseases in different crops such as cereals, oilseed rape or peanuts. The biochemical mode of action is the inhibition of the demethylation of lanosterol or 24-methylene-dihydrolanosterol, which are precursors of sterols in fungi. The chemical has been submitted for joint EPA and PMRA review by Bayer CropScience. The crops proposed for joint review include barley, the dried shell and bean subgroup, the oilseed crop group, and wheat. Uses on peanuts and rice are being proposed for the U.S. only.

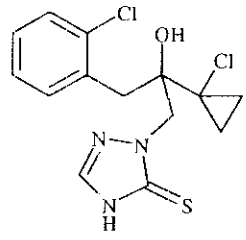
TABLE A.1. Test Compound Nomenclature.	
Chemical structure	
Common name	Prothioconazole (ISO accepted)
Company experimental name	JAU6476
IUPAC name	2-[(2*RS*)-2-(1-chlorocyclopropyl)-3-(2-chlorophenyl)-2-hydroxypropyl]-2*H*-1,2,4-triazole-3(4*H*)-thione
CAS name	2-[2-(1-chlorocyclopropyl)-3-(2-chlorophenyl)-2-hydroxyprcpyl]-1,2-dihydro-3*H*-1,2,4-triazole-3-thione
CAS registry number	178928-70-6
PC Code	113961
End-use product (EP)	Proline® 480 SC (4 lb/gal suspension concentrate), EPA File Symbol 264-IEL

TABLE A.2. Physicochemical Properties of the Prothioconazole		
Parameter	Value	References
Melting range	139.1 to 144.5°C	MRID 46246003/CES
pH	5.8 (1% solution)	MRID 46246003/CES
Density at 20°C	1.36 g/mL	MRID 46246003/CES
Water solubility at 20°C	pH / mg/L 4 / 5 8 / 300 9 / 2000	MRID 46246003/CES
Solvent solubility at 20°C	Solvent / g/L Acetone / >250 Acetonitrile / 69 Dichloromethane / 88 Dimethysulfoxide / 126 Ethyl acetate / >250 n-Heptane / <0.1 1-Octanol / 58 Polyethylene glycol / >250 2-Propanol / 87 Xylene / 8	MRID 46246003/CES
Vapor pressure at 20 or 25°C	$<4 \times 10^{-7}$ Pa (calculated from determinations at 70°C)	MRID 46246003/CES
Dissociation constant, pK_a	6.9 (calculated from K_{OW})	MRID 46246003/CES
Octanol/water partition coefficient, at 20°C	pH / $Log(K_{OW})$ unbuffered water / 4.05 4 / 4.16 7 / 3.82 9 / 2.00	MRID 46246003/CES
UV/visible absorption spectrum	Peak maxima at 257 nm. No absorption at > 300 nm.	MRID 46246003/CES

CES - Chemistry Evaluation Section of PMRA

B. EXPERIMENTAL DESIGN

B.1. Livestock

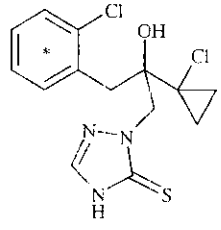
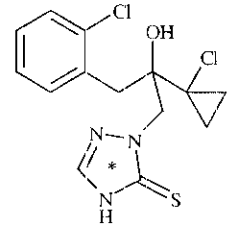
TABLE B.1.1. General Test Animal Information.					
Species	Breed	Age	Weight at study initiation (kg)	Health Status	Description of housing/holding area
Phenyl-label Study (MRID 46246150):					
Lactating goat	"Bunte Deutsche Edelziege"	~30 months	39.0	No observable toxicological signs.	Stainless steel metabolism cage in air-conditioned room (20 ± 1°C) with 18 hours of illumination.
Triazole-label Study (MRID 46246149):					
Lactating goat	"Deutsche Edelziege"	~19 months	29.5	No observable toxicological signs.	Stainless steel metabolism cage in air-conditioned room (22 ± 1°C) with 18 hours of illumination.

TABLE B.1.2. Test Animal Dietary Regime.				
Composition of Diet	Feed consumption (g/day)	Water	Acclimation period	Predosing
Ruminant feed, apples, and hay	~2000 g feed + apples offered per day; hay offered *ad libitum*; actual feed consumption was not reported.	Tap water, *ad libitum*	6 days (triazole study). Approximately 1 week (phenyl study).	None

TABLE B.1.3. Test Animal Dosing Regime.			
Treatment Type	Feeding Level (ppm test material in food)	Vehicle	Timing/Duration
Oral	246 [1] (phenyl study) 195 [2] (triazole study)	Oral intubation of test substance in 0.5% aqueous tragacanth suspension.	Once per day after morning milking for three consecutive days.

[1] Based on an experimentally determined feed consumption of 4.1% of body weight.
[2] Based on an experimentally determined feed consumption of 5.1% of body weight.

B.2. Test Materials

TABLE B.2.1. Test Material Characteristics.		
Phenyl-label Study		**Triazole-label Study**
Chemical structure		
Radiolabel position	[phenyl-UL-^{14}C]-prothioconazole	[triazole-UL-^{14}C]-prothioconazole
Lot No.	12106/1	14512/1
Purity	>99% radiochemical purity; >98% chemical purity	>99% (chemical purity and radio-chemical purity)
Specific activity [1]	0.381 MBq/mg (2.29 x 10^7 dpm/mg; 10.3 µCi/mg; 3.55 Ci/mole)	369.6 kBq/mg (22,176,000 dpm/mg; 9.99 µCi/mg; 3.44 Ci/mole)

[1] Bq = disintegrations per second

B.3. Sampling Information

TABLE B.3.1. Sample Collection Information.			
Milk collected	Urine and feces collected	Interval from last dose to sacrifice	Tissues harvested and analyzed
Milk was collected twice daily, immediately prior to dosing and then approximately 8 hours later. The final sample was collected directly before sacrifice. The amounts of milk collected were not reported.	Urine and feces were collected as quantitatively as possible in 24 hour intervals.	5 hours	Liver, kidney, muscle (round, flank, loin), and fat (perirenal, omental, subcutaneous)

B.4 Identification/Characterization of Residues

B.4.1. Sample Handling and Preparation

Milk was collected twice daily. An aliquot of each sample was radioassayed. All milk samples collected over the duration of the dosing period were combined with the other samples from that study and stored frozen (~-18°C) until extraction and analysis. In the triazole-label study, a portion of milk was immediately subjected to extraction procedures. Tissue samples were minced or disintegrated (fat) after collection and then stored frozen (~-18°C) until extraction and analysis. Portions of round, loin, and flank muscle were combined to make a composite sample, and portions of perirenal, subcutaneous, and omental fat were similarly combined.

The applicant noted that cysteine HCl was added to milk samples and to the extraction mixture for tissues during sample extraction in order to stabilize the parent compound and the metabolites.

Phenyl-label Study:

Milk: A subsample of milk was and extracted three times with methanol, and the extracts were combined and concentrated. The extract was mixed with buffer solution (pH 3) and cleaned up on an XAD 7 column. The effluent from the column was collected, the column was rinsed with water, and the retained radioactive compounds were eluted with methanol. The methanol eluate was concentrated and redissolved in methanol/water for HPLC analyses.

Liver, kidney, and muscle: Subsamples of liver, kidney, and muscle were extracted three times with acetonitrile (ACN):water (80:20, v:v) and then twice with ACN:water (50:50, v:v). The 80:20 ACN:water extracts were combined and the 50:50 ACN:water extracts were discarded because of low radioactivity. The combined extracts were concentrated, diluted with ACN, and partitioned with hexane. The hexane phase was concentrated and mixed with methanol. The ACN phase was concentrated, diluted with buffer solution (pH 3), and then cleaned up on an XAD 7 column, which was rinsed with buffer solution and water and then eluted with methanol. The methanol eluate was evaporated to dryness and redissolved in methanol for HPLC analysis.

Fat: A subsample of fat was extracted three times with ACN:water (80:20, v:v) and then twice with ACN:water (50:50, v:v). All extracts were combined, concentrated to aqueous, diluted with methanol, and partitioned with hexane. The methanol phase was concentrated, diluted with buffer solution (pH 3), and then cleaned up on an XAD 7 column, which was rinsed with buffer solution and water and then eluted with methanol. The methanol eluate was evaporated to dryness and redissolved in methanol/water for HPTLC/TLC analysis.

To confirm metabolite identification, the applicant subjected the methanol extract of milk to treatment with boiling HCl (conditions not described) to cleave glucuronic acid conjugates, then compared the HPLC profile of the resulting hydrolysate with the profile prior to hydrolysis.

The extraction procedures for milk and tissue samples are summarized in FIGURES B.4.1.1-B.4.1.3. Please note that flow charts for kidney and muscle were not included; the liver flow chart is representative of the extraction procedures used for liver, kidney, and muscle.

FIGURE B.4.1. Extraction procedures for milk.

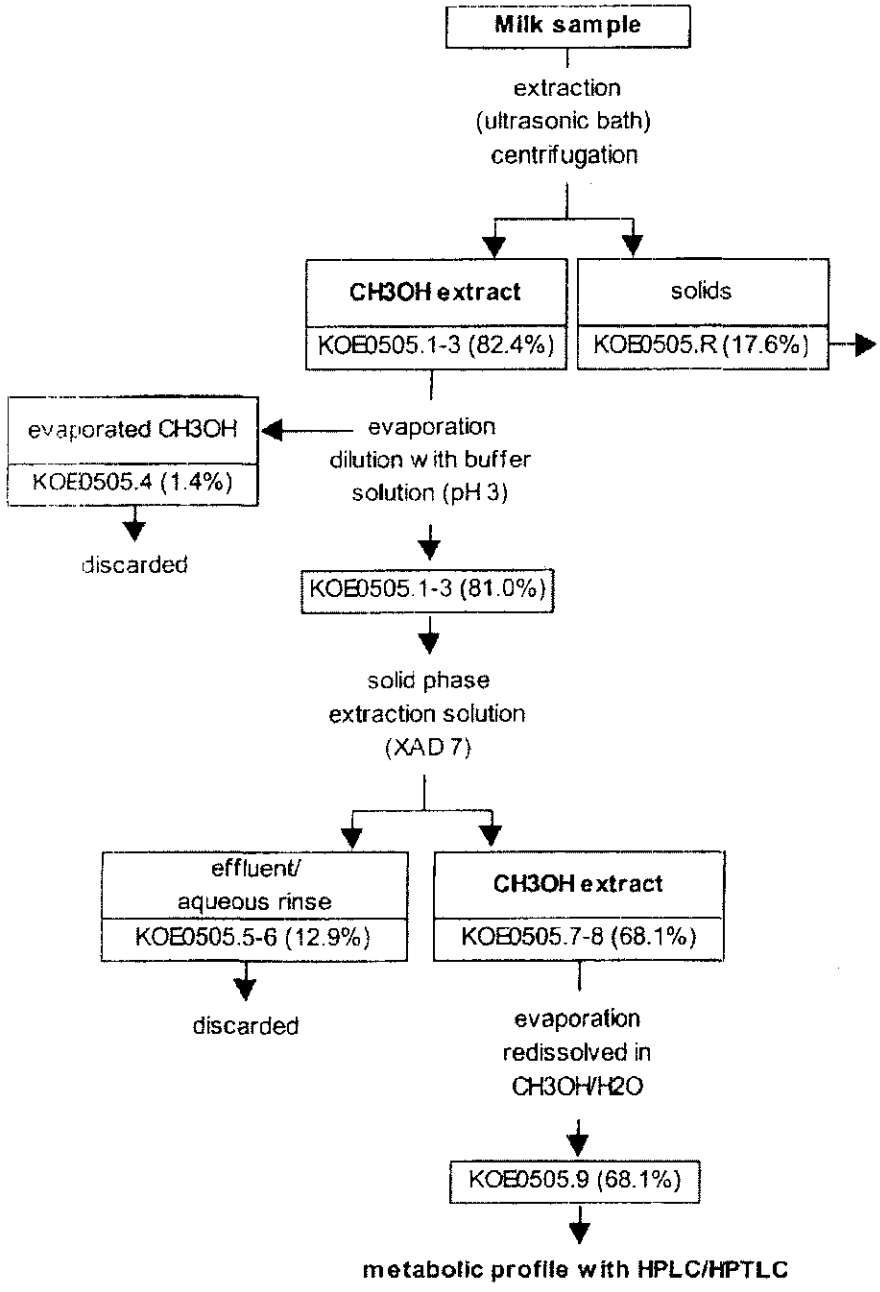


FIGURE B.4.1.2. Extraction procedure for fat.

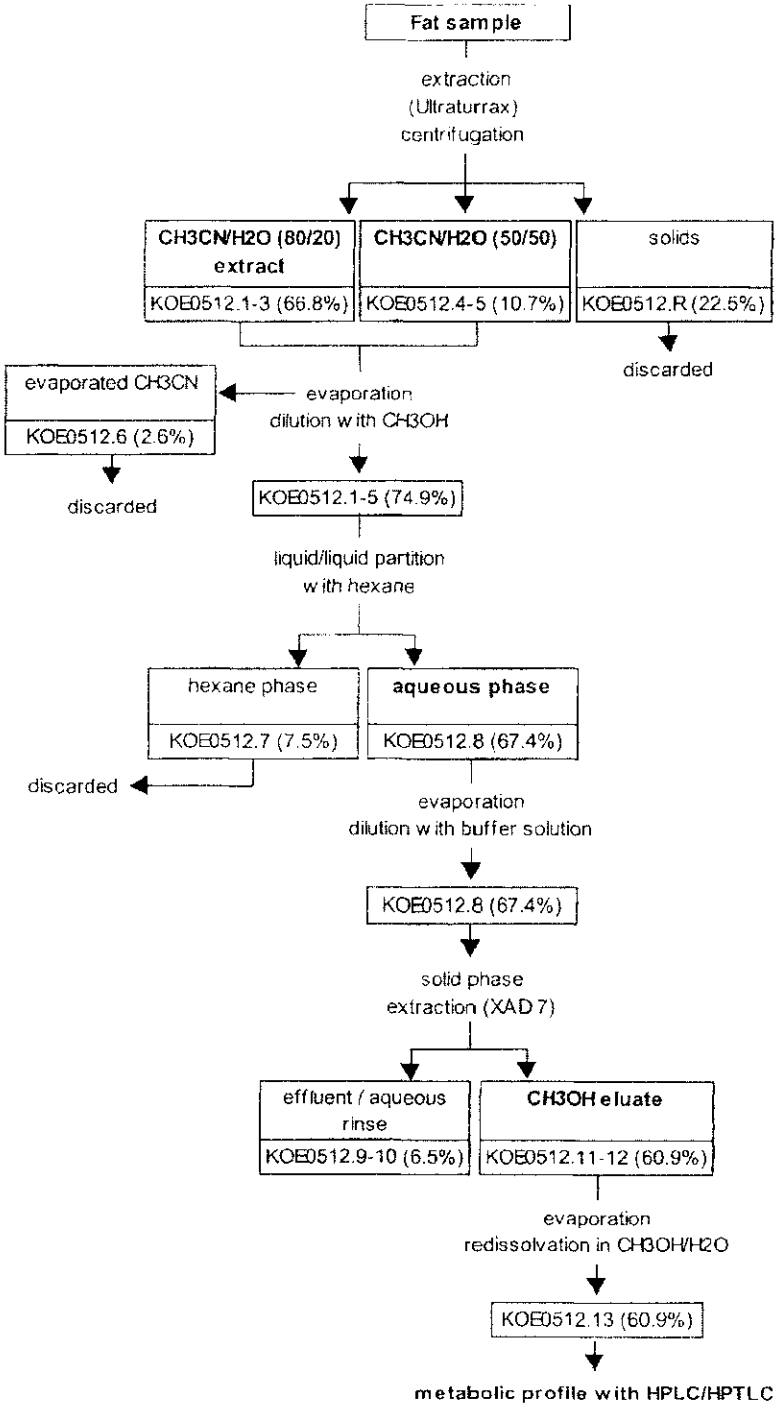
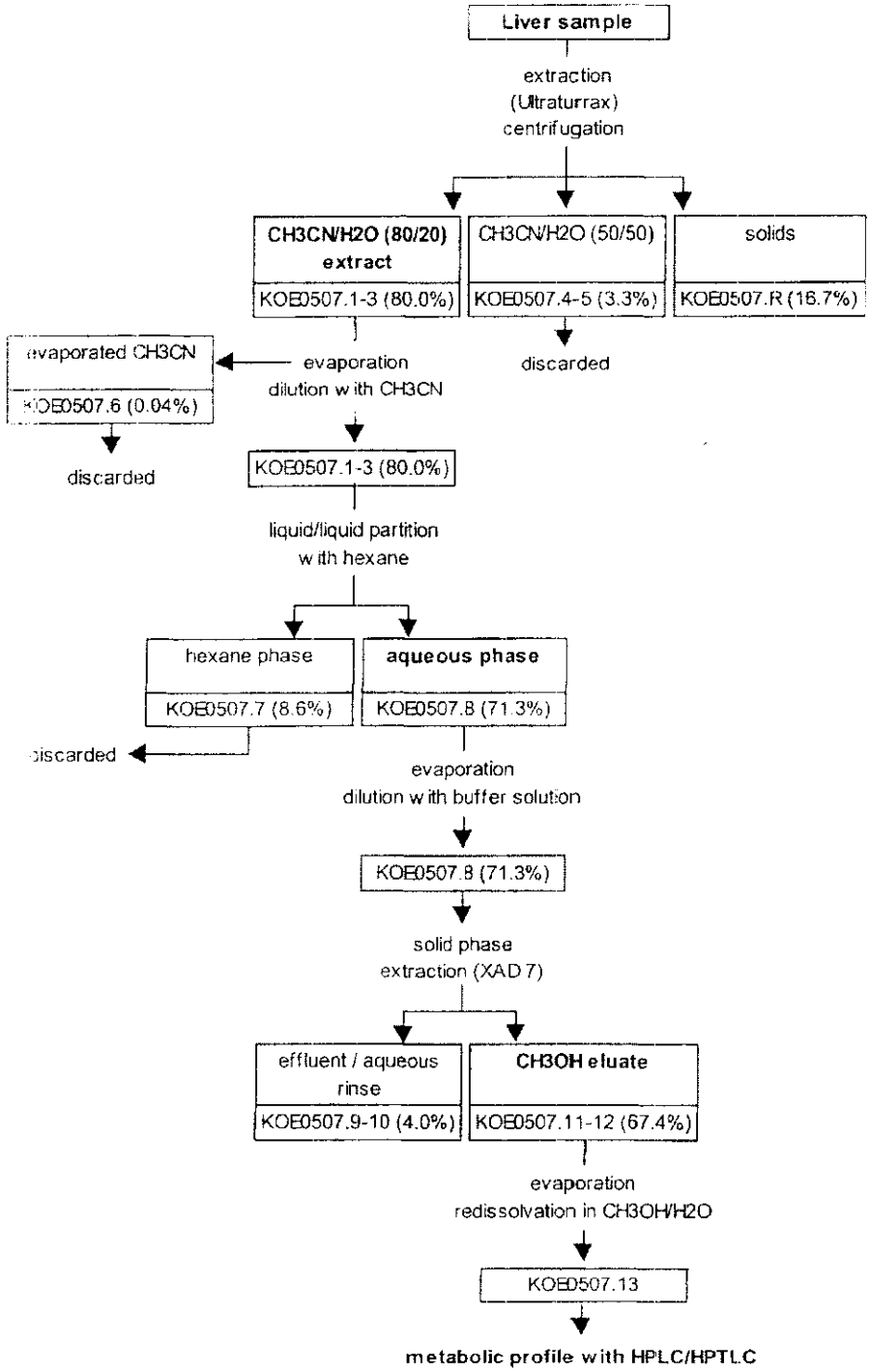


FIGURE B.4.1.3. Extraction procedures for liver (also used for kidney and muscle).



Triazole-label Study:

Milk: A subsample of milk was extracted three times with methanol, and the extracts were isolated by centrifugation, combined, and concentrated. The extract was mixed with acetonitrile (ACN) and cleaned up by C18 solid phase extraction (SPE); the effluent of the columns was collected and then the columns were rinsed with ACN:water (80:20, v:v). The effluent and rinse were combined, which yielded two phases. The effluent phase was concentrated to aqueous, diluted with buffer solution (pH 3), and then cleaned up on an XAD 7 column; the effluent from the column was collected. The second phase was diluted with buffer solution and applied to the same XAD 7 column. The column was rinsed with water, and the retained radioactive compounds were eluted with methanol; the methanol eluate was concentrated and diluted with water/methanol for HPLC analyses. The effluent and rinse from the XAD 7 column were combined and concentrated, which yielded a precipitate (lactose). Acetone was added, and the mixture was subjected to ultrasound; this step was repeated twice and the acetone phases were decanted, evaporated to dryness, and redissolved in water for HPLC analysis. The remaining lactose was diluted with water and reserved for HPLC analysis.

Extraction of milk was repeated to isolate a metabolite for HPLC-MS analyses. The solids remaining from the second extraction were subjected to microwave extraction. The solids were mixed with ACN:water (1:1, v:v) and then microwaved at 130°C for 3 minutes then 140°C for 15 minutes; the microwave extraction procedure was repeated.

Liver, kidney, and muscle: Subsamples of liver, kidney, and muscle were extracted three times with ACN:water (80:20, v:v) and then once with ACN:water (50:50, v:v). The first three extracts were combined, and the fourth extract was discarded. The combined extracts were cleaned up by C18 SPE. The effluent of the column was collected, concentrated to aqueous, diluted with buffer solution (pH 3), and then cleaned up on an XAD 7 column, which was rinsed with water and then eluted with methanol. The effluent and the water rinse from the XAD column were combined and concentrated for HPLC analysis. The methanol eluate was concentrated for HPLC analysis.

The extraction of liver was repeated, and the solids remaining after extraction were subjected to microwave extraction (using ACN:water, 1:1, v:v); the extract was reserved for HPLC analysis.

Fat: A subsample of fat was extracted three times with ACN:water (80:20, v:v) and the extracts were combined and cleaned up by C18 SPE. The effluent of the column was collected, and the column was eluted with methanol and dichloromethane; the methanol/dichloromethane eluate was discarded. The effluent was concentrated to aqueous, diluted with buffer solution (pH 3), and then cleaned up on an XAD 7 column, which was rinsed with water and then eluted with methanol. The effluent and the water rinse from the XAD column were combined and concentrated for HPLC analysis. The methanol eluate was concentrated for HPLC analysis.

To confirm metabolite identification, the applicant subjected the methanol extracts of milk, liver, kidney, and muscle to treatment with HCl at 100°C (for 4-8 hours) and compared the HPLC profile of the resulting hydrolysate with the profile prior to hydrolysis.

The extraction procedures for milk and tissue samples are summarized in FIGURES B.4.1.4-B.4.1.8.

FIGURE B.4.1.4. Extraction procedure for milk.

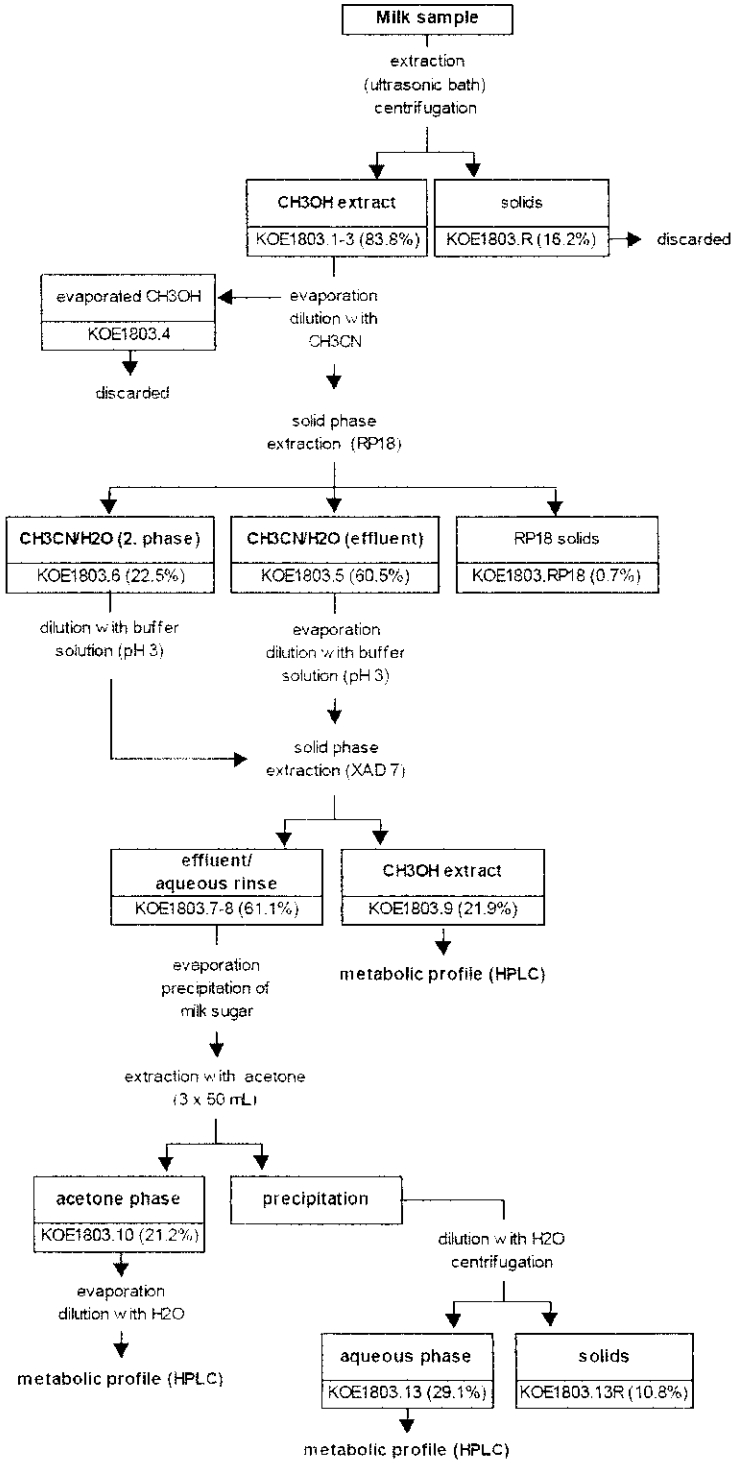


FIGURE B.4.1.5. Extraction procedure for liver.

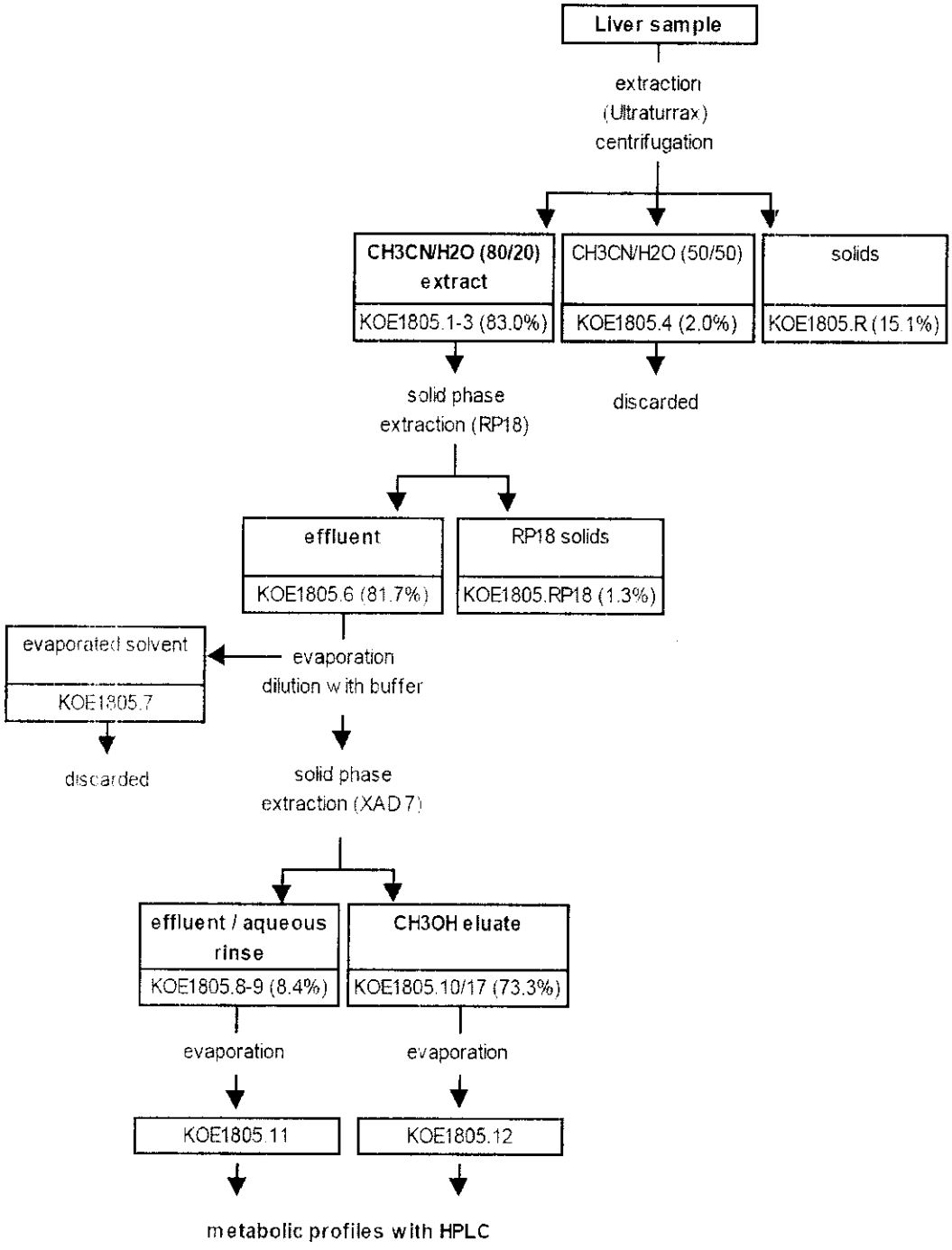


FIGURE B.4.1.6. Extraction procedure for kidney.

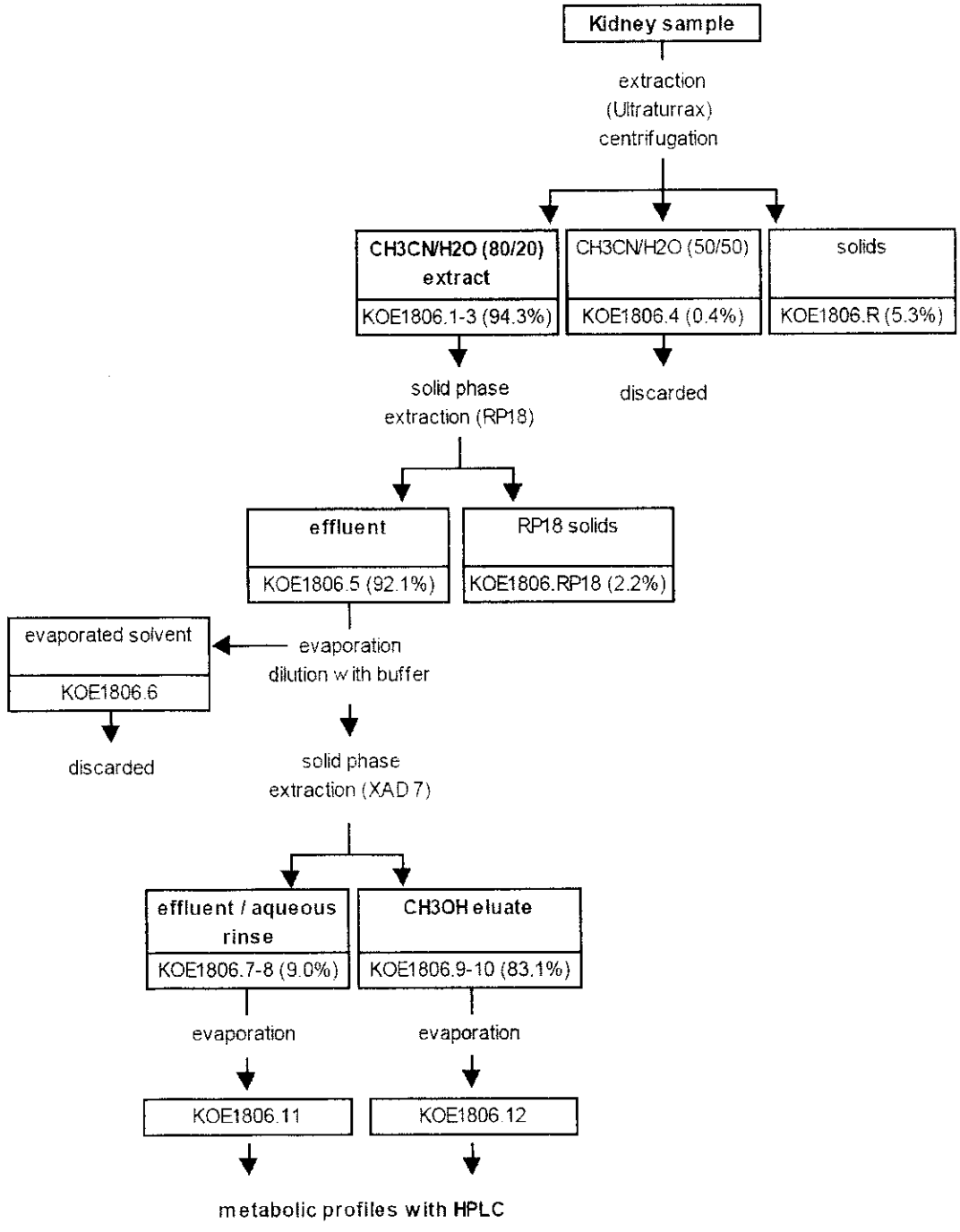


FIGURE B.4.1.7. Extraction procedure for muscle.

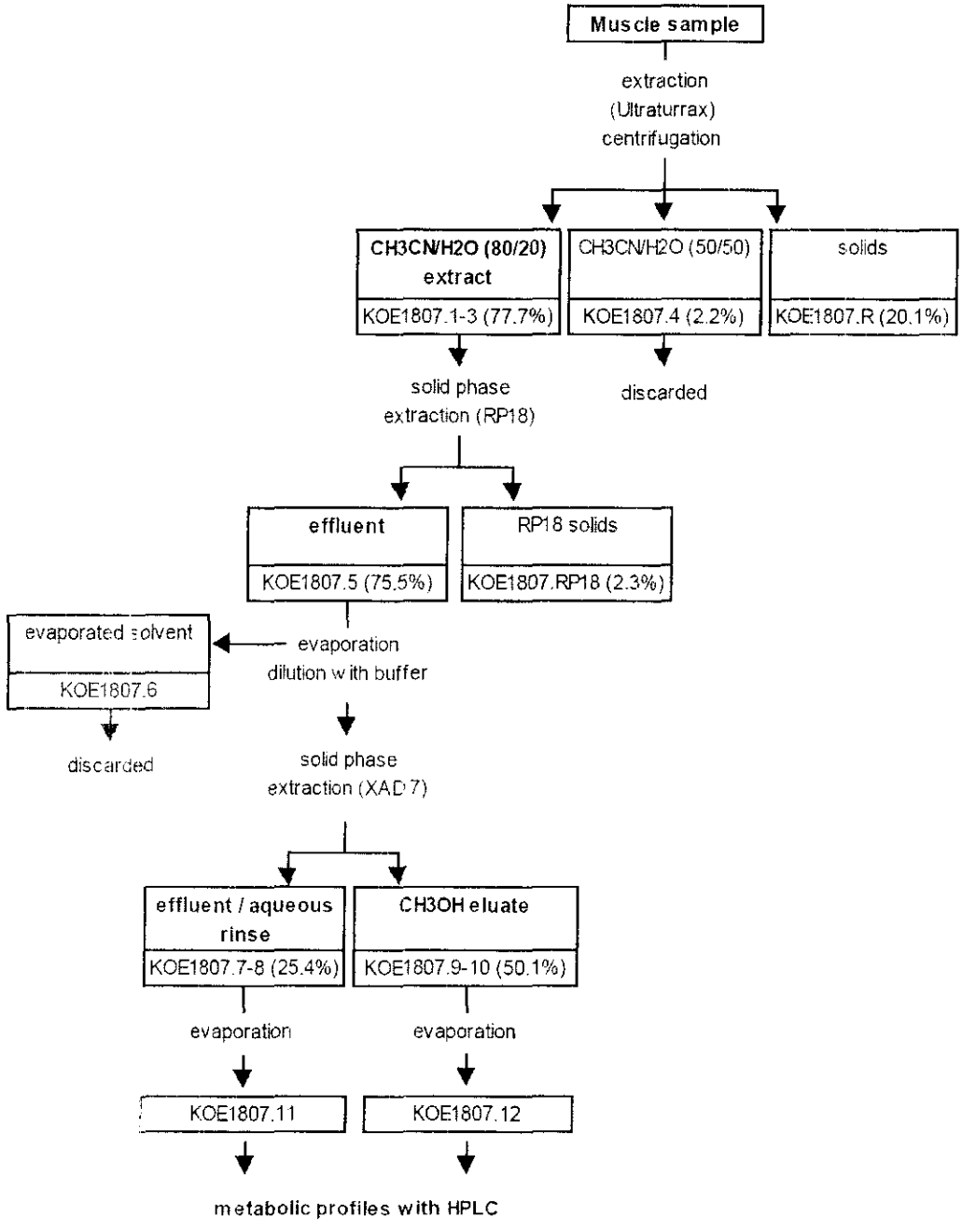
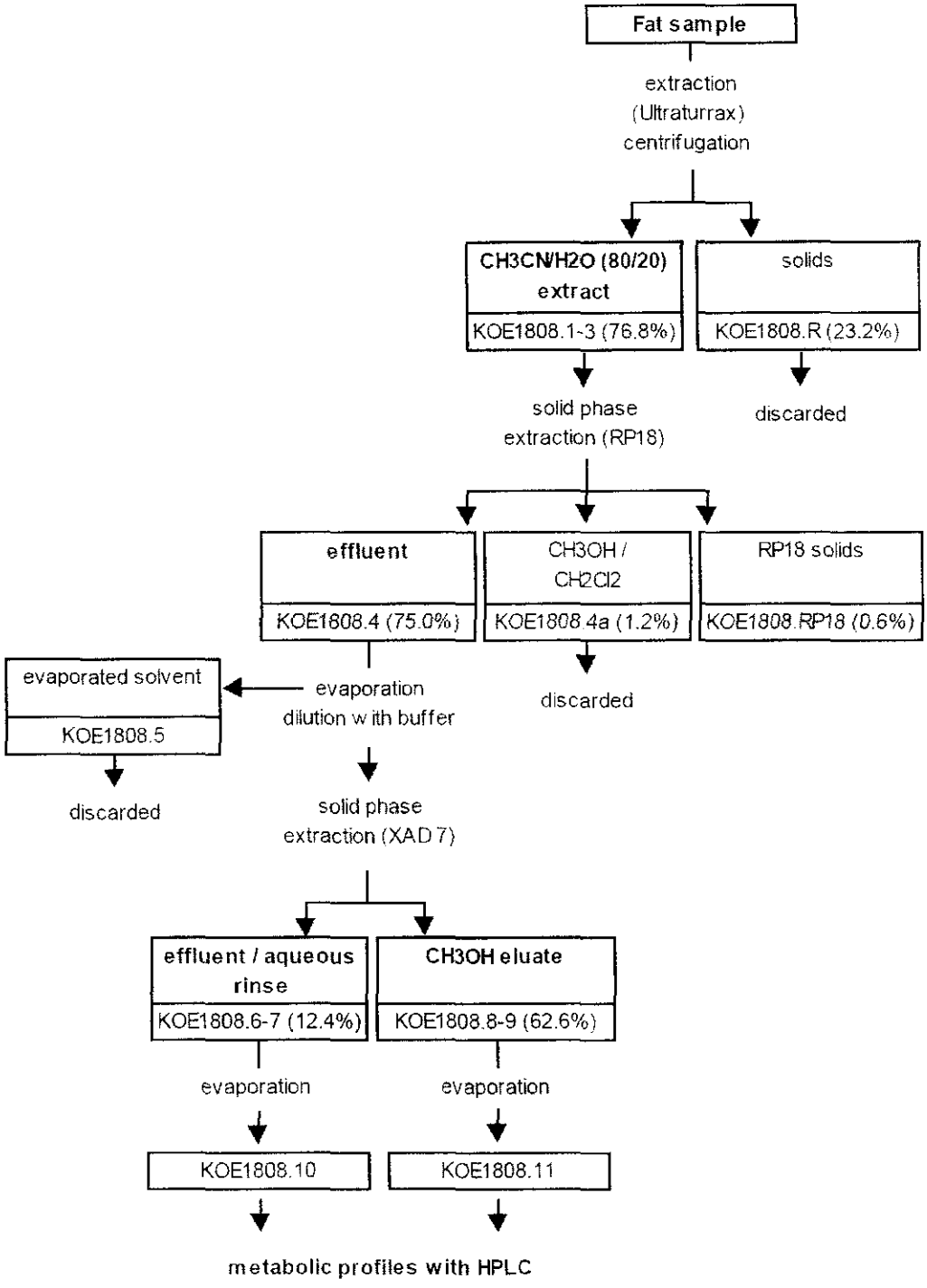


FIGURE B.4.1.8. Extraction procedure for fat.



B.4.2. Analytical Methodology

Total radioactive residues (TRRs) were measured (in duplicate) in milk samples by liquid scintillation counting (LSC). Tissue samples were freeze dried, and TRRs were determined by combustion/LSC (in triplicate). The limits of quantitation (LOQs) for TRR determination in the phenyl-label study were 0.001 ppm for milk, 0.002 ppm for liver, 0.001 ppm for kidney, 0.001-0.003 ppm for muscle, and 0.005-0.009 ppm for fat. For the triazole-label study, the LOQs were 0.001 ppm for milk, 0.004 ppm for liver, 0.003 ppm for kidney, 0.003-0.004 ppm for muscle, and 0.006-0.010 ppm for fat.

Phenyl-label Study: Extracts and hydrolysates were analyzed by HPLC using a system equipped with a UV detector, a radiodetector, a fraction collector, and one of the following column/mobile phase combinations: (1) a C18 column and a gradient mobile phase of water and ACN, each containing acetic acid; (2) a C18 column and a gradient mobile phase of 0.1 M ammonium acetate and ACN; or (3) a Diol column and a gradient mobile phase of hexane, ethanol, and ammonium hydroxide. The third system was used to isolate metabolites for further identification. For the phenyl-label study, the extracts used for HPLC profiling were fractionated and subject to HPTLC analyses. TLC analyses were conducted on silica 60 F_{254} plates using methanol, dichloromethane and ammonium hydroxide.

LC-MS or LC-MS/MS analyses were used for metabolite confirmation or for identification of metabolites which could not be identified by HPLC or HPTLC. Analyses were conducted using a reverse phase column and MS or MS/MS detection with electrospray ionization; a gradient mobile phase of 1% acetic acid and ACN, or water and ACN, each containing 0.1% acetic acid, was used. ^{1}H NMR analyses were also used for structure elucidation. In addition, the following metabolites were isolated from urine in the current study, identified by LC-MS/MS and ^{1}H NMR, and used for metabolite identification: JAU6476-4-hydroxy; JAU6476-desthio-3,4-dihydroxy-diene; JAU6476-dihydroxy-diene (two diastereomers); and JAU6476-4-hydroxy-glucuronide.

Triazole-label Study: Extracts and hydrolysates were analyzed by HPLC using a system equipped with a UV detector, a radiodetector, a fraction collector, and one of the following column/mobile phase combinations: (1) a C18 column and a gradient mobile phase of water and ACN, each containing acetic acid at 1%; (2) a Diol column and a gradient mobile phase of water and ACN, each containing acetic acid at 1%; or (3) a C8 column with a gradient mobile phase of water and methanol, each containing ion pairing reagent tetrabutylammonium-hydrogensulfate at 0.005 M. The second system was used to isolate metabolites for further identification. For the triazole-label study, The extracts used for HPLC profiling were fractionated and subjected to HPTLC analyses. TLC analyses were conducted on silica 60 F_{254} plates using a solvent system of dichloromethane:methanol:aqueous ammonia (80:20:5, v:v:v), or using AMD with methanol and dichloromethane.

LC-MS or LC-MS/MS analyses were used for metabolite confirmation or for identification of metabolites which could not be identified by HPLC or HPTLC. Analyses were conducted using

a reverse phase column, a gradient mobile phase of water and acetonitrile, each containing 0.1% formic acid, and MS or MS/MS detection with electro-spray ionization. In addition, the following metabolites were isolated from urine in the current study, identified by LC-MS/MS and ^{1}H NMR, and used for metabolite identification: JAU6476-*S*-glucuronide and JAU6476-hydroxy-glucuronide (two isomers). Of the two hydroxy-glucuronide isomers, one was assigned to be 4-hydroxy-glucuronide based on the results of the [phenyl-^{14}C]-prothioconazole study.

For both prothioconazole studies: The applicant noted that prothioconazole reference standard (non-labeled) and cysteine HCl were spiked to each TLC origin before applying sample solutions to minimize decomposition of prothioconazole and metabolites. Non-labeled standards were visualized under a UV lamp. Radioactive zones were detected using radioluminography. Metabolites were identified by co-chromatography.

In all studies, metabolites were identified by comparison of retention times or co-chromatography with reference standards; the chemical names and structures of the reference standards used in this study are presented in Appendix I.

C. RESULTS AND DISCUSSION

The storage intervals and conditions for the goat metabolism studies are presented in TABLE C.1. Analysis of the methanol (organic) extracts was completed within 3 months (phenyl-label) and within 6 months (triazole-label study) of sample collection for all matrices. For the triazole-label study, analysis of the aqueous extracts was completed approximately 8 months after sample collection; however TLC analyses of the extracts indicated that the profiles were stable in these extracts during storage. Additional extractions and analyses of milk, liver and kidney samples were conducted within 19-28 months (phenyl-label study), and 7-11 months (triazole-label study) of sample collection to allow for metabolite identification; comparison of the HPLC profiles of the organic extracts with those of the initial extracts indicated that the metabolite profile was stable during storage. The submitted storage stability information and data are adequate to support the goat metabolism studies.

Phenyl-label Study: Total radioactive residues (TRRs) in goat milk and tissues are reported in TABLE C.2.1a. TRRs were 0.020-0.071 ppm in milk, 6.092 ppm in liver, 6.762 ppm in kidney, 0.084-0.106 ppm in muscle, and 0.149-0.172 ppm in fat from a goat dosed orally with [phenyl-UL-^{14}C]-prothioconazole at 246 ppm in the diet for 3 consecutive days. Radioactivity was highest in liver and kidney and lowest in milk. Residues in milk were generally highest in samples collected 8 hours after dosing; a graph of the residue levels in milk over the course of the study is presented in FIGURE C.2.1a. The dosing period was too short to determine whether milk residues had reached a plateau by the end of the dosing period. Approximately 67% of the total administered dose was excreted in urine and feces. Approximately one-third of the administered dose was not accounted for in the excreta and tissues. Given the high concentrations determined in the liver and kidney, it was assumed that this fraction was absorbed in the contents of the gastrointestinal tract prior to excretion. The total clearance (CL) in plasma

was calculated as 11.3 mL/min/kg bw, as calculated from plasma curve analysis from a two compartment disposition model assuming a complete absorption process. The absorption process of the administered radioactivity was characterized by a lag-time (t_{lag}) of ~7 minutes, followed by a half-life of absorption (t_a) of ~14 minutes. The radioactivity was monophasically eliminated from the plasma with a half-life of 5.3 hours. The total mean residence time (MRT) was 8.2 hours, based on an analysis of the concentration time plot in plasma.

The distribution of the radioactivity in goat matrices is presented in TABLE C.2.2a. The majority of the radioactivity (~77-97% of the TRRs) was extracted using methanol (milk) or ACN/water (tissues). Non-extractable residues accounted for 16-23% of the TRRs (≤0.04 ppm) in milk, muscle, and fat; <3% of the TRRs (0.166 ppm) in kidney; and 16.7% of the TRRs (1.018 ppm) in liver. The applicant normalized the extraction results; however, accountabilities prior to normalization were 93-116%. Residues were characterized primarily by HPLC analysis, using TLC analyses for confirmation and LC-MS/MS analyses for identification of metabolites. These methods successfully identified the predominant residues in goat matrices.

The characterization and identification of residues in goat matrices is summarized in TABLE C.2.3a. Approximately 29-71% of the TRRs were identified in goat matrices. Prothioconazole was found to be a major residue in liver, kidney, muscle, and fat at 12.94-17.97% of the TRRs (0.012-1.215 ppm). Prothioconazole was found at lower levels in milk (0.89% of the TRRs, <0.001 ppm). Two co-eluting metabolites, JAU6476-*O*- or *S*-glucuronide (position of glucuronidation could not be determined) and JAU6476-3-hydroxy-desthio were found to be major metabolites in milk and tissues at 34.32% of the TRRs (2.321 ppm) in kidney, and 10.02-14.80% of the TRRs (0.004-0.610 ppm) in milk, liver, muscle, and fat. HPTLC analyses indicated that JAU6476-3-hydroxy-desthio accounted for <2% of the TRRs in each of these matrices. JAU6476-desthio was a major metabolite in fat at 18.98% of the TRRs (0.032 ppm), but was found at lower levels in other goat matrices (<3% of the TRRs; <0.087 ppm). One additional metabolite found at significant levels was JAU6476-4-hydroxy, at 11.21% of the TRRs (0.683 ppm) in liver (found at <5% of the TRRs (or ≤0.210 ppm) in other goat matrices). Several additional metabolites were identified in goat matrices, each at <8% of the TRRs (<0.51 ppm): JAU6476-4-hydroxy-glucuronide and JAU6476-hydroxy-glucuronide in liver, kidney, muscle, and fat; JAU6476-4-hydroxy-desthio in liver; and JAU6476-*N*-glucuronide in all matrices. Polar metabolites (glucuronic acid conjugates of JAU6476-desthio, JAU6476-hydroxy-desthio, JAU6476-dihydroxy-desthio, and JAU6476-hydroxymethoxy-desthio, as well as small amounts of JAU6476-dihydroxy-diene and JAU6476-desthio-dihydroxy-diene) were tentatively identified or characterized in all matrices. Unknowns accounted for <8% of the TRRs (<0.182 ppm) in milk, liver, and fat.

The applicant noted that metabolites were identified in milk, muscle, and fat extracts by comparison of the HPLC profile to that of the liver extract. Metabolites were identified in the liver extract by co-chromatography with reference standards. The identification of the following metabolites was confirmed by HPTLC: prothioconazole, JAU6476-glucuronide, JAU6476-3-

hydroxy-desthio, JAU6476-*N*-glucuronide, JAU6476-4-hydroxy, and JAU6476-desthio in all matrices, and JAU6476-4-hydroxy-desthio in liver.

To identify the JAU6476-hydroxy-glucuronide metabolites, the applicant extracted a second subsample of kidney and isolated the fractions containing these metabolites. LC-MS/MS analyses of the isolated components indicated the presence of two isomers of JAU6476-hydroxy-glucuronides. One of the isomers was determined to be JAU6476-4-hydroxy-glucuronide based on comparison of the retention time with that of the metabolite isolated from urine (and identified by LC-MS/MS and NMR). These metabolites were identified in liver, muscle, and fat based on comparison with the kidney extract.

The applicant subjected the methanol XAD eluate of milk to treatment with boiling HCl. The resulting HPLC profile indicated the presence of JAU6476-4-hydroxy-desthio and an isomer of JAU6476-dihydroxy-desthio, confirming the identification of JAU6476-4-hydroxy-desthio-glucuronide and JAU6476-dihydroxy-desthio-glucuronide in milk.

The applicant isolated the JAU6476-glucuronide metabolite from liver. Previous ^{1}H NMR analyses had indicated that the metabolite was an *O*- or *S*-glucuronide. The isolated metabolite was subjected to acid hydrolysis (100°C, 4 hours) which resulted in complete cleavage and formation of JAU6476-*S*-methyl. The applicant concluded that the metabolite was likely JAU6476-*S*-glucuronide, but that unambiguous spectroscopic assignment could not be made; therefore, the metabolite was referred to as JAU6476-*O*- or *S*-glucuronide.

The applicant noted that JAU6476-sulfonic acid may have been present in liver; the compound may have co-eluted with JAU6476-4-hydroxy during HPLC analyses, and had the same R_f value as prothioconazole under the HPTLC conditions used for analysis.

In the liver, the hexane fraction accounted for 8.62% of the TRRs (0.525 ppm). Analysis of the fraction (resulting from the partitioning of the initial extracts) by TLC (and confirmed by HPTLC) indicated that non-label specific metabolites (prothioconazole, JAU6476-desthio and JAU6476-4-hydroxy) comprised 6.5% of those TRRs (0.395 ppm). Each metabolite peak was <3.2% of the TRRs (<0.2 ppm). Unknown components comprised the other ~2.2% of the TRRs (0.131 ppm). The applicant did not attempt to release the non-extractable residues in liver (16.72% of the TRRs; 1.018 ppm) in the phenyl-label study, but conducted it in the triazole-label study.

The aqueous effluents/rinses of the XAD cleanup in kidney (14.59% of the TRRs; 0.986 ppm) were not further analyzed. The profiles for both radiolabels were identical, and did not indicate the presence of label specific metabolites. However, it is believed that a large component of the effluent/rinse was comprised of the same compounds as found in the profiles of the column eluates.

Triazole-label Study: Total radioactive residues (TRRs) in goat milk and tissues are reported in TABLE C.2.1b. TRRs were 0.080-0.249 ppm in milk, 6.248 ppm in liver, 4.507 ppm in kidney, 0.115-0.142 ppm in muscle, and 0.109-0.213 ppm in fat from a goat dosed orally with [triazole-UL-^{14}C]-prothioconazole at 195 ppm in the diet for 3 consecutive days. Radioactivity was highest in liver and kidney and lowest in milk. Residues in milk were generally highest in samples collected 8 hours after dosing, and did not appear to have reached a plateau by the end of the dosing period; a graph of the residue levels in milk over the course of the study is presented in FIGURE C 2.1b. Approximately 59% of the total administered dose was excreted in urine and feces. Approximately 40% of the administered dose was not accounted for in the excreta and tissues. Given the high concentrations determined in the liver and kidney, it was assumed that this fraction was absorbed in the contents of the gastrointestinal tract prior to excretion. The total clearance (CL) in plasma was calculated as 8.8 mL/min/kg bw, assuming complete absorption. The absorption process was characterized by a lag-time (t_{lag}) of ~4 minutes, followed by a half-life of absorption (t_a) of ~16 minutes. The radioactivity was monophasically eliminated from the plasma with a half-life of 7.7 hours, using a two compartment disposition model. The total mean residence time (MRT) was 10.6 hours, based on an analysis of the concentration time course in plasma.

The distribution of the radioactivity in goat matrices is presented in TABLE C.2.2b. The majority of the radioactivity (~77-94% of the TRRs) was extracted using methanol (milk) or ACN/water (tissues). The non-extractable residues of liver were subjected to microwave extraction which released an additional 4.1% of the TRRs (0.256 ppm). Non-extractable residues accounted for 16-23% of the TRRs (≤0.04 ppm) in milk, muscle, and fat; <6% of the TRRs (0.237 ppm) in kidney; and ~10% of the TRRs (0.662 ppm) in liver. The applicant normalized the extraction results; however, accountabilities prior to normalization were 94-104% for milk, liver, kidney, and muscle, and 72% for fat. Residues were characterized primarily by HPLC analysis, using TLC analyses for confirmation and LC-MS/MS analyses for identification of metabolites. These methods successfully identified the predominant residues in goat matrices.

The characterization and identification of residues in goat matrices is summarized in TABLE C.2.3b. Approximately 61-84% of the TRRs were identified in goat matrices. Prothioconazole was found to be a major residue in liver, kidney, and fat at 16.12-19.50% of the TRRs (0.028-1.047 ppm). Prothioconazole was found at lower levels in milk (3.18% of the TRRs, 0.005 ppm) and muscle (7.17% of the TRRs, 0.008 ppm). Thiocyanate was found to account for a major portion of radioactivity in milk (41.12% of the TRRs, 0.061 ppm), kidney (9.01% of the TRRs, 0.406 ppm), muscle (29.62% of the TRRs, 0.035 ppm), and fat (12.38% of the TRRs, 0.022 ppm). Thiocyanate was found at lower levels in liver (2.04% of the TRRs, 0.128 ppm). Radioactivity corresponding to 10.76% of the TRRs (0.016 ppm) was attributed to lactose in milk. Two co-eluting metabolites, JAU6476-*S*-glucuronide and JAU6476-3-hydroxy-desthio, were found to be major metabolites in kidney at 33.85% of the TRRs (1.526 ppm); in muscle at 13.62% of the TRRs (0.016 ppm); and fat at 11.92% of the TRRs (0.021 ppm). These metabolites were also found in milk and liver at <6.1% of the TRRs (<0.38 ppm). JAU6476-desthio was a major metabolite in fat at 15.11% of the TRRs (0.026 ppm), but was found at

lower levels in other goat matrices (<5% of the TRRs; <0.31 ppm). Additional metabolites found at significant levels were JAU6476-4-hydroxy at 10.97% of the TRRs (0.686 ppm) in liver (found at <8.5% of the TRRs (<0.164 ppm) in other goat matrices), and JAU6476-hydroxy-glucuronide at 11.15% of the TRRs (0.019 ppm) in fat (<7% of the TRRs (<0.32 ppm) in other goat matrices). Several additional metabolites were identified in goat matrices, each at <7% of the TRRs (<0.41 ppm): JAU6476-4-hydroxy-glucuronide in kidney and muscle; JAU6476-hydroxy sulfate and sulfate conjugate in liver; JAU6476-lactoside in milk; JAU6476-4-hydroxy-desthio in liver; JAU6476-*N*-glucuronide in liver, kidney, muscle, and fat; and JAU6476-*S*-methyl in liver. Unknown metabolites accounted for 5.1-11.0% of the TRRs (<0.32 ppm) in liver and kidney. However, HPLC analyses indicated that individual unknowns were ≤3.1% of the TRRs.

The applicant noted that preliminary structure assignments were achieved for most metabolites in the milk and tissue organic extracts by comparison of the HPLC profile with that of the respective extracts from the corresponding goat metabolism study with [phenyl-^{14}C]-prothioconazole. HPTLC analyses with reference standards were used to confirm the following metabolite identifications: prothioconazole in all matrices; thiocyanate in all matrices; one isomer of JAU6476-hydroxy-glucuronide in milk, liver, and fat; two isomers of JAU6476-hydroxy-glucuronide in kidney and muscle; JAU6476-*S*-glucuronide in all matrices; JAU6476-3-hydroxy-desthio in milk and fat; JAU6476-4-hydroxy-desthio in liver; JAU6476-*N*-glucuronide in kidney and muscle (using liver extract); JAU6476-4-hydroxy in all matrices; and JAU6476-desthio in all matrices. LC-MS/MS analyses were used to confirm the identification of JAU6476-4-hydroxy in liver and to identify JAU6476-*N*-glucuronide in liver.

In milk, JAU6476-lactoside was identified using LC-MS and LC-MS/MS analyses. The analyses indicated that the metabolite was a disaccharide conjugate of prothioconazole. The applicant concluded that because the metabolite was isolated from milk, the disaccharide was most likely lactose. The applicant additionally concluded that the radioactivity associated with the solids that precipitated during concentration of the aqueous phase could be attributed to lactose.

The applicant subjected the methanol XAD eluate of milk to treatment with boiling HCl. The resulting HPLC profile indicated that cleavage of the glucuronides and JAU6476-lactoside occurred. The stability of prothioconazole was also tested; HPLC analyses indicated that prothioconazole was unchanged after HCl hydrolysis at 100°C for 4 or 8 hours. Acid hydrolyses of the extracts of liver, kidney, and muscle similarly indicated that cleavage of glucuronide and sulfate conjugates occurred (with the exception of JAU6476-*N*-glucuronide which was not cleaved under the conditions used), with corresponding increases in the concentrations of prothioconazole and JAU6476-4-hydroxy.

In liver, LC-MS analyses were used to identify JAU6476-hydroxy-glucuronide (one isomer), a sulfate conjugate of JAU6476-hydroxy, and a further sulfate conjugate of an unknown compound. Unambiguous assignment of HPLC peaks to each of these compounds could not be made; however, the applicant assumed that the compound which eluted first was JAU6476-

hydroxy-glucuronide. Metabolite JAU6476-*S*-methyl was identified in liver by comparing the HPLC profile with that of a liver extract from a hen metabolism study with [phenyl-^{14}C]-prothioconazole (MRID 46246202); JAU6476-*S*-methyl was conclusively identified in the hen metabolism study.

Based on TLC analyses of XAD aqueous eluates using co-chromatography with reference standards, the applicant excluded the presence of the following triazole-related compounds: *1H*-1,2,4-triaxial in milk, liver, kidney, and fat; triaxial acetic acid in milk and liver; and triazolyl alanine in liver.

The non-extractable residues of milk and liver accounted for approximately 16% and 15% of the TRRs, respectively. The extraction of milk and liver was repeated, and the solids remaining after extraction were subjected to microwave extraction (using ACN:water, 1:1, v:v), which released 4.1% of the TRRs (0.256 ppm) from liver and 7.0% of the TRRs (0.011 ppm) from milk; non-extractable residues were 10.6% of the TRRs (0.661 ppm) in liver and 11.1% of the TRRs (<0.02 ppm) in milk after microwave extraction. The HPLC profile of the released radioactivity from liver indicated the presence of small amounts of the compounds found in the liver methanol extract. The extracts released from the solids in the liver sample (by microwave extraction) were comprised of prothioconazole, JAU6476-desthio, JAU6476-*N*-glucuronide and JAU6476-4-hydroxy. Since these metabolites were non-label specific, they would be expected to be found in the phenyl-label sample as well. Also, the initial extraction schemes were similar between the 2 radiolabels. Because these residues were expected to be the same metabolites as found in the solvent extracts, no further extraction was conducted.

C.1. Storage Stability

Phenyl-label: The initial extraction and analysis (HPLC and HPTLC) of the major extracts of goat matrices was completed within three months of sample collection (sacrifice of the goat). The metabolite profiles used for identification and characterization were based on these initial analyses. For liver, a second and third extraction were conducted approximately 19 and 28 months after sample collection, for the purposes of co-chromatography with JAU6476-desthio metabolites (isolated and identified in a separate goat metabolism study, MRID 46246201) and JAU6476-sulfonic acid. The HPLC profile of the extracts of the stored liver samples were found to be similar to the initial liver extract, with the exception of the polar metabolites, indicating that the metabolite profile was stable in liver for up to ~25 months. For kidney, a second extraction was conducted approximately 21 months following sample collection, for the purposes of metabolite isolation and structure elucidation. The HPLC profile of the extract of the stored sample was similar to that of the initial extract, indicating that the metabolite profile was stable in kidney for up to ~18 months.

Triazole-label Study: The initial extraction and analysis (HPLC and TLC) of the organic (methanol) extracts of goat matrices was completed within two months of sample collection (sacrifice of the goat). For liver, kidney, and muscle, the metabolite profiles used for

identification and characterization were based on these initial analyses. For milk and fat, the organic extracts were reanalyzed after approximately 5 and 6 months of storage, respectively, after the initial analyses. Because these extracts were sonicated prior to analysis, the extracts were more homogeneous than the aliquots used for initial analyses. For the milk extract, the second analysis revealed an additional peak that was not observed in the first analysis; structure elucidation of the initial peak indicated that it was not a degradation product. For the fat extract, metabolite concentrations were found to be higher in the second analysis than the first, with no change in the metabolite pattern. Therefore, the applicant used the results from the second analyses for metabolic profiling of the organic extracts of milk and fat.

The aqueous extracts of goat matrices were subjected to HPLC analyses for metabolite profiling approximately 8 months after sample collection. To demonstrate the stability of the metabolite profiles in these extracts during storage, the applicant analyzed the aqueous extracts by TLC within two months of sample collection and again at the time of HPLC analyses of these extracts. These analyses indicated that the metabolite profile did not change in the aqueous extract during storage.

Subsamples of milk, liver, and muscle were subjected to additional extractions conducted approximately 8, 7, and 11 months, respectively, after sample collection. The additional extractions and analyses were conducted for milk and liver to isolate metabolites for structure elucidation; the additional extraction and analysis for muscle was conducted to obtain sufficient organic extract to conduct acid hydrolyses. Comparison of the organic extracts of the stored samples with those of the initial extracts indicated that the metabolite profiles were stable in the samples during storage. There were some differences observed in the concentrations of polar metabolites in liver after storage; however, the differences were not significant.

TABLE C.1. Summary of Storage Conditions.			
Matrix	Storage Temp. (°C)	Actual Storage Duration	Interval of Demonstrated Storage Stability
Phenyl-label Study			
Milk	~-18	~3 months	None provided.
Liver		~3, 19, 28 months	~25 months from first analysis
Kidney		~3, 21 months	~18 months from first analysis
Muscle		~3 months	None provided.
Fat		~3 months	None provided.
Triazole-label Study			
Milk	~-18	**RAC:** ~2, 8 months **Methanol extract:** ~2-5 months **Aqueous extract:** ~6 months	**RAC:** ~8 months **Aqueous extract:** ~6 months
Liver		**RAC:** ~2, 7 months **Methanol extract:** ~2 months **Aqueous extract:** ~6 months	**RAC:** ~7 months **Aqueous extract:** ~6 months
Kidney		**RAC:** ~2 months **Methanol extract:** ~2 months **Aqueous extract:** ~6 months	**Aqueous extract:** ~6 months
Muscle		**RAC:** ~2, 11 months **Methanol extract:** ~2 months **Aqueous extract:** ~6 months	**RAC:** ~11 months **Aqueous extract:** ~6 months
Fat		**RAC:** ~2 months **Methanol extract:** ~2-6 months **Aqueous extract:** ~6 months	**Aqueous extract:** ~6 months

C.2. Identification, Characterization, and Distribution of Residues

TABLE C.2.1a. Total Radioactive Residues (TRRs) in Milk, Tissue and Excreta from the Phenyl-label Study.

Matrix	Collection Timing (hours after first dose)	Radioactivity	
		ppm	% of administered dose
Urine	24	--	15.88
	48	--	17.44
	53	--	9.12
	Total	--	42.44
Feces	24	--	9.92
	48	--	11.26
	53	--	2.97
	Total	--	24.15
Milk	8	0.042	0.004
	24	0.020	0.003
	32	0.071	0.004
	48	0.026	0.003
	53	0.061	0.003
Milk (composite sample)	Over duration of study	0.037	--
Liver	At sacrifice	6.092	0.442
Kidney	At sacrifice	6.762	0.067
Round muscle	At sacrifice	0.084	--
Flank muscle	At sacrifice	0.106	--
Loin muscle	At sacrifice	0.100	--
Body muscle (composite sample)	At sacrifice	0.097 [1] (0.088)	0.270 [2]
Perirenal fat	At sacrifice	0.162	--
Subcutaneous fat	At sacrifice	0.149	--
Omental fat	At sacrifice	0.172	--
Body fat (composite sample)	At sacrifice	0.167 [1] (0.169)	0.180 [2]
Total % of Administered Dose	--	--	67.57

[1] Average of the three different types of tissue. Value in parentheses is the reported TRRs for the composite sample used for extraction/analysis.

[2] Calculated using goat body weight and assuming muscle and fat account for 30% and 12%, respectively, of body weight.

FIGURE C.2.1a. TRRs in milk, tissues and excreta from the phenyl and triazole-label studies.

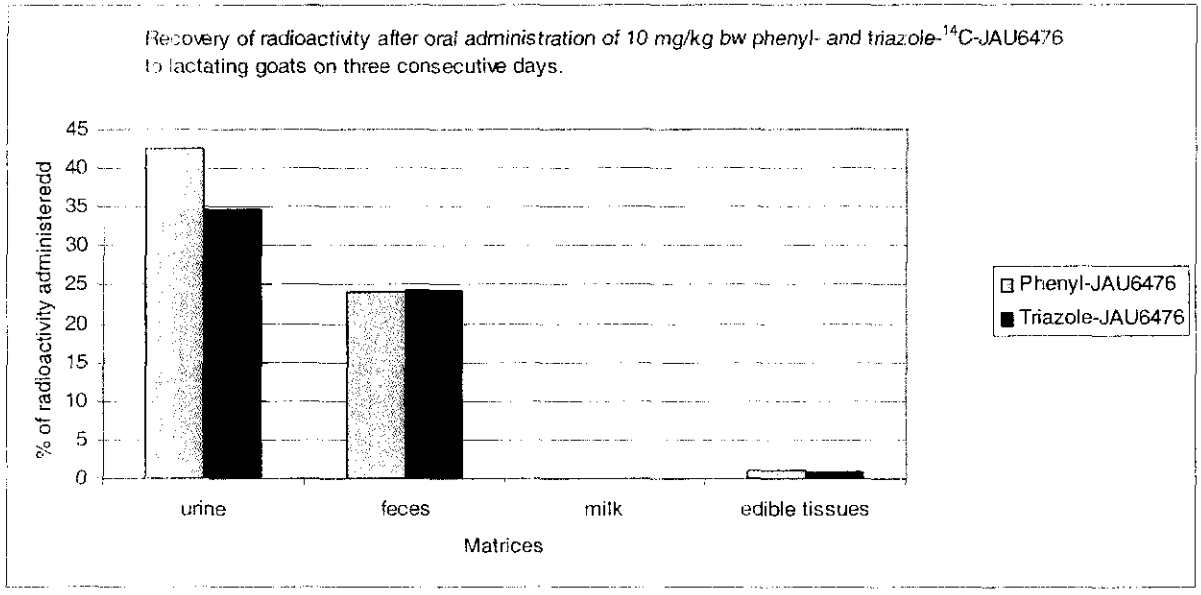


FIGURE C.2.1.1a. TRRs (ppm) in milk, tissues/organs from the phenyl and triazole-label studies.

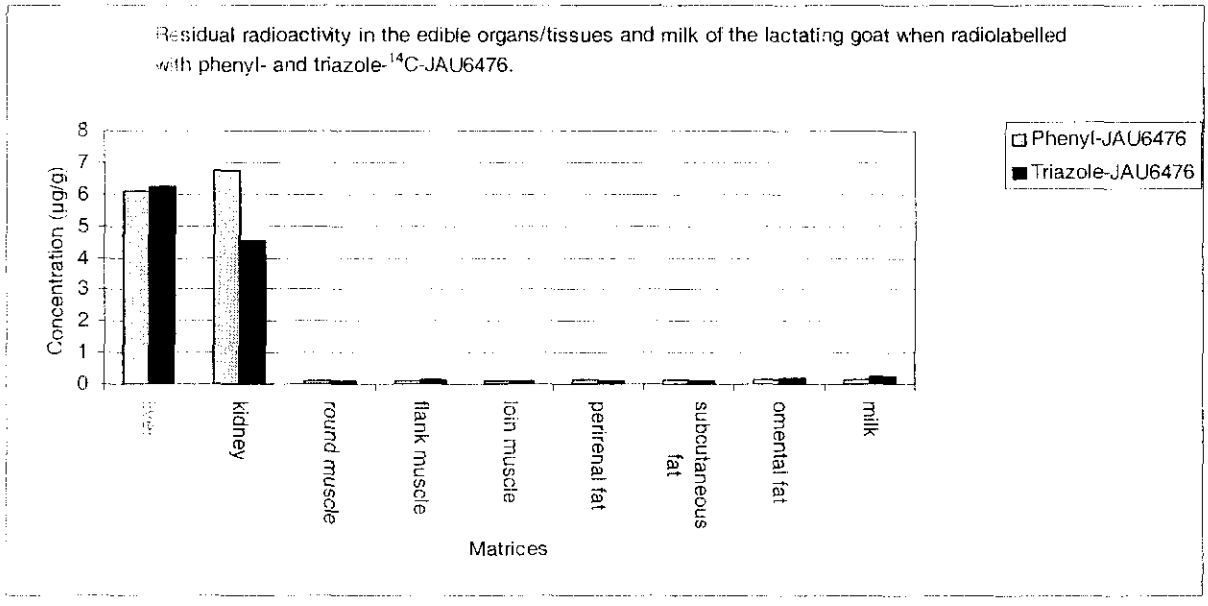


FIGURE C.2.1.2a. Pharmacokinetics of [Phenyl-UL-^{14}C]-Prothioconazole in Milk of Lactating Goat.

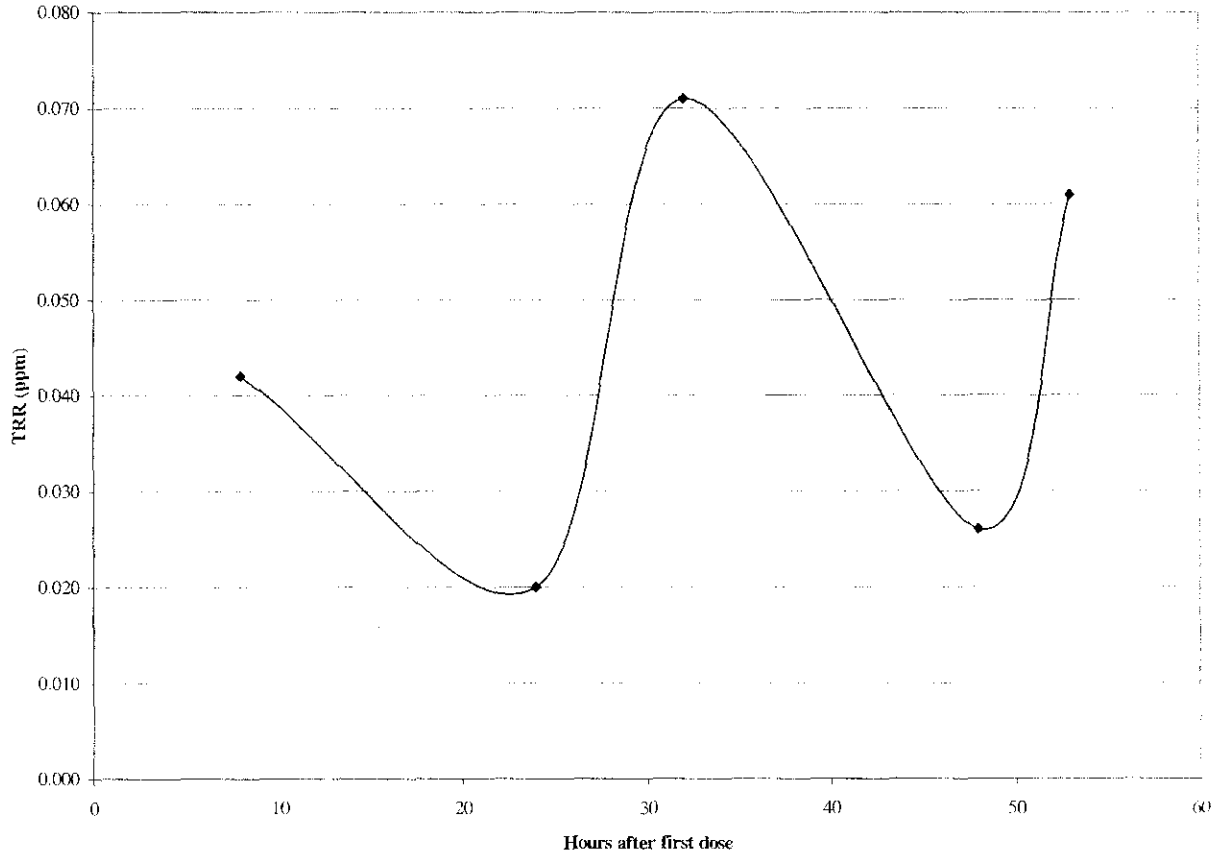


TABLE C.2.2a	**Distribution of the Parent and the Metabolites in Goat Matrices Following Dosing with [Phenyl-UL-^{14}C]-Prothioconazole.** [1]									
Metabolite Fraction	Milk		Liver		Kidney		Muscle		Fat	
	TRR = 0.037 ppm		TRR = 6.092 ppm		TRR = 6.762 ppm		TRR = 0.088 ppm		TRR = 0.169 ppm	
	%TRR	ppm	%TRR	ppm	%TRR	ppm	%TRR	ppm	%TRR	ppm
Methanol extract	82.41	0.031								
ACN/water (80:20) extract			79.99	4.873	96.63	6.534	79.78	0.071	77.48	0.131
-Methanol (evaporated)	1.39	0.001								
-ACN (evaporated)			0.04	0.002	0.17	0.012	--	--	2.59	0.004
-Hexane phase			8.62	0.525	3.96	0.268	11.91	0.011	7.52	0.013
-Aqueous phase			71.33	4.345	92.50	6.255	67.87	0.060	67.37	0.114
--Methanol XAD eluate	68.12	0.025	67.36	4.104	77.90	5.268	56.70	0.050	60.90	0.103
Prothioconazole	0.89	0.0003	12.94	0.788	17.97	1.215	13.37	0.012	13.31	0.022
JAU6476-desthio-3,4-dihydroxy-dienyl-glucuronide	--	--	4.96	0.302	--	--	--	--	--	--
JAU6476-desthio-dihydroxy-diene	2.36	0.0009	1.48	0.090	--	--	--	--	--	--
JAU6476-4-hydroxy-desthio-glucuronide	3.75	0.0014	--	--	--	--	--	--	--	--
JAU6476-dihydroxy-desthio-glucuronide										
JAU6476-hydroxymethoxy-desthio-glucuronide										
JAU6476-4-hydroxy-glucuronide	--	--	2.39	0.146	4.01	0.271	2.05	0.002	2.46	0.004
JAU6476-hydroxy-glucuronide	--	--	5.05	0.307	7.44	0.503	5.42	0.005	3.17	0.005
JAU6476-dihydroxy-diene	1.97	0.0007	--	--	--	--	--	--	--	--
JAU6476-desthio-glucuronide	2.04	0.0008	--	--	--	--	--	--	--	--
JAU6476-3-hydroxy-desthio [2]	11.96	0.0045	10.02	0.610	34.32	2.321	14.80	0.013	10.09	0.017
JAU6476-glucuronide										
JAU6476-4-hydroxy-desthio	--	--	1.52	0.092	--	--	--	--	--	--
JAU6476-N-glucuronide	1.27	0.0005	2.80	0.170	2.64	0.179	1.14	0.001	0.80	0.001
JAU6476-4-hydroxy	2.10	0.0008	11.21	0.683	3.10	0.210	4.94	0.004	3.61	0.006
JAU6476-desthio	2.83	0.0011	1.24	0.076	1.29	0.087	2.95	0.003	18.98	0.032
Polar metabolites/ conjugates	31.30 [3]	0.0117	10.78 [4]	0.657	7.13 [5]	0.482	12.01 [5]	0.011	4.25 [5]	0.007

TABLE C.2.2a.	**Distribution of the Parent and the Metabolites in Goat Matrices Following Dosing with [Phenyl-UL-^{14}C]-Prothioconazole.** [1]									
Metabolite Fraction	Milk		Liver		Kidney		Muscle		Fat	
	TRR = 0.037 ppm		TRR = 6.092 ppm		TRR = 6.762 ppm		TRR = 0.088 ppm		TRR = 0.169 ppm	
	%TRR	ppm	%TRR	ppm	%TRR	ppm	%TRR	ppm	%TRR	ppm
Unknowns	7.64	0.0029	2.99 [6]	0.182	--	--	--	--	4.24	0.007
--XAD effluent/rinse	12.90	0.005	3.97	0.242	14.59	0.986	11.17	0.010	6.47	0.011
ACN/water (50:50) extract			3.30	0.201	0.92	0.063	3.76	0.003	Combined with 80:20 extract	
Non-extractable	17.59	0.007	16.72	1.018	2.45	0.166	16.46	0.015	22.52	0.038

[1] Shading indicates that the extraction step and/or characterization analysis was not conducted for the matrix in question.
[2] HPTLC analyses indicated that JAU6476-3-hydroxy-desthio accounted for <0.7% of the TRRs in milk, 1.3% of the TRRs in liver, 1.6% of the TRRs in kidney, 1.1% of the TRRs in muscle, and 1.8% of the TRRs in fat.
[3] A total of 12 peaks, each ≤8.58% of the TRRs (≤0.0032 ppm).
[4] A total of 3 peaks, each ≤4.69% of the TRRs (0.286 ppm). Based on a comparison of the retention times with those of isolated polar metabolites of urine and metabolites isolated in a goat separate metabolism study (refer to the DER for MRID 46246201), the applicant concluded that this fraction contained glucuronic acid conjugates of JAU6476-desthio, JAU6476-hydroxy-desthio, JAU6476-dihydroxy-desthio, and JAU6476-hydroxymethoxy-desthio, as well as small amounts of JAU6476-dihydroxy-diene and JAU6476-desthio-dihydroxy-diene.
[5] Based on a comparison of the retention times with those of isolated polar metabolites of urine, this fraction was characterized as containing glucuronic acid conjugates, JAU6476-dihydroxy-diene, and JAU6476-desthio-dihydroxy-diene.
[6] One peak.

TABLE C.2.3a. Summary of Characterization and Identification of Radioactive Residues in Goat Matrices *Following Dosing with [Phenyl-UL-^{14}C]-Prothioconazole at 246 ppm in the Diet.*

Compound	Milk		Liver		Kidney		Muscle		Fat	
	TRR = 0.037 ppm		TRR = 6.092 ppm		TRR = 6.762 ppm		TRR = 0.088 ppm		TRR = 0.169 ppm	
	%TRR	ppm	%TRR	ppm	%TRR	ppm	%TRR	ppm	%TRR	ppm
Prothioconazole	0.89	0.0003	12.94	0.788	17.97	1.215	13.37	0.012	13.31	0.022
JAU6476-4-hydroxy-glucuronide	--	--	2.39	0.146	4.01	0.271	2.05	0.002	2.46	0.004
JAU6476-hydroxy-glucuronide	--	--	5.05	0.307	7.44	0.503	5.42	0.005	3.17	0.005
JAU6476-*O*- or *S*-glucuronide	11.96	0.0045	10.02	0.610	34.32	2.321	14.80	0.013	10.09	0.017
JAU6476-3-hydroxy-desthio										
JAU6476-4-hydroxy-desthio	--	--	1.52	0.092	--	--				
JAU6476-*N*-glucuronide	1.27	*0.0005*	2.80	0.170	2.64	0.179	1.14	0.001	0.80	0.001
JAU6476-4-hydroxy	2.10	0.0008	11.21	0.683	3.10	0.210	4.94	0.004	3.61	0.006
JAU6476-desthio	2.83	0.0011	1.24	0.076	1.29	0.087	2.95	0.003	18.98	0.032
Polar metabolites/conjugates:										
JAU6476-desthio-3,4-dihydroxy-dienyl glucuronide	--	--	4.96	0.302	--	--	--	--	--	--
JAU6476-desthio-dihydroxy-diene	2.36	*0.0009*	1.48	*0.090*	--	--	--	--	--	--
JAU6476-4-hydroxy-*desthio-glucuronide*	3.75	0.0014	--	--	--	--	--	--	--	--
JAU6476-dihydroxy-desthio-glucuronide										
JAU6476-hydroxymethoxy-desthio-glucuronide										
JAU6476-dihydroxy-diene	1.97	0.0007	--	--	--	--	--	--	--	--
JAU6476-desthio-glucuronide	2.04	0.0008	--	--	--	--	--	--	--	--
Total identified	29.17	0.011	53.61	3.264	70.77	4.786	44.67	0.040	52.42	0.087
Characterized polar metabolites/conjugates	31.30	0.0117	10.78	0.657	7.13	0.482	12.01	0.011	4.25	0.007
Unknowns	7.64	0.0029	2.99	0.182	--	--	--	--	4.24	0.007
Fractions not analyzed	14.29	0.005	15.93	0.970	19.64	1.329	26.84	0.024	16.58	0.028
Total extractable	82.41	0.023	83.29	5.074	97.55	6.597	83.54	0.074	77.48	0.131
Unextractable (PES) [1]	17.59	0.007	16.72	1.018	2.45	0.166	16.46	0.015	22.52	0.038
Accountability [2]	100.0		100.0		100.0		100.0		100.0	

[1] Residues remaining after exhaustive extractions.

[2] Accountability = (Total extractable + Total unextractable)/(absolute values of the TRRs (ppm)) * 100.

TABLE C.2.1b. Total Radioactive Residues (TRRs) in Milk, Tissue and Excreta from the Triazole-Label Study.

Matrix	Collection Timing (hours after first dose)	Radioactivity	
		ppm	% of administered dose
Urine	24	--	14.46
	48	--	15.44
	53	--	4.63
	Total	--	34.53
Feces	24	--	10.14
	48	--	12.50
	53	--	1.58
	Total	--	24.22
Milk	8	0.127	0.005
	24	0.080	0.005
	32	0.242	0.008
	48	0.151	0.008
	53	0.249	0.006
Milk (composite sample)	Over duration of study	0.150	--
Liver	At sacrifice	6.248	0.507
Kidney	At sacrifice	4.507	0.053
Round muscle	At sacrifice	0.115	--
Flank muscle	At sacrifice	0.142	--
Loin muscle	At sacrifice	0.115	--
Body muscle (composite sample)	At sacrifice	0.124 [1] (0.117)	0.124 [2]
Perirenal fat	At sacrifice	0.112	--
Subcutaneous fat	At sacrifice	0.109	--
Omental fat	At sacrifice	0.213	--
Body fat (composite sample)	At sacrifice	0.145 [1] (0.174)	0.057 [2]
Total % of Administered Dose	--	--	59.52

[1] Average of the three different types of tissue. Value in parentheses is the reported TRRs for the composite sample used for extraction/analysis.

[2] Calculated using goat body weight and assuming muscle and fat account for 30% and 12%, respectively, of body weight.

FIGURE C.2.1b. Pharmacokinetics of [Triazole-UL-^{14}C]-Prothioconazole in Milk of Lactating Goat.

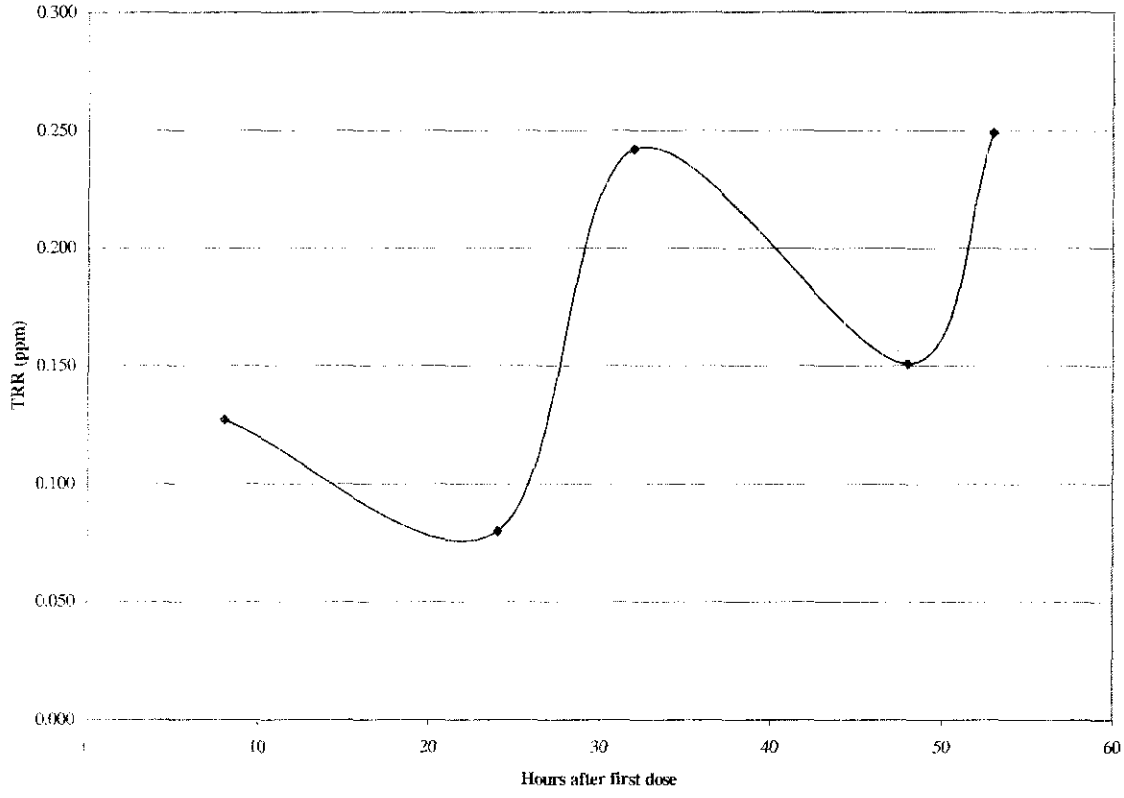


TABLE C.2.2b. Distribution of the Parent and the Metabolites in Goat Matrices Following Dosing with [Triazole-UL-^{14}C]-Prothioconazole at 195 ppm in the Diet. [1]

Metabolite Fraction	Milk		Liver		Kidney		Muscle		Fat	
	TRR = 0.150 ppm		TRR = 6.248 ppm		TRR = 4.507 ppm		TRR = 0.117 ppm		TRR = 0.174 ppm	
	%TRR	ppm	%TRR	ppm	%TRR	ppm	%TRR	ppm	%TRR	ppm
Methanol extract	83.76	0.126								
ACN/water (80:20) extract			82.96	5.183	94.32	4.252	77.72	0.091	76.80	0.134
-ACN/water C18 effluent	83.03	0.125	81.69	5.105	92.14	4.153	75.47	0.088	74.99	0.131
-Solids (C18 SPE)	0.73	0.001	1.26	0.079	2.18	0.098	2.25	0.003	0.62	0.001
--Methanol XAD eluate	21.92	0.033	73.29	4.579	83.13	3.747	50.10	0.059	62.60	0.109
Prothioconazole	3.18	0.005	14.09	0.881	19.50	0.879	7.17	0.008	16.12	0.028
Thiocyanate	1.74	0.003	--	--	--	--	4.25	0.005	--	--
JAU6476-hydroxy-glucuronides [2]	3.63	0.005	5.03	0.315	5.55 [4]	0.250	3.28 [4]	0.004	11.15	0.019
					6.09 [5]	0.275	4.67 [5]	0.005		
JAU6476-lactoside	4.35	0.007	--	--	--	--	--	--	--	--
JAU6476-hydroxy-sulfate/sulfate conjugate [3]	--	--	6.53	0.408	--	--	--	--	--	--
	--	--	3.86	0.241	--	--	--	--	--	--
JAU6476-3-hydroxy-desthio	4.35	0.007	6.07	0.379	33.85	1.526	13.62	0.016	11.92	0.021
JAU6476-*S*-glucuronide										
JAU6476-4-hydroxy-desthio	--	--	2.86	0.179	--	--	--	--	--	--
JAU6476-*N*-glucuronide	--	--	4.55	0.284	3.44	0.155	5.28	0.006	8.31	0.014
JAU6476-4-hydroxy	3.30	0.005	10.97	0.686	3.64	0.164				
JAU6476-desthio	1.37	0.002	4.94	0.309	3.00	0.135	0.90	0.001	15.11	0.026
JAU6476-*S*-methyl	--	--	0.60	0.038	--	--	--	--	--	--
Polar metabolites/ conjugates	--	--	12.39 [6]	0.773	8.05	0.363	10.93	0.013	--	--
Unknown	--	--	1.39	0.087	--	--	--	--	--	--
--XAD effluent/rinse	--	--	8.40	0.525	9.01	0.406	25.36	0.030	12.38	0.022
Prothioconazole			2.66	0.166	--	--	--	--	--	--
Thiocyanate			2.04	0.128	9.01	0.406	25.36	0.030	12.38	0.022
Unknown(s)			3.70	0.231	--	--	--	--	--	--
---Acetone phase	21.24	0.032								
Thiocyanate	21.24	0.032								
---Aqueous phase	29.11	0.044								
Thiocyanate	18.14	0.027								
Unknown	10.97	0.016								
---Solids (lactose)	10.76	0.016								
ACN:water (50:50) extract			1.95	0.122	0.43	0.019	2.20	0.003		

TABLE C.2.2b. Distribution of the Parent and the Metabolites in Goat Matrices Following Dosing with [Triazole-UL-^{14}C]-Prothioconazole at 195 ppm in the Diet. [1]

Metabolite Fraction	Milk		Liver		Kidney		Muscle		Fat	
	TRR = 0.150 ppm		TRR = 6.248 ppm		TRR = 4.507 ppm		TRR = 0.117 ppm		TRR = 0.174 ppm	
	%TRR	ppm	%TRR	ppm	%TRR	ppm	%TRR	ppm	%TRR	ppm
-Methanol/DCM C18 eluate									1.19	0.002
Non-extractable	16.24	0.024	15.09	0.943	5.25	0.237	20.08	0.023	23.20	0.040
Microwave extract [8]			4.10 [9]	0.256						
Non-extractable			10.58	0.661						

[1] Shading indicates that the extraction step and/or characterization analysis was not conducted for the matrix in question.
[2] Likely to be two isomers.
[3] Tentatively identified compounds; two compounds were identified by HPLC-MS, but unambiguous assignment to the single peaks was not possible.
[4] Isomer 1, most probably JAU6476-4-hydroxy-glucuronide.
[5] Isomer 2.
[6] A total of 6 peaks, each ≤3.08% of the TRRs (≤0.192 ppm). Based on the peaks observed after acid treatment of the extract, this fraction contained conjugates and dienes.
[7] Microwave extraction of non-extractable residues from a second extraction of liver.
[8] The HPLC profile of this fraction had small amounts of the compounds found in the methanol extract of liver.

TABLE C.2.3b. Summary of Characterization and Identification of Radioactive Residues in Goat Matrices Following Dosing with [Triazole-UL-^{14}C]-Prothioconazole at 195 ppm in the Diet.

Compound	Milk		Liver		Kidney		Muscle		Fat	
	TRR = 0.150 ppm		TRR = 6.248 ppm		TRR = 4.507 ppm		TRR = 0.117 ppm		TRR = 0.174 ppm	
	%TRR	ppm	%TRR	ppm	%TRR	ppm	%TRR	ppm	%TRR	ppm
Prothioconazole	3.18	0.005	16.75	1.047	19.50	0.879	7.17	0.008	16.12	0.028
Thiocyanate	41.12	0.061	2.04	0.128	9.01	0.406	29.62	0.035	12.38	0.022
JAU6476-4-hydroxy-glucuronide	--	--	--	--	5.55	0.250	3.28	0.004	--	--
JAU6476-hydroxy-glucuronide	3.63 [1]	0.005	5.03	0.315	6.09	0.275	4.67	0.005	11.15 [1]	0.019
JAU6476-hydroxy-sulfate/sulfate conjugate	--	--	6.53	0.408	--	--	--	--	--	--
	--	--	3.86	0.241	--	--	--	--	--	--
JAU6476-lactoside	4.35	0.007	--	--	--	--	--	--	--	--
JAU6476-*S*-glucuronide	4.35	0.007	6.07	0.379	33.85	1.526	13.62	0.016	11.92	0.021
JAU6476-3-hydroxy-desthio										
JAU6476-4-hydroxy-desthio	--	--	2.86	0.179	--	--	--	--	--	--
JAU6476-*N*-glucuronide	--	--	4.55	0.284	3.44	0.155	5.28	0.006	8.31	0.014
JAU6476-4-hydroxy	3.30	0.005	10.97	0.686	3.64	0.164				
JAU6476-desthio	1.37	0.002	4.94	0.309	3.00	0.135	0.90	0.001	15.11	0.026
JAU6476-*S*-methyl	--	--	0.60	0.038	--	--	--	--	--	--
Total identified	61.30	0.092	64.21	4.014	84.08	3.790	64.54	0.075	74.99	0.130
Polar metabolites/ conjugates	--	--	12.39	0.773	8.05	0.363	10.93	0.013	--	--
Unknowns	10.97	0.016	5.09	0.318	--	--	--	--	--	--
Fractions not analyzed	11.49	0.017	7.31	0.457	2.61	0.117	4.45	0.006	1.81	0.003
Total extractable	83.76	0.126	89.01	5.561	94.75	4.270	79.92	0.094	76.80	0.134
Unextractable (PES) [2]	16.24	0.024	10.58	0.6617	5.25	0.237	20.08	0.023	23.20	0.040
Accountability [3]	100.0		100.0		100.0		100.0		100.0	

[1] Likely to be two isomers of JAU6476-hydroxy-glucuronide.
[2] Residues remaining after exhaustive extractions.
[3] Accountability = (Total extractable + Total unextractable)/(absolute values of the TRRs (ppm)) * 100.

C.3. Proposed Metabolic Profile

The metabolic pathway for [phenyl-UL-^{14}C]-JAU6476 or [triazole-UL-^{14}C]-JAU6476 in lactating goats is shown in FIGURE C.3.1. JAU6476 was extensively metabolized following the oral administration of [phenyl-UL-^{14}C]-JAU6476 or [triazole-UL-^{14}C]-JAU6476 to lactating goats. The major metabolic pathways for JAU6476 in goats were:

- conjugation of the unchanged parent compound with glucuronic acid forming an *S*-glucuronide,
- hydroxylation of JAU6476 followed by glucuronidation,
- cleavage of the triazolinthione moiety of JAU6476 to yield thiocyanate, and
- desulfuration of JAU6476 yielding JAU6476-desthio

Several minor metabolic processes were also elucidated. These minor pathways were:

1. methylation of the sulfur atom to form JAU6476-*S*-methyl,
2. conjugation of JAU6476 to the *N*-glucuronide,
3. hydroxylations of JAU6476 and JAU6476-desthio (probably through epoxide intermediates) leading to the formation of the corresponding dihydroxy and dihydroxy-dienes,
4. glucuronidation and/or methylation of the di-hydroxylated JAU6476-desthio metabolites, and
5. conjugation of JAU6476-dihydroxy with glucuronic acid.

FIGURE C.3.1 Proposed Metabolic Profile of [phenyl-UL-^{14}C] and [triaxial -UL-^{14}C]-Prothioconazole in Lactating Goat.

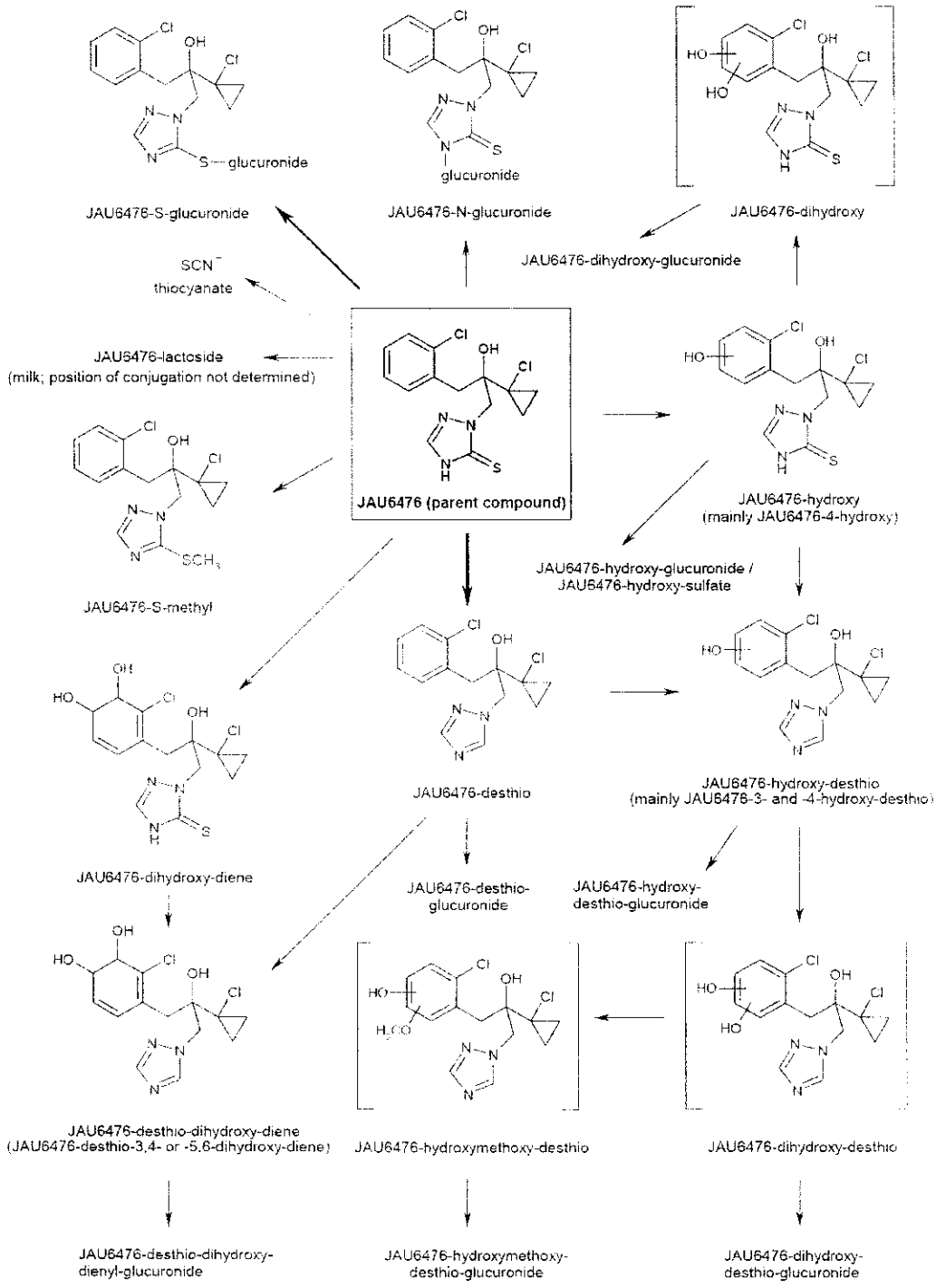


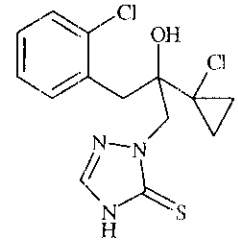
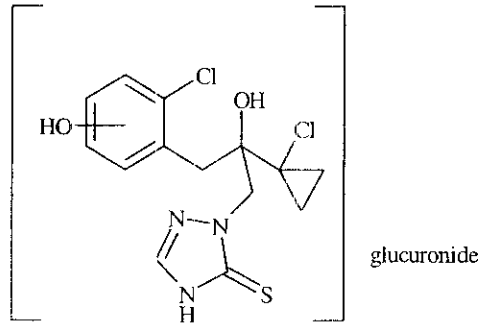
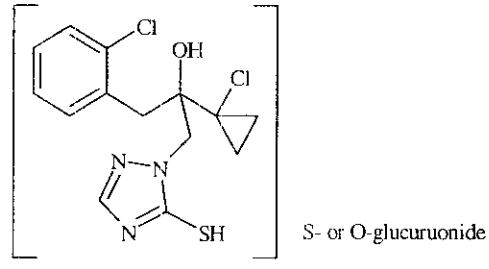
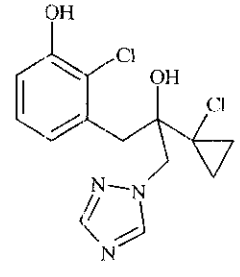
TABLE C.3.1. Identification of Compounds from Metabolism Study		
Common name/code FIGURE C.3.1 ID No.	Chemical name	Chemical structure
Prothioconazole: JAU6476	2-[2-(1-chlorocyclopropyl)-3-(2-chlorophenyl)-2-hydroxypropyl]-1,2-dihydro-3*H*-1,2,4-triazole-3-thione	
JAU6476-hydroxy-glucuronide		 glucuronide
JAU6476-*O*- or *S*-glucuronide		 S- or O-glucuruonide
JAU6476-3-hydroxy-desthio	α-(1-chlorocyclopropyl)-α-[(2-chloro-3-hydroxyphenyl)methyl]-1*H*-1,2,4-triazole-1-ethanol	

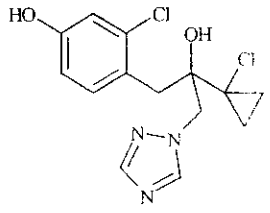
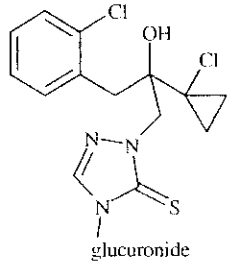
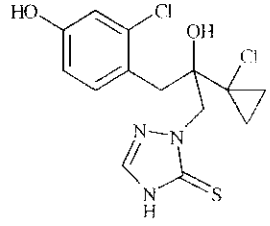
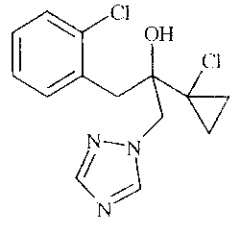
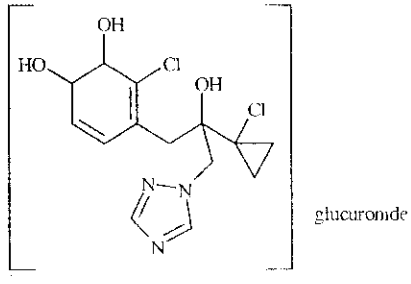
TABLE C.3.1. Identification of Compounds from Metabolism Study		
Common name/code FIGURE C.3.1 ID No.	Chemical name	Chemical structure
JAU6476-4-hydroxy-desthio	α-(1-chlorocyclopropyl)-α-[(2-chloro-4-hydroxyphenyl)methyl]-1*H*-1,2,4-triazole-1-ethanol	
JAU6476-*N*-glucuronide		
JAU6476-4-hydroxy	2-[2-(1-chlorocyclopropyl)-3-(2-chloro-4-hydroxyphenyl)-2-hydroxypropyl]-1,2-dihydro-3*H*-1,2,4-triazole-3-thione	
JAU6476-desthio	α-(1-chlorocyclopropyl)-α-[(2-chlorophenyl)methyl]-1*H*-1,2,4-triazole-1-ethanol	
JAU6476-desthio-3,4-dihydroxy-dienyl-glucuronide		

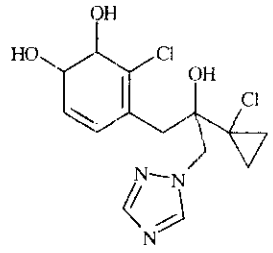
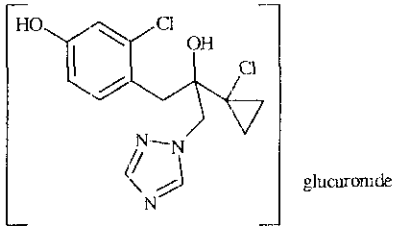
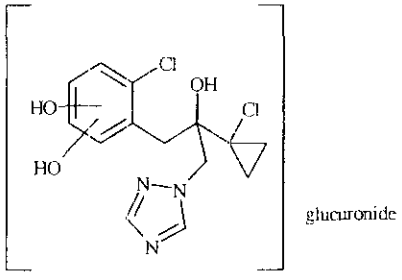
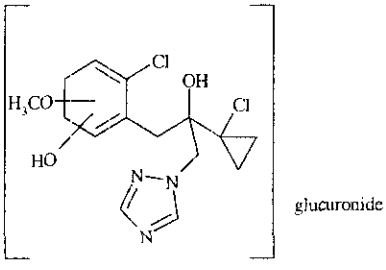
TABLE C.3.1. Identification of Compounds from Metabolism Study		
Common name/code FIGURE C.3.1 ID No.	Chemical name	Chemical structure
JAU6476-desthio-dihydroxy-diene also referred to as JAU6476-desthio-3,4-dihydroxy-diene	3-chloro-4-[2-(1-chlorocyclopropyl)-2-hydroxy-3-(1*H*-1,2,4-triazol-1-yl)propyl]cyclohexa-3,5-diene-1,2-diol [1]	
JAU6476-4-hydroxy-desthio-glucuronide		
JAU6476-dihydroxy-desthio-glucuronide		
JAU6476-hydroxy-methoxy-desthio-glucuronide		

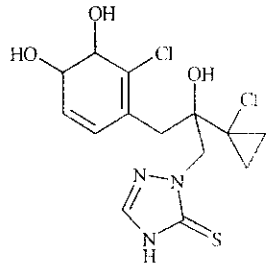
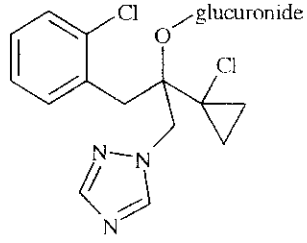
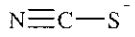
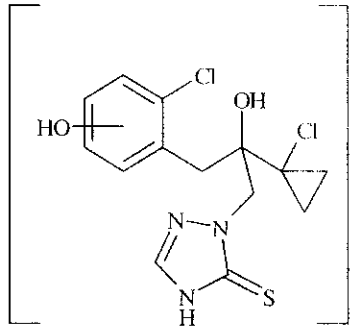
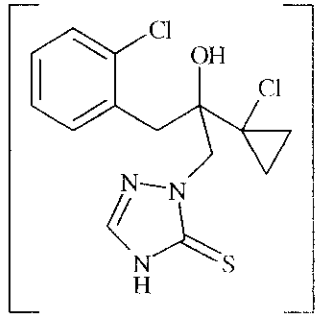
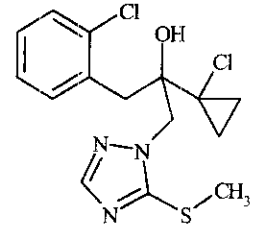
TABLE C.3.1. Identification of Compounds from Metabolism Study		
Common name/code FIGURE C.3.1 ID No.	Chemical name	Chemical structure
JAU6476-dihydroxy-diene	2-[2-(1-chlorocyclopropyl)-3-(2-chloro-3,4-dihydroxycyclohexa-1,5-dien-1-yl)-2-hydroxypropyl]-2,4-dihydro-3*H*-1,2,4-triazole-3-thione [1]	
JAU6476-desthio-glucuronide		
Thiocyanate	thiocyanate ion	
Sulfate conjugate of JAU6476-hydroxy		
JAU6476-lactoside		

TABLE C.3.1. Identification of Compounds from Metabolism Study		
Common name/code FIGURE C.3.1 ID No.	Chemical name	Chemical structure
JAU6476-*S*-methyl	α-(1-chlorocyclopropyl)-α-[(2-chlorophenyl)methyl]-3-(methylthio)-1*H*-1,2,4-triazole-1-ethanol	

[1] Chemical name generated using ACD chemical naming software.

D. CONCLUSION

One goat was dosed orally with either [phenyl-UL-^{14}C]-prothioconazole at 246 ppm in the diet, or [triazole-UL-^{14}C]-prothioconazole at 195 ppm in the diet for 3 consecutive days. The majority of the administered dose was eliminated in the urine and feces (~67% for the phenyl-label, and ~59% for the triazole-label). Only minor amounts of the administered dose were detected in milk and tissues (<1% of the TRRs for both radiolabels). In the phenyl-label study, prothioconazole was found to be a major residue in liver, kidney, muscle, and fat. JAU6476-*O*- or *S*-glucuronide and JAU6476-3-hydroxy-desthio were major metabolites in milk and tissues. JAU6476-desthio was a major metabolite in fat. JAU6476-4-hydroxy was a major metabolite in liver. In the triazole-label study, prothioconazole was found to be a major residue in liver, kidney, and fat. Thiocyanate was found to account for a major portion of radioactivity in milk, kidney, muscle, and fat. Lactose comprised ~11% of the TRRs in milk. JAU6476-*S*-glucuronide and JAU6476-3-hydroxy-desthio were major metabolites in kidney, muscle, and fat. JAU6476-desthio was also a major metabolite in fat.

Based on the results of the phenyl- and triazole-label studies, the applicant concluded that prothioconazole is metabolized in goats via several steps: conjugation of the triazolinethione moiety of the parent compound with glucuronic acid to form the *S*-glucuronide and N-glucuronide of the parent; elimination of sulfur to form the metabolite JAU6476-desthio; oxidative hydroxylation of the phenyl moiety in prothioconazole and JAU6476-desthio to monohydroxy, dihydroxy, and dihydroxy-diene compounds, partly followed by conjugation with glucuronic acid; conjugation of the triazolinethione moiety of the parent compound with lactose; conjugation of hydroxylated metabolites of prothioconazole with sulfate; methylation of the triazolinethione moiety of prothioconazole to form JAU6476-*S*-methyl; and cleavage of the parent compound to form thiocyanate.

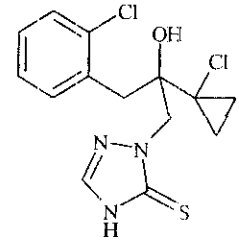
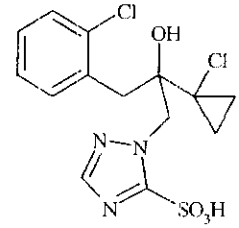
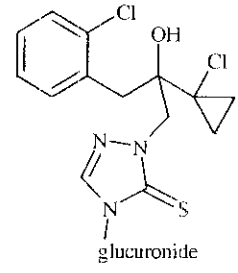
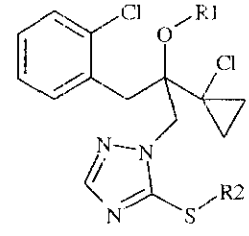
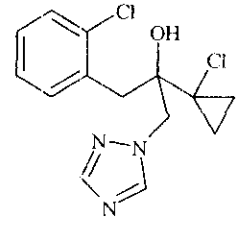
E. REFERENCES

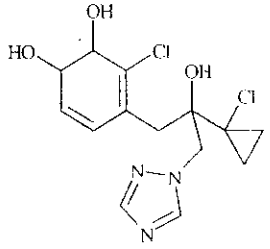
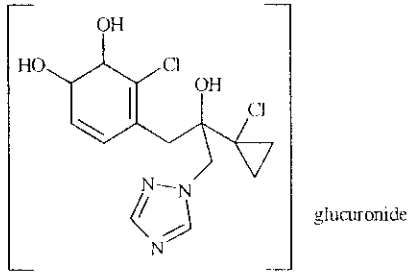
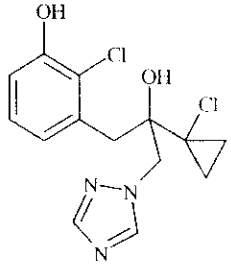
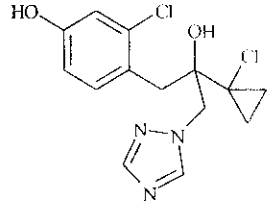
46246202 Weber, H.; Spiegel, K. (2001) [Phenyl-UL-(Carbon 14)]JAU6476: Absorption, Distribution, Excretion, and Metabolism in Laying Hens. Project Number: M81819090, MR-309/01. Unpublished study prepared by Bayer CropScience Ag Development. 142 p.

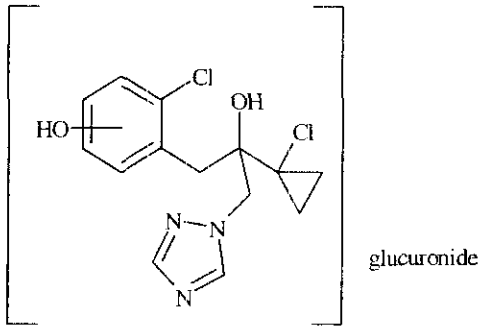
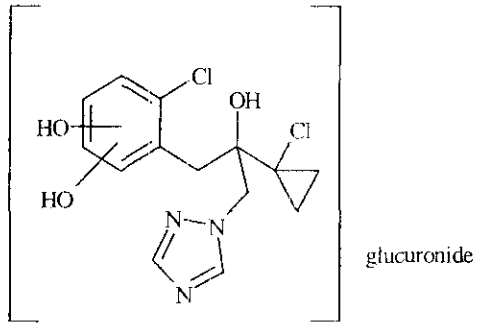
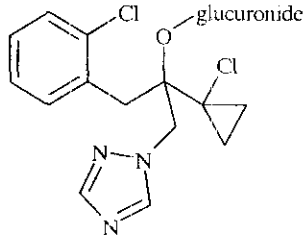
F. DOCUMENT TRACKING

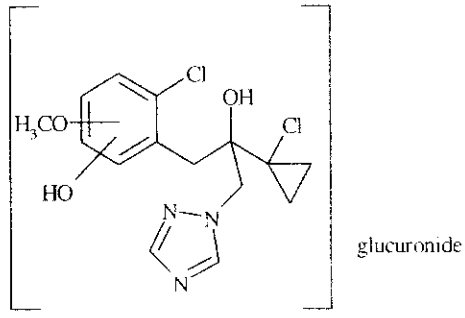
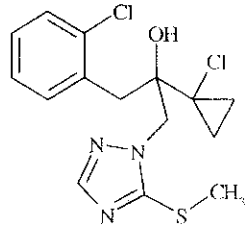
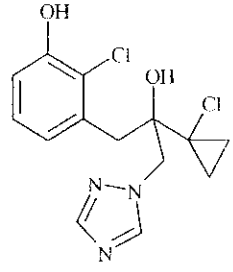
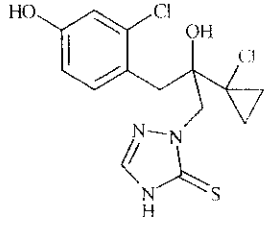
RDI: Louise Croteau, Suzan Mathew (PMRA) 23/01/2006; Henri Bietlot (PMRA) 24/01/2006; Stephen Funk (IO)13/03/2006; Leung Cheng (RAB3).
Petition Number: PP#4F6830
DP Barcode: D303508
PC Code: 113961

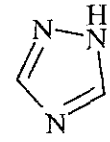
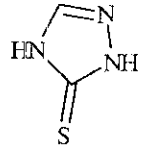
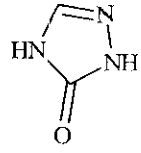
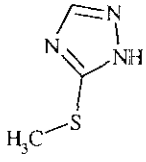
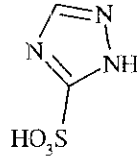
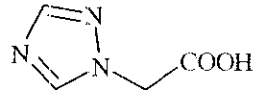
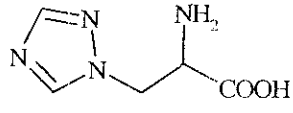
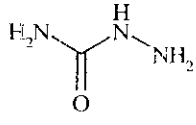
Template Version September 2003

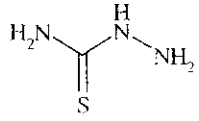
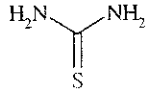
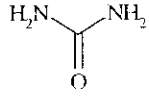
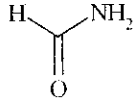
APPENDIX I. Chemical Names and Structures of Reference Standards Used in Goat Metabolism Study.		
Common name; Company code	Chemical name	Chemical structure
Prothioconazole: JAU6476 [1]	2-[2-(1-chlorocyclopropyl)-3-(2-chlorophenyl)-2-hydroxypropyl]-1,2-dihydro-3*H*-1,2,4-triazole-3-thione	
JAU6476-sulfonic acid [1] (also as potassium salt)	1-[2-(1-chlorocyclopropyl)-3-(2-chlorophenyl)-2-hydroxypropyl]-1*H*-1,2,4-triazole-5-sulfonic acid [2]	
JAU6476-*N*-glucuronide [3]		
JAU6476-glucuronide [3] (position of conjugation not determined definitely)	[JAU6476-*S*-glucuronide: R1 = H, R2 = glucuronic acid JAU6476-*O*-glucuronide: R1 = glucuronic acid, R2 = H]	
JAU6476-desthio [3] (unlabeled standard also used)	α-(1-chlorocyclopropyl)-α-[(2-chlorophenyl)methyl]-1*H*-1,2,4-triazole-1-ethanol	

APPENDIX I. Chemical Names and Structures of Reference Standards Used in Goat Metabolism Study.		
Common name; Company code	Chemical name	Chemical structure
JAU6476-desthio-3,4-dihydroxy-diene [4]	3-chloro-4-{2-(1-chlorocyclopropyl)-2-hydroxy-3-(1*H*-1,2,4-triazol-1-yl)propyl}cyclohexa-3,5-diene-1,2-diol [2]	
JAU6476-desthio-3,4-dihydroxy-dienyl-glucuronide [4]		 glucuronide
JAU6476-3-hydroxy-desthio [3] (unlabeled standard also used)	α-(1-chlorocyclopropyl)-α-[(2-chloro-3-hydroxyphenyl)methyl]-1*H*-1,2,4-triazole-1-ethanol	
JAU6476-4-hydroxy-desthio [3] (unlabeled standard also used)	α-(1-chlorocyclopropyl)-α-[(2-chloro-4-hydroxyphenyl)methyl]-1*H*-1,2,4-triazole-1-ethanol	

APPENDIX 1. Chemical Names and Structures of Reference Standards Used in Goat Metabolism Study.		
Common name; Company code	Chemical name	Chemical structure
JAU6476-hydroxy-desthio-glucuronide [4]		
JAU6476-dihydroxy-desthio-glucuronide [4]		
JAU6476-desthio-glucuronide [4]		

APPENDIX I. Chemical Names and Structures of Reference Standards Used in Goat Metabolism Study.		
Common name; Company code	Chemical name	Chemical structure
JAU6476-hydroxy-methoxy-desthio-glucuronide [4]		 glucuronide
JAU6476-*S*-methyl [3]	α-(1-chlorocyclopropyl)-α-[(2-chlorophenyl)methyl]-3-(methylthio)-1*H*-1,2,4-triazole-1-ethanol	
JAU6476-3-hydroxy-desthio	α-(1-chlorocyclopropyl)-α-[(2-chloro-3-hydroxyphenyl)methyl]-1*H*-1,2,4-triazole-1-ethanol	
JAU6476-4-hydroxy	2-[2-(1-chlorocyclopropyl)-3-(2-chloro-4-hydroxyphenyl)-2-hydroxypropyl]-1,2-dihydro-3*H*-1,2,4-triazole-3-thione	
Thiocyanate (potassium salt) [1]	thiocyanate ion	N≡C—S
Cyanide (potassium salt) [1]	cyanide ion	N≡C

APPENDIX I. Chemical Names and Structures of Reference Standards Used in Goat Metabolism Study.		
Common name; Company code	Chemical name	Chemical structure
Free Triazole [1]	1*H*-1,2,4-triazole [2]	
Triazolin-thione; triazole-*S*	2,4-dihydro-3*H*-1,2,4-triazole-3-thione [2]	
Oxo-triazole; triazolione	2,4-dihydro-3*H*-1,2,4-triazole-3-one [2]	
S-methyl triazole	5-(methylthio)-1*H*-1,2,4-triazole [2]	
Triazolyl sulfonic acid	1*H*-1,2,4-triazole-5-sulfonic acid [2]	
Triazolyl acetic acid	1*H*-1,2,4-triazol-1-ylacetic acid [2]	
Triazolyl alanine	2-amino-3-(1*H*-1,2,4-triazol-1-yl)propanoic acid [2]	
Semicarbazide	hydrazinecarboxamide [2]	

APPENDIX I. Chemical Names and Structures of Reference Standards Used in Goat Metabolism Study.		
Common name; Company code	Chemical name	Chemical structure
Thio-semicarbazide	hydrazinecarbothioamide [2]	
Thiourea	thiourea	
Urea	urea	
Formamide	formamide	

[1] Standard was radiolabeled.
[2] Chemical name generated using ACD chemical naming software.
[3] Isolated and identified in rat metabolism study (MRID 46246421).
[4] Isolated and identified in goat metabolism study with desthio metabolite (refer to the DER for MRID 46246201).
[5] Isolated and identified in spring wheat metabolism study (refer to the DER for MRID 46246141).

Primary Evaluator	Stephen Funk, Chemist, HED/IO	Date: 23/06/2006
Approved by	Leung Cheng, Ph.D., Team Leader, HED/RAB3	Date: 23/06/2006

In the absence of signatures, this document is considered to be a draft with deliberative material for internal use only.

This DER was originally prepared under contract by Dynamac Corporation (20440 Century Boulevard, Suite 100; Germantown, MD 20874; submitted 04/13/2005). The DER has been reviewed by the Health Effects Division (HED) and revised to reflect current Office of Pesticide Programs (OPP) policies.

STUDY REPORT:

46246201 Weber, H.; Weber, E.; Spiegel, K. (2002) ((Phenyl-UL-(Carbon 14))JAU6476-desthio: Absorption, Distribution, Excretion, and Metabolism in the Lactating Goat Including the Validation of the Residue Analytical Method for the Determination of JAU6476-desthio, JAU6476-3-hydroxy-desthio. Project Number: M91819091, SXX1, SXX2. Unpublished study prepared by Bayer Ag Institut fuer Ruckstands-Analytik. 399 p.

EXECUTIVE SUMMARY:

Bayer CropScience has submitted a study investigating the metabolism of [phenyl-UL-^{14}C]JAU6476-desthio (specific activity 0.397 MBq/mg) in a lactating goat; JAU6476-desthio is a metabolite of prothioconazole. The test substance was administered orally to a single goat at 195 ppm in the diet. The goat was dosed once per day for 3 consecutive days. Milk was collected twice daily throughout the study, and tissues (muscle, fat, liver, and kidney) were collected at sacrifice. The in-life and analytical phases of the study were conducted by Bayer CropScience (Monheim, Germany).

Total radioactive residues (TRR) were 0.074-0.314 ppm in milk, 18.422 ppm in liver, 18.986 ppm in kidney, 0.232-0.277 ppm in muscle, and 0.216-0.240 ppm in fat. Radioactivity was highest in liver and kidney and lowest in fat. Residues in milk were generally highest in samples collected 8 hours after dosing, and did not appear to have reached a plateau by the end of the dosing period. A large portion of the administered dose was excreted, with urine and feces

accounting for a total of ~74% of the administered dose. The majority of the radioactivity (~81-97%) was extracted using methanol (milk) or acetonitrile/water (tissues). Nonextractable residues accounted for ≤0.030 ppm in milk, muscle, and fat, <3% TRR in kidney, and 18.4% TRR (3.40 ppm) in liver. The petitioner normalized the extraction results; however, accountabilities prior to normalization were 89-101%. Residues were characterized primarily by HPLC analysis, using TLC analyses for confirmation and LC-MS/MS analyses for identification of metabolites. These methods successfully identified the predominant residues in goat matrices, except for liver. Although microwave extraction was conducted on the nonextractable residues of liver (different extraction sample), only an additional ~2% TRR was released. Adequate storage stability data were submitted demonstrating the stability of the metabolite profile in goat samples and extracts for the duration of the study.

Approximately 60-75% TRR were identified (or tentatively identified) in goat matrices. The test substance, JAU6476-desthio, was found to be a major residue in liver and fat, at 31.18% TRR (5.744 ppm) and 13.88% TRR (0.032 ppm), respectively. JAU6476-desthio was not found in milk and was found in kidney and muscle at <8% TRR. JAU6476-desthio-glucuronide was the major metabolite in kidney, at 24.07% TRR (4.567 ppm); this metabolite was also found in milk, muscle, and fat, at <7% TRR. Sulfate conjugates of JAU6476-dihydroxy-desthio, JAU6476-hydroxy-methoxy-desthio, and JAU6476-hydroxy-desthio together accounted for 44.03% TRR in milk; these conjugates were not detected in goat tissues. One diastereomer of JAU6476-desthio-3,4-dihydroxy-dienyl-glucuronide was a major residue in kidney, muscle, and fat, at 12.77-15.02% TRR (0.034-2.610 ppm), and was a minor residue in milk and liver (<4% TRR); a second diastereomer of this metabolite was found in all goat matrices, at <9% TRR. JAU6476-4-hydroxy-desthio was a major metabolite in fat, at 14.6% TRR (0.034 ppm); this metabolite was also found in liver, kidney, and muscle, at <9% TRR. Several additional metabolites were identified in goat matrices, each at <8% TRR: JAU6476-desthio-3,4-dihydroxy-diene in all matrices; glucuronides of JAU6476-dihydroxy-desthio, JAU6476-4,5-dihydroxy-desthio, JAU6476-3-hydroxy-desthio, JAU6476-4-hydroxy-desthio, and JAU6476-hydroxy-methoxy-desthio in all matrices; JAU6476-dihydroxy-desthio in milk, liver, muscle, and fat; JAU6476-4,5-dihydroxy-desthio in milk, liver, and muscle; and JAU6476-3-hydroxy-desthio in liver, kidney, and muscle. Unknown metabolites accounted for a significant portion of the radioactivity in liver and kidney (11.0-14.5% TRR); however, HPLC analyses indicated that individual unknowns were ≤5.1% TRR.

Based on the results of the study, the petitioner concluded that JAU6476-desthio is metabolized in goats via several steps: conjugation with glucuronic acid to form JAU6476-desthio-glucuronide; hydroxylation of JAU6476-desthio to form the isomers JAU6476-3-hydroxy-desthio and JAU6476-4-hydroxy-desthio, which was partly followed by conjugation with glucuronic acid; further hydroxylation of the chlorohydroxyphenyl moiety to form JAU6476-3,4- and JAU6476-4,5-dihydroxy-desthio, which was also followed partly by conjugation with glucuronic acid; and oxidation of the chlorophenyl moiety of the isomers of JAU6476-hydroxy-desthio to form JAU6476-desthio-dihydroxy-dienes, which was followed by conjugation with glucuronic acid to some extent. The observation of JAU6476-hydroxy-methoxy-desthio-

glucuronide indicated that JAU6476-hydroxy-methoxy-desthio may have formed as an intermediate.

STUDY/WAIVER ACCEPTABILITY/DEFICIENCIES/CLARIFICATIONS:

The acceptability of this study for regulatory purposes is addressed in the forthcoming U.S. EPA Residue Chemistry Summary Document, DP Barcode D303508.

COMPLIANCE:

Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided. No deviations from regulatory requirements were reported which would have an impact on the validity of the study.

A. BACKGROUND INFORMATION

Prothioconazole is a broad-spectrum systemic fungicide intended for the control of ascomycetes, basidiomycetes, and deuteromycetes fungi in barley, canola, the dried shelled and bean crop subgroup, the oilseed crop group, peanuts, rice, and wheat. Prothioconazole is a systemic demethylation inhibitor fungicide (Group 3 fungicide) of the triazolinthione chemical class. The chemical has been submitted for joint EPA and PMRA review by Bayer CropScience. The crops proposed for joint review include barley, the dried shelled and bean subgroup, the oilseed crop group, and wheat. Uses on peanuts and rice are being proposed for the U.S. only.

TABLE A.1. Test Compound Nomenclature.

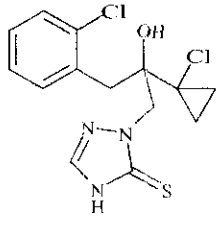
Chemical structure	
Common name	Prothioconazole
Company experimental name	JAU6476
IUPAC name	(*RS*)-2-[2-(1-chlorocyclopropyl)-3-(2-chlorophenyl)-2-hydroxypropyl]-2,4-dihydro-1,2,4-triazole-3-thione
CAS name	2-[2-(1-chlorocyclopropyl)-3-(2-chlorophenyl)-2-hydroxypropyl]-1,2-dihydro-3H-1,2,4-triazole-3-thione
CAS registry number	178928-70-6
PC Code	113961
End-use product (EP)	Proline® 480 SC (4 lb/gal suspension concentrate)

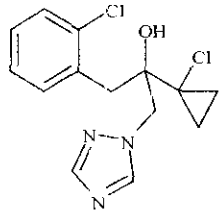
TABLE A.1. Test Compound Nomenclature.	
Chemical structure	
Common name	Prothioconazole-desthio
Company experimental name	JAU6476-desthio
IUPAC name	2-(1-chlorocyclopropyl)-1-(2-chlorophenyl)-3-(1H-1,2,4-triazol-1-yl)propan-2-ol
CAS name	α-(1-chlorocyclopropyl)-α-(2-chlorophenyl)methyl-1H-1,2,4-triazole-1-ethanol
CAS registry number	120983-64-4
PC Code	613962
End-use product (EP)	Not applicable

TABLE A.2. Physicochemical Properties of the Technical Grade Test Compound.		
Parameter	Value	Reference
Prothioconazole		
Melting point/range	139.1 to 144.5° C	MRID 46246003
pH	5.8 (1% solution)	MRID 46246003
Density	1.36 g/mL at 20° C	MRID 46246003
Water solubility	mg/L (20° C) pH 4 5 pH 8 300 pH 9 2000	MRID 46246003
Solvent solubility	g/L at 20° C Acetone >250 Acetonitrile 69 *Dichloromethane* 88 Dimethylsulfoxide 126 Ethyl acetate >250 n-Heptane <0.1 *1-Octanol* 58 Polyethylene glycol >250 2-Propanol 87 Xylene 8	MRID 46246003
Vapor pressure	<<4 x 10^{-7} Pa at 20 or 25 EC (calculated from determinations at 70° C)	MRID 46246003
Dissociation constant, pK_a	6.9 (calculated from K_{OW})	MRID 46246003
Octanol/water partition coefficient, Log(K_{OW})	20° C unbuffered water 4.05 pH 4 4.16 pH 7 3.82 pH 9 2.00	MRID 46246003

TABLE A.2. Physicochemical Properties of the Technical Grade Test Compound.		
Parameter	Value	Reference
UV/visible absorption spectrum	Peak maxima at 257 nm	MRID 46246003
Prothioconazole-desthio		
Melting point	108.5° C	MRID 46477704
pH	Not provided	
Density	Not provided	
Water solubility	g/L (20° C) unbuffered, pH 5.5 0.051	MRID 46477704
Solvent solubility	g/L at 20° C Hexane 3.0 Toluene 84.0 Dichloromethane >200.0 2-Propanol 35.0 Acetone 100.0 Acetonitrile 43.0	MRID 46477704
Vapor pressure	2.7×10^{-7} Pa at 20° C	MRID 46477704
Dissociation constant, pK_a	Not provided	
Octanol/water partition coefficient, $Log(K_{OW})$	Not provided	
UV/visible absorption spectrum	Not provided	

B. EXPERIMENTAL DESIGN

B.1. Livestock

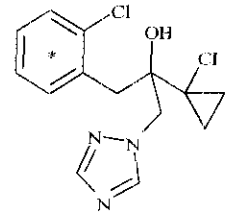
TABLE B.1.1. General Test Animal Information.					
Species	Breed	Age	Weight at study initiation (kg)	Health Status	Description of housing/holding area
Lactating goat	"Bunte Deutsche Edelziege"	~30 months	39	No observable toxicological signs	Stainless steel metabolism cage in air-conditioned room (20 ± 1° C) with 18 hours of illumination

TABLE B.1.2. Test Animal Dietary Regime.				
Composition of Diet	Feed consumption (g/day)	Water	Acclimation period	Predosing
Ruminant feed, apples, and hay	~2000 g feed + apples offered per day; hay offered *ad libitum*; actual feed consumption was not reported	Tap water, *ad libitum*	7 days	None

TABLE B.1.3. Test Animal Dosing Regime			
Treatment Type	Feeding Level (ppm test material in food)	Vehicle	Timing/Duration
Oral	195 [1]	Oral intubation of test substance in 0.5% aqueous tragacanth suspension	Once per day after morning milking for three consecutive days

[1] Based on an experimentally determined daily feed consumption of 5.1% of body weight.

B.2. Test Materials

TABLE B.2.1. Test Material Characteristics.		
Chemical structure		
Radiolabel position	[phenyl-UL-^{14}C]JAU6476-desthio	
Lot No.	12087/1	
Purity	>99% radiochemical and chemical purity	
Specific activity [1]	**Before radiodilution:**	5.96 MBq/mg (3.576 x 10^8 dpm/mg; 161 :Ci/mg; 1.86 x 10^6 MBq/mole; 1.116 x 10^{14} dpm/mole; 50.26 Ci/mole)
	After radiodilution:	0.397 MBq/mg (2.384 x 10^7 dpm/mg; 10.73 :Ci/mg; 1.24 x 10^5 MBq/mole; 7.443 x 10^{12} dpm/mole; 3.35 Ci/mole)

[1] Bq = disintegrations per second

B.3. Sampling Information

TABLE B.3.1. Sample Collection Information			
Milk collected	Urine and feces collected	Interval from last dose to sacrifice	Tissues harvested and analyzed
Milk was collected twice daily, immediately prior to dosing and then approximately 8 hours later. The final sample was collected directly before sacrifice. The amounts of milk collected were not reported.	Urine and feces were collected as quantitatively as possible in 24- hour intervals.	5 hours	Liver, kidney, muscle (round, flank, loin), and fat (perirenal, omental, subcutaneous)

B.4. Identification/Characterization of Residues

B.4.1. Sample Handling and Preparation

Milk was collected twice daily. An aliquot of each sample was radioassayed. All milk samples

collected in the morning during the dosing period were combined, and all samples collected during the evening milking, including the sample collected before sacrifice, were combined. One portion of each milk composite sample was immediately subjected to extraction procedures; the remainder was stored frozen (~ -18° C) until extraction and analysis. Tissue samples were minced or disintegrated (fat) after collection and then stored frozen (~-18° C) until extraction and analysis. Portions of round, loin, and flank muscle were combined to make a composite sample, and portions of perirenal, subcutaneous, and omental fat were similarly combined.

Milk: Both the morning and evening milk composite samples were subjected to extraction procedures. A subsample of milk was extracted three times with methanol, and the extracts were combined and concentrated. The extract was diluted with methanol and partitioned with hexane. The methanol phase was evaporated to dryness, redissolved in buffer solution (pH 3), and cleaned up on an XAD 7 column. The effluent from the column was collected, the column was rinsed with water, and the retained radioactive compounds were eluted with methanol. The methanol eluate was concentrated and redissolved in methanol/water for HPLC analyses.

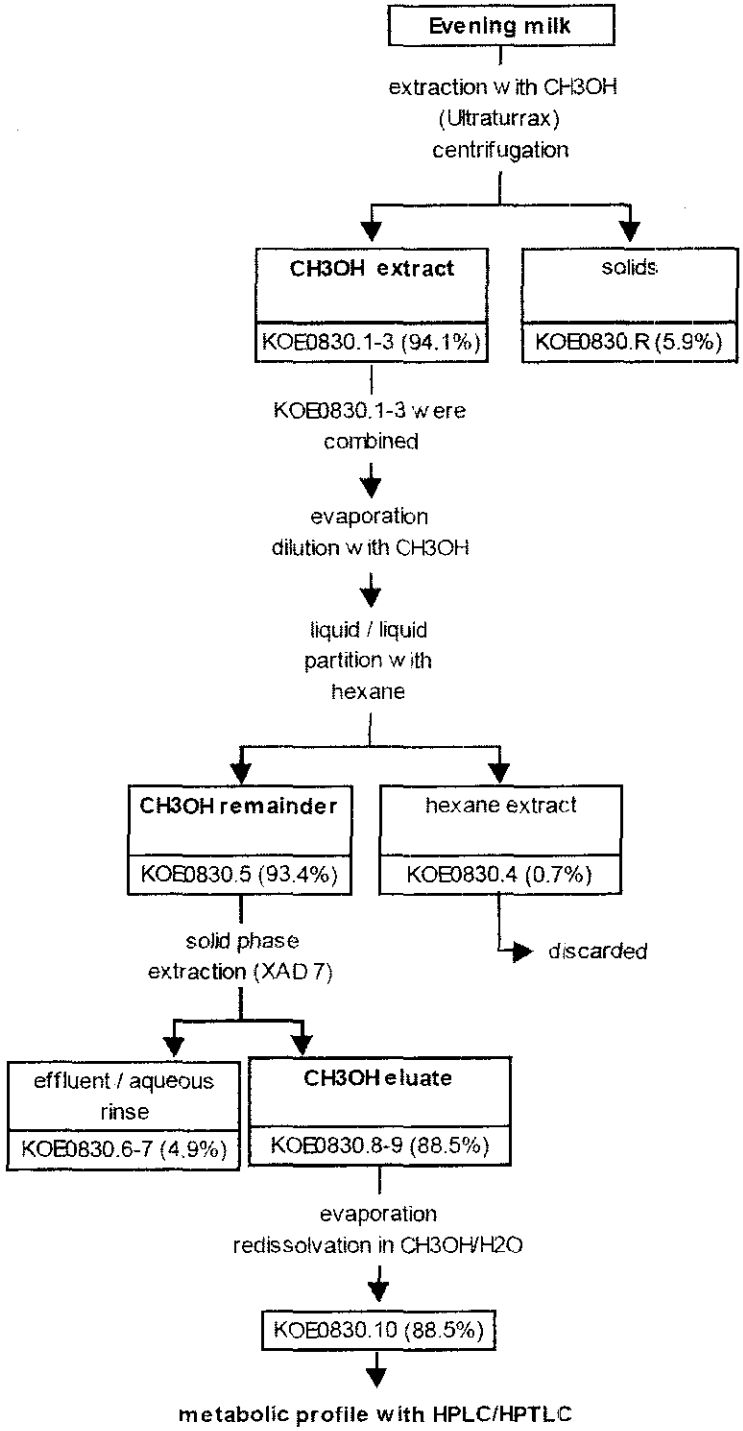
Liver, kidney, muscle, and fat: Subsamples of liver, kidney, muscle, and fat were extracted three times with acetonitrile (ACN):water (80:20, v:v) and then once (fat sample) or twice (liver, kidney, and muscle samples) with ACN:water (50:50, v:v). For liver and muscle, the first four extracts were combined, and the fifth extract was discarded due to low radioactivity. For kidney, the first three extracts were combined, and the fourth and fifth extracts were discarded. For fat, the four extracts were combined. The combined extracts were concentrated, diluted with methanol, and partitioned with n-hexane. The methanol phase was concentrated, diluted with buffer solution (pH 3), and then cleaned up on an XAD 7 column, which was rinsed with buffer solution and water and then eluted with methanol. The methanol eluate was evaporated to dryness and redissolved in methanol for HPLC analysis.

The extraction of liver was repeated, and the solids remaining after extraction were subjected to microwave extraction (using ACN:water, 80/50, v/v); all extracts were combined and reserved for HPLC analysis.

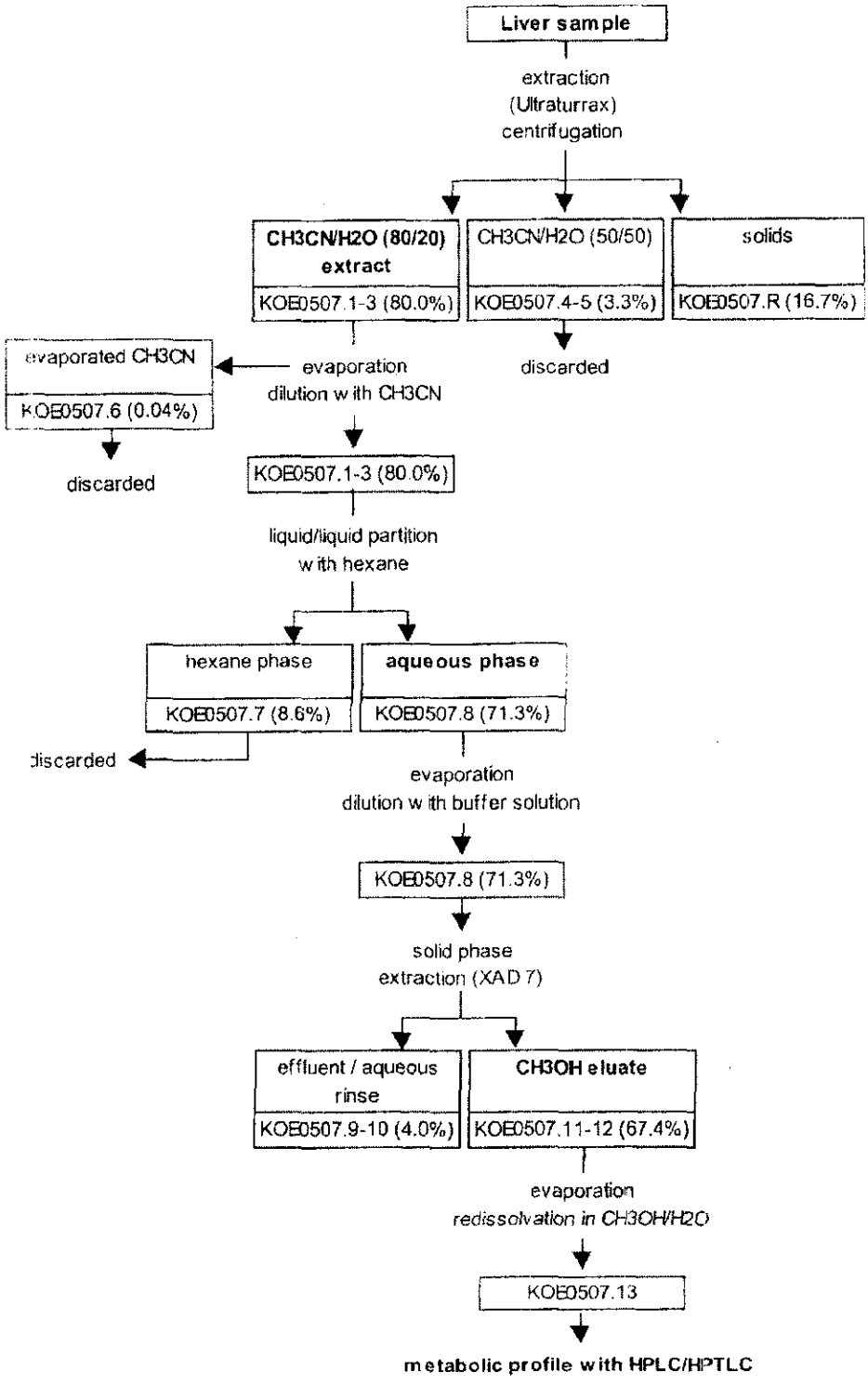
To confirm metabolite identification, the petitioner subjected the extracts of goat matrices to treatment with acid (5 N HCl, 100° C, 4 h) and compared the HPLC profile of the resulting hydrolysate with the profile prior to hydrolysis.

The extraction procedures for milk and tissue samples are summarized in the flow charts below, which were copied without alteration from MRID 46246201. We note that flow charts for kidney, muscle and fat were not included; the liver flow chart is representative of the extraction procedures used for liver, kidney, muscle, and fat.

Extraction procedures for milk:



Extraction procedures for liver (also used for kidney, muscle, and fat):



B.4.2. Analytical Methodology

Total radioactive residues (TRR) were measured in milk samples by LSC (in duplicate). Tissue samples were freeze dried, and TRR were determined by combustion/LSC (in triplicate). The LOQs for TRR determination were 0.001 ppm for milk, 0.002 ppm for liver, 0.001 ppm for kidney, 0.001-0.002 ppm for muscle, and 0.003-0.006 ppm for fat. To determine background radioactivity levels for LOQ calculations, the petitioner used samples of milk collected during the acclimation period and samples of tissue collected from a goat from a different metabolism study.

Extracts and hydrolysates were analyzed by HPLC using a system equipped with a UV detector (230 nm), a radiodetector, a fraction collector, and one of the following column/mobile phase combinations: (1) a C18 column and a gradient mobile phase of water and ACN, each containing acetic acid at 1%; (2) a Diol column and a gradient mobile phase of hexane and ethanol containing ammonium hydroxide; or (3) a reverse-phase column with a gradient mobile phase of pH 7 buffer, ACN, and 0.005 M ion-pairing reagent tetrabutylammonium-hydrogensulfate. The second system was used to isolate metabolites from urine for further identification. Metabolites were identified by comparison of retention times or cochromatography with reference standards; the chemical names and structures of the reference standards used in this study are presented in Appendix I.

The extracts used for HPLC profiling were fractionated and subjected to HPTLC analyses. TLC analyses were conducted on silica 60 F_{254} plates using automated multiple development (AMD) with methanol and dichloromethane. Non-labeled standards were visualized under a UV lamp. Radioactive zones were detected using radioluminography. Metabolites were identified by cochromatography.

LC-MS or LC-MS/MS analyses were used for metabolite confirmation or for identification of metabolites which could not be identified by HPLC or HPTLC. Analyses were conducted using a reverse phase column, a gradient mobile phase of water and ACN, each containing 0.1% acetic acid, or 1% acetic acid and ACN, and MS or MS/MS detection with electrospray ionization. ^{1}H NMR analyses were also used for structure elucidation. To identify metabolites in this study, the petitioner isolated the majority of metabolites found in urine (in sample collected 24 hours after the first dose), purified the isolated compounds, and then used LC-MS/MS and ^{1}H NMR analyses for structure elucidation. In addition, the isolated metabolites were subjected to enzymatic cleavage (using a mixture of β-glucuronidase and arylsulfatase) or acid hydrolysis (5 N HCl, 100° C, 4 h), and the resulting products were analyzed by LC-MS/MS and NMR. In the case of glucuronic acid and sulfate conjugates and hydroxylated metabolites, the position of conjugation or hydroxylation could not always be unambiguously assigned using LC-MS/MS and NMR. The following metabolites were isolated from urine in the current study, identified by LC-MS/MS and NMR, and then used as reference standards for metabolite identification in milk and tissues: two diastereomers of JAU6476-desthio-3,4-dihydroxy-dienyl-glucuronide; two diastereomers of JAU6476-desthio-3,4-dihydroxy-diene (identified as cleavage products of the

corresponding glucuronides); JAU6476-desthio-4,5-dihydroxy-dienyl-glucuronide; JAU6476-dihydroxy-desthio-glucuronide (two diastereomers, likely to be 3,4-dihydroxy); JAU6476-4,5-dihydroxy-desthio-glucuronide; JAU6476-3-hydroxy-desthio-glucuronide; JAU6476-4-hydroxy-desthio-glucuronide; JAU6476-hydroxy-methoxy-desthio-glucuronide; an isomer of JAU6476-hydroxy-desthio-glucuronide; JAU6476-desthio-glucuronide; JAU6476-4,5-dihydroxy-desthio (identified after acid hydrolysis of glucuronide); JAU6476-3-hydroxy-desthio (identified after acid hydrolysis of glucuronide); and JAU6476-4-hydroxy-desthio (identified after acid hydrolysis of glucuronide).

C. RESULTS AND DISCUSSION

The storage intervals and conditions for the goat metabolism study are presented in Table C.1. The petitioner did not provide any dates of sample extraction or analysis, but stated the approximate intervals between sample collection (sacrifice of the goat) and analysis. Analysis of the methanol (organic) extracts was completed within 3 months of sample collection for all matrices except milk; analysis of the evening milk extract was completed within 6 months of sample collection. Additional extractions and analyses of milk and muscle samples were conducted 17-19 months after sample collection to allow for metabolite identification; comparison of the HPLC or HPTLC profiles of the organic extracts with those of the initial extracts indicated that the metabolite profile was stable during storage. The submitted storage stability information and data are adequate to support this goat metabolism study.

Total radioactive residues (TRR) in goat milk and tissues are reported in Table C.2.1. TRR were 0.074-0.314 ppm in milk, 18.422 ppm in liver, 18.986 ppm in kidney, 0.232-0.277 ppm in muscle, and 0.216-0.240 ppm in fat from a goat dosed orally with [phenyl-UL-^{14}C]JAU6476-desthio, a metabolite of prothioconazole, at 195 ppm in the diet for 3 consecutive days. Radioactivity was highest in liver and kidney and lowest in fat. Residues in milk were generally highest in samples collected 8 hours after dosing, and did not appear to have reached a plateau by the end of the dosing period; a graph of the residue levels in milk over the course of the study is presented in Figure C.2.1. A large portion of the administered dose was excreted, with urine and feces accounting for a total of ~74% of the administered dose.

The distribution of the radioactivity in goat matrices is presented in Table C.2.2. The majority of the radioactivity (~81-97%) was extracted using methanol (milk) or ACN/water (tissues). Nonextractable residues accounted for ≤0.030 ppm in milk, muscle, and fat, <3% TRR in kidney, and 18.45% TRR (3.398 ppm) in liver. The petitioner normalized the extraction results; however, accountabilities prior to normalization were 89-101%. Residues were characterized primarily by HPLC analysis, using TLC analyses for confirmation and LC-MS/MS analyses for identification of metabolites. These methods successfully identified the predominant residues in goat matrices. The characterization and identification of residues in goat matrices is summarized in Table C.2.3. Approximately 60-75% TRR were identified (or tentatively identified) in goat matrices. The test substance, JAU6476-desthio, was found to be a major residue in liver and fat, at 31.18% TRR (5.744 ppm) and 13.88% TRR (0.032 ppm), respectively. JAU6476-desthio was not found in milk and was found in kidney and muscle at <8% TRR. JAU6476-desthio-

glucuronide was the major metabolite in kidney, at 24.07% TRR (4.567 ppm); this metabolite was also found in milk, muscle, and fat, at <7% TRR. Sulfate conjugates of JAU6476-dihydroxy-desthio, JAU6476-hydroxy-methoxy-desthio, and JAU6476-hydroxy-desthio together accounted for 44.03% TRR in milk; these conjugates were not detected in goat tissues. One diastereomer of JAU6476-desthio-3,4-dihydroxy-dienyl-glucuronide was a major residue in kidney, muscle, and fat, at 12.77-15.02% TRR (0.034-2.610 ppm), and was a minor residue in milk and liver (<4% TRR); a second diastereomer of this metabolite was found in all goat matrices, at <9% TRR. JAU6476-4-hydroxy-desthio was a major metabolite in fat, at 14.6% TRR (0.034 ppm); this metabolite was also found in liver, kidney, and muscle, at <9% TRR. Several additional metabolites were identified in goat matrices, each at <8% TRR: JAU6476-desthio-3,4-dihydroxy-diene in all matrices; glucuronides of JAU6476-dihydroxy-desthio, JAU6476-4,5-dihydroxy-desthio, JAU6476-3-hydroxy-desthio, JAU6476-4-hydroxy-desthio, and JAU6476-hydroxy-methoxy-desthio in all matrices; JAU6476-dihydroxy-desthio in milk, liver, muscle, and fat; JAU6476-4,5-dihydroxy-desthio in milk, liver, and muscle; and JAU6476-3-hydroxy-desthio in liver, kidney, and muscle. Unknown metabolites accounted for a significant portion of the radioactivity in liver and kidney (11.0-14.5% TRR); however, HPLC analyses indicated that individual unknowns were ≤5.1% TRR.

HPTLC analyses were used to confirm the following metabolite identifications: JAU6476-desthio in liver; two diastereomers of JAU6476-desthio-3,4-dihydroxy-dienyl-glucuronide in all matrices (with the exception of liver, in which the presence of only one diastereomer was confirmed); two diastereomers of JAU6476-desthio-3,4-dihydroxy-diene in muscle; JAU6476-desthio-glucuronide in milk, kidney, and muscle; and JAU6476-3-hydroxy-desthio and JAU6476-4-hydroxy-desthio in goat tissues (these compounds co-eluted under the conditions used for HPTLC analyses).

In milk, the sulfate conjugates were identified by LC-MS/MS analyses after isolation of the metabolite fraction. LC-MS/MS analyses indicated the presence of sulfate conjugates of JAU6476-hydroxy-desthio, JAU6476-dihydroxy-desthio, and JAU6476-hydroxy-methoxy-desthio.

The petitioner subjected the evening milk extract to treatment with boiling HCl. The resulting HPLC profile indicated the presence of JAU6476-3-hydroxy-desthio (which may have resulted from JAU6476-3-hydroxy-desthio-glucuronide, JAU6476-3-hydroxy-desthio-sulfate, and/or metabolites with the 3,4-dihydroxy-diene moiety), JAU6476-4-hydroxy-desthio (may have formed from the corresponding sulfate and glucuronide conjugates), and JAU6476-dihydroxy-desthio (resulting from the corresponding glucuronide and sulfate conjugates). Similar results were observed when the major extracts of liver, kidney, muscle, and fat were subjected to acid hydrolysis. The petitioner noted that acid treatment of the extract converted most metabolites to a total of five metabolites (marker compounds; JAU6476-desthio, JAU6476-dihydroxy-desthio, JAU6476-4,5-dihydroxy-desthio, JAU6476-3-hydroxy-desthio, and JAU6476-4-hydroxy-desthio).

A comparison of the HPLC profiles of the extracts of morning and evening milk samples indicated that the metabolite profile of morning milk was similar to that of evening milk.

The nonextractable residues of liver accounted for approximately 18% TRR. The extraction of liver was repeated, and the solids remaining after extraction were subjected to microwave extraction (using ACN:water, 80/50, v/v), which released 2.1% TRR (0.393 ppm), leaving 15.6% TRR (2.86 ppm) as nonextractable. The petitioner combined all extracts, including the microwave extract, and compared the HPTLC profile of the combined extracts with that of the initial extract. The HPTLC analyses indicated that the profiles were similar in the two extracts. No additional attempts to further release radioactivity from the nonextractable residues of liver were made. The petitioner surmised that the residues that were not extracted would be the same metabolites as found in the solvent extracts.

C.1. Storage Stability

The petitioner did not provide any dates of sample extraction or analysis. It was stated that initial extraction and analysis (HPLC and HPTLC) of the major extracts of goat matrices was completed within 3 months of sample collection (sacrifice of the goat), with the exception of milk. The metabolite profiles used for identification and quantification were based on these initial analyses. The HPLC analysis used for metabolite profiling in milk was completed within approximately 6 months of sample collection.

For evening milk, three extractions were conducted, at approximately 3, 6, and 17 months following sample collection. A comparison of the HPLC profiles of the three extracts indicated that the profiles were not identical; however, the differences in the profiles were likely due to different matrix loads. The petitioner demonstrated that if the milk metabolites were split into three metabolite groups, the TRR values corresponding to each of the groups were similar in all three extracts. In addition, acid hydrolyses of the extracts from the second and third extractions were found to yield the same hydrolysis products.

For muscle, a second extraction was conducted approximately 19 months after sample collection, for the purposes of conducting acid hydrolysis of the extract. The HPLC profile of the extract of the stored sample was found to be similar to that of the initial muscle extract, indicating that the profile was stable in muscle for approximately 19 months. For liver, a second extraction was conducted within approximately 3 months following sample collection, for the purposes of further HPTLC confirmation of metabolites. The HPTLC profile of the second extract of the stored sample was similar to that of the initial extract.

The petitioner should note for future submissions that the dates of sample extraction and analyses should be provided for all samples.

TABLE C.1. Summary of Storage Conditions			
Matrix	Storage Temp. (° C)	Actual Storage Duration	Interval of Demonstrated Storage Stability
Milk	~-18	~3, 6, 17 months	~17 months
Liver		~3 months	~3 months
Kidney		~3 months	None provided
Muscle		~3, 19 months	~19 months
Fat		~3 months	None provided

C.2. Identification, Characterization, and Distribution of Residues

TABLE C.2.1. Total Radioactive Residues (TRR) in Milk, Tissue and Excreta			
Matrix	Collection Timing (hours after first dose)	Radioactivity	
		ppm	% of administered dose
Urine	24	--	21.01
	48	--	22.99
	53	--	9.13
	Total	--	53.13
Feces	24	--	8.15
	48	--	11.40
	53	--	1.12
	Total	--	20.67
Milk	8	0.270	0.012
	24	0.074	0.006
	32	0.282	0.011
	48	0.084	0.007
	53	0.314	0.010
Milk (morning composite sample)	Over duration of study	0.079	--
Milk (evening composite sample	Over duration of study	0.286	--
Liver	At sacrifice	18.4	1.326
Kidney	At sacrifice	19.0	0.180
Round muscle	At sacrifice	0.277	--
Flank muscle	At sacrifice	0.232	--
Loin muscle	At sacrifice	0.232	--

TABLE C.2.1. Total Radioactive Residues (TRR) in Milk, Tissue and Excreta			
Matrix	Collection Timing (hours after first dose)	Radioactivity	
		ppm	% of administered dose
Body muscle (composite sample)	At sacrifice	0.247 [1] (0.266)	0.262 [2]
Perirenal fat	At sacrifice	0.216	--
Subcutaneous fat	At sacrifice	0.233	--
Omental fat	At sacrifice	0.240	--
Body fat (composite sample)	At sacrifice	0.230 [1]	0.097 [2]
Total % of Administered Dose	--	--	75.72

[1] Average of the three different types of tissue. Value in parentheses is the reported TRR for the composite sample used for extraction/analysis.
[2] Calculated using goat body weight and assuming muscle and fat account for 30% and 12%, respectively, of body weight.

FIGURE C.2.1. Pharmacokinetics of Prothioconazole Metabolite JAU6476-Desthio in Milk of Lactating Goat.

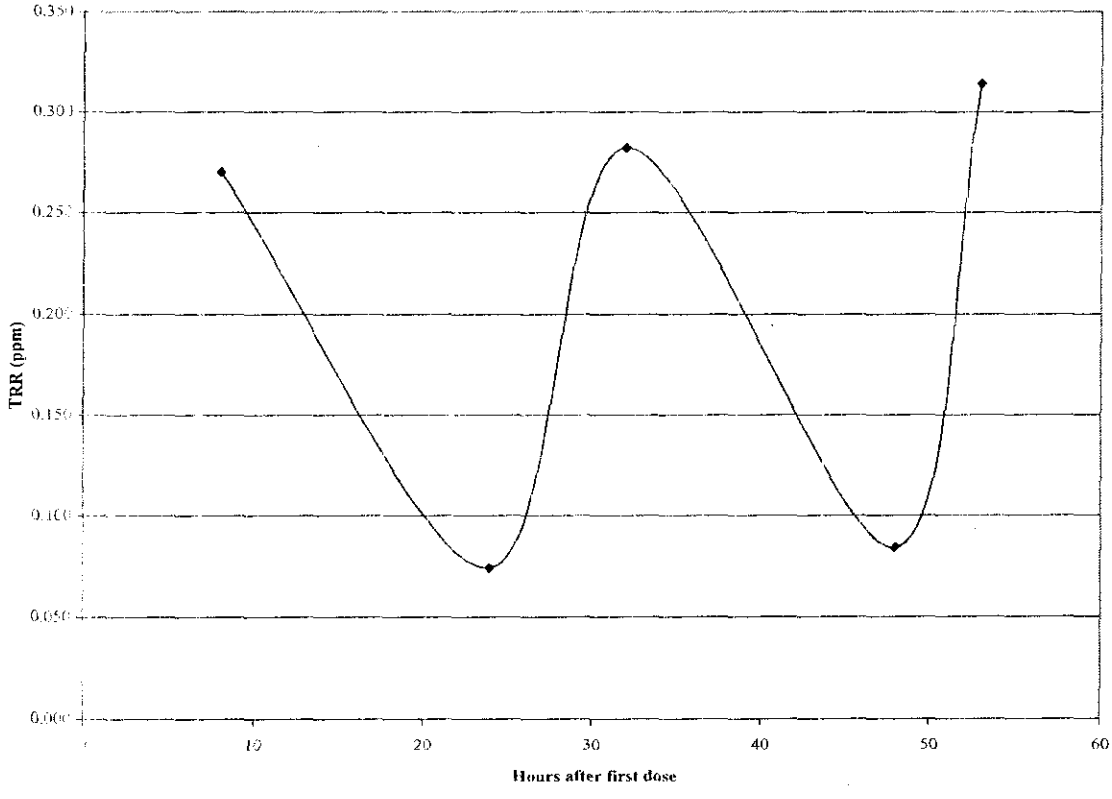


TABLE C.2.2. Distribution of the Parent and the Metabolites in Goat Matrices Following Dosing with *Prothioconazole Metabolite* [phenyl-UL-^{14}C]JAU6476-Desthio at 195 ppm in the Diet. [1]

Metabolite Fraction	Milk (evening)		Liver		Kidney		Muscle		Fat	
	TRR=0.286 ppm		TRR=18.421 ppm		TRR=18.975 ppm		TRR=0.266 ppm		TRR=0.231 ppm	
	%TRR	ppm	%TRR	ppm	%TRR	ppm	%TRR	ppm	%TRR	ppm
Methanol extract	94.12	0.269								
ACN/water extract			81.00	14.921	96.59	18.328	87.92	0.234	88.55	0.205
-ACN (evaporated)	--	--	0.04	0.007	0.03	0.005	0.39	0.001	0.0	0.00
-Hexane phase	0.69	0.002	3.99	0.736	1.51	0.286	2.41	0.006	3.11	0.007
-Aqueous phase	93.43	0.267	76.97	14.179	95.06	18.037	85.12	0.227	85.44	0.198
--Methanol XAD eluate	88.51	0.253	70.88	13.057	86.38	16.392	77.01	0.205	84.14	0.195
JAU6476-desthio	--	--	31.18	5.744	7.66	1.454	1.76	0.005	13.88	0.032
JAU6476-desthio-3,4-dihydroxy-dienyl-glucuronide (D1)	2.35	0.007	1.98	0.366	7.28	1.382	8.17	0.022	7.83	0.018
JAU6476-desthio-3,4-dihydroxy-dienyl-glucuronide (D2)	3.01	0.009	3.81	0.703	13.75	2.610	12.77	0.034	15.02	0.035
JAU6476-desthio-3,4-dihydroxy-diene (D1)	2.44	0.007	1.16 [2,3]	0.213	1.62 [2]	0.307	3.64	0.010	4.31 [2]	0.010
JAU6476-desthio-3,4-dihydroxy-diene (D2)	3.04 [2]	0.009					7.11	0.019		
JAU6476-3-hydroxy-desthio-glucuronide	2.63 [2]	0.008	2.74 [2]	0.504	4.92 [2]	0.933	--	--	5.30 [2]	0.012
JAU6476-dihydroxy-desthio-glucuronide [4]							5.88 [2]	0.016		
JAU6476-4,5-dihydroxy-desthio-glucuronide							--	--		
JAU6476-desthio-glucuronide	6.22	0.018	--	--	24.07	4.567	3.57 [5]	0.009	4.17	0.010
JAU6476-hydroxy-methoxy-desthio-glucuronide	5.11 [2]	0.015	2.77 [2]	0.511	7.32 [2]	1.388	5.20 [2]	0.014		
JAU6476-4-hydroxy-desthio-glucuronide							5.84 [2]	0.016	4.68 [2]	0.011
JAU6476-dihydroxy-desthio [4]	1.56 [2]	0.004	2.15 [6]	0.396	--	--	1.72	0.005	5.36	0.012
JAU6476-4,5-dihydroxy-desthio	1.38	0.004	4.76 [7]	0.878	--	--	2.80	0.007	--	--
JAU6476-3-hydroxy-desthio	--	--	0.96	0.178	1.22	0.231	4.80	0.013	--	--
JAU6476-4-hydroxy-desthio	--	--	8.37	1.542	4.06	0.770	3.03	0.008	14.55 [8]	0.034
Sulfate conjugates [9]	44.03	0.126	--	--	--	--	--	--	--	--

TABLE C.2.2. Distribution of the Parent and the Metabolites in Goat Matrices Following Dosing with Prothioconazole Metabolite [phenyl-UL-^{14}C]JAU6476-Desthio at 195 ppm in the Diet. [1]

Metabolite Fraction	Milk (evening)		Liver		Kidney		Muscle		Fat	
	TRR=0.286 ppm		TRR=18.421 ppm		TRR=18.975 ppm		TRR=0.266 ppm		TRR=0.231 ppm	
	%TRR	ppm	%TRR	ppm	%TRR	ppm	%TRR	ppm	%TRR	ppm
Unknowns	16.74 [10]	0.048	10.99 [11]	2.024	14.50 [12]	2.750	10.73 [13]	0.029	9.04 [14]	0.021
---Acid hydrolysate of methanol XAD eluate	(88.51)	(0.253)	(70.88)	(13.057)	(86.38)	(16.392)	(77.01)	(0.205)	(84.14)	(0.195)
JAU6476-desthio	–	-	36.12	6.653	18.95	3.595	2.90	0.008	12.27	0.028
JAU6476-desthio-glucuronide	5.07	0.015	--	--	14.23	2.700	1.51	0.004	2.25	0.005
JAU6476-dihydroxy-desthio [4]	31.14	0.089	5.34	0.983	4.22	0.802	8.55	0.023	6.83	0.016
JAU6476-4,5-dihydroxy-desthio	17.72	0.051	2.12	0.390	2.34	0.444	2.36	0.006	2.30	0.005
JAU6476-3-hydroxy-desthio	10.99	0.031	4.61	0.850	22.68	4.303	35.13	0.093	28.90	0.067
JAU6476-4-hydroxy-desthio	19.72	0.056	10.23	1.884	17.05	3.236	18.50	0.049	21.12	0.049
Unknowns	3.87	0.011	12.47	2.296	6.91	1.311	8.07	0.021	10.47	0.024
--XAD effluent/rinse	4.92	0.014	6.09	1.121	8.67	1.645	8.11	0.021	1.29	0.003
ACN/water (50:50)	--	--	0.55	0.101	0.62	0.116	0.92	0.002	--	--
Nonextractable	5.88	0.017	18.45	3.398	2.80	0.531	11.16	0.030	11.45	0.026

[1] Dashes indicates that the extraction step and/or characterization analysis was not conducted for the matrix in question. D: *diastereomer*; I: *isomer*.
[2] Tentative identification. The petitioner considered a metabolite to be tentatively identified when it was identified by retention time comparison only.
[3] May have co-eluted with JAU6476-4,5-dihydroxy-dienyl-glucuronide.
[4] NMR spectroscopy indicated that hydroxy groups may be in 3,4-, 3,6-, and/or 5,6-position.
[5] May have co-eluted with JAU6476-dihydroxy-desthio-glucuronide and/or JAU6476-3-hydroxy-desthio-glucuronide.
[6] May have co-eluted with JAU6476-alpha-hydroxy-desthio.
[7] May have co-eluted with JAU6476-desthio-glucuronide.
[8] Contained trace amounts of JAU6476-3-hydroxy-desthio.
[9] Sulfate conjugates of JAU6476-dihydroxy-desthio, JAU6476-hydroxy-methoxy-desthio, and JAU6476-hydroxy-desthio.
[10] A total of 5 peaks, each #7.69% TRR (#0.022 ppm).
[11] A total of 9 peaks, each #1.63% TRR (#0.300 ppm).
[12] A total of 6 peaks, each #5.13% TRR (#0.974 ppm).
[13] A total of 4 peaks, each #4.18% TRR (#0.011 ppm).
[14] Two peaks, 4.12% and 4.91% TRR (0.010 and 0.011 ppm).

TABLE C.2.3. Summary of Characterization and Identification of Radioactive Residues in Goat Matrices Following Dosing with Prothioconazole Metabolite [phenyl-*UL*-^{14}C]JAU6476-Desthio at 195 ppm in the Diet.

Compound [1]	Milk		Liver		Kidney		Muscle		Fat	
	TRR=0.286 ppm		TRR=18.42 ppm		TRR=18.98 ppm		TRR=0.266 ppm		TRR=0.231 ppm	
	%TRR	ppm	%TRR	ppm	%TRR	ppm	%TRR	ppm	%TRR	ppm
JAU6476-desthio	–	-	31.18	5.744	7.66	1.454	1.76	0.005	13.88	0.032

TABLE C.2.3. Summary of Characterization and Identification of Radioactive Residues in Goat Matrices Following Dosing with Prothioconazole Metabolite [phenyl-UL-^{14}C]JAU6476-Desthio at 195 ppm in the Diet.

Compound [1]	Milk		Liver		Kidney		Muscle		Fat	
	TRR=0.286 ppm		TRR=18.42 ppm		TRR=18.98 ppm		TRR=0.266 ppm		TRR=0.231 ppm	
	%TRR	ppm	%TRR	ppm	%TRR	ppm	%TRR	ppm	%TRR	ppm
JAU6476-desthio-3,4-dihydroxy-diene-glucuronide (D1)	2.35	0.007	1.98	0.366	7.28	1.382	8.17	0.022	7.83	0.018
JAU6476-desthio-3,4-dihydroxy-diene-glucuronide (D2)	3.01	0.009	3.81	0.703	13.75	2.610	12.77	0.034	15.02	0.035
JAU6476-desthio-3,4-dihydroxy-diene (D1)	2.44	0.007	*1.16* [2]	*0.213*	*1.62*	*0.307*	3.64	0.010	*4.31*	*0.010*
JAU6476-desthio-3,4-dihydroxy-diene (D2)	*3.04*	*0.009*					7.11	0.019		
JAU6476-dihydroxy-desthio-glucuronide (I1, D1) [3]	--	--	--	--	--	--	*5.88*	*0.016*	--	--
JAU6476-dihydroxy-desthio-glucuronide (I1, D2) [3]	*2.63*	*0.008*	*2.74*	*0.504*	*4.92*	*0.933*	--	--	*5.30*	*0.012*
JAU6476-4,5-dihydroxy-desthio-glucuronide (I2)										
JAU6476-3-hydroxy-desthio-glucuronide										
JAU6476-4-hydroxy-desthio-glucuronide	*5.11* [4]	*0.015*	*2.77* [4]	*0.511*	*7.32* [4]	*1.388*	*5.84*	*0.016*	*4.68*	*0.011*
JAU6476-hydroxy-methoxy-desthio-glucuronide	--	--	--	--	--	--	*5.20*	*0.014*	--	--
JAU6476-desthio-glucuronide	6.22	0.018	--	--	24.07	4.567	3.57 [5]	0.009	4.17 [4]	0.010
JAU6476-dihydroxy-desthio (I1) [3]	*1.56*	*0.004*	2.15 [6]	0.396	--	--	1.72 [6]	0.005	5.36 [6]	0.012
JAU6476-4,5-dihydroxy-desthio (I2)	*1.38*	*0.004*	4.76 [7]	0.878	--	--	2.80	0.007	--	--
JAU6476-3-hydroxy-desthio	--	--	0.96	0.178	1.22	0.231	4.80	0.013	--	--
JAU6476-4-hydroxy-desthio	--	-	8.37	1.542	4.06	0.770	3.03	0.008	14.55	0.034
Sulfate conjugates [8]	44.03	0.126	--	--	--	--	--	--	--	--
Unknowns	16.74	0.048	10.99	2.024	14.50	2.750	10.73	0.029	9.04	0.021
Fractions not analyzed	5.61	0.016	10.67	1.965	10.83	2.052	11.83	0.030	4.40	0.010
Total identified	58.05	0.167	53.21	9.807	58.04	11.014	49.37	0.132	60.81	0.141
Total tentatively identified [11]	13.72	0.040	6.67	1.228	13.86	2.628	16.92	0.046	14.29	0.033
-Total	71.77	0.207	59.88	11.035	71.9	13.642	66.29	0.178	75.10	0.174
Total characterized	22.35	0.064	21.66	3.989	25.33	4.802	22.56	0.059	13.44	0.031
Total extractable	94.12	0.269	81.55	15.022	97.21	18.444	88.84	0.236	88.55	0.205

TABLE C.2.3. Summary of Characterization and Identification of Radioactive Residues in Goat Matrices Following Dosing with Prothioconazole Metabolite [phenyl-UL-^{14}C]JAU6476-Desthio at 195 ppm in the Diet.

Compound [1]	Milk		Liver		Kidney		Muscle		Fat	
	TRR=0.286 ppm		TRR=18.42 ppm		TRR=18.98 ppm		TRR=0.266 ppm		TRR=0.231 ppm	
	%TRR	ppm	%TRR	ppm	%TRR	ppm	%TRR	ppm	%TRR	ppm
Unextractable (PES) [9]	5.88	0.017	18.45	3.398	2.80	0.531	11.16	0.030	11.45	0.026
Accountability [10]	100.0		100.0		100.0		100.0		100.0	

[1] D: diastereomer; I: isomer.
[2] May have co-eluted with JAU6476-desthio-4,5-dihydroxy-dienyl-glucuronide.
[3] Based on NMR results, 3,4-; 5,6-; and/or 3,6-dihydroxy positions were possible.
[4] May have co-eluted with JAU6476-hydroxy-methoxy-desthio.
[5] May have co-eluted with JAU6476-dihydroxy-desthio-glucuronide and JAU6476-hydroxy-desthio-glucuronide.
[6] May have co-eluted with *JAU6476-alpha-hydroxy-desthio.*
[7] May have co-eluted with JAU6476-desthio-glucuronide.
[8] *Sulfate conjugates of* JAU6476-dihydroxy-desthio, JAU6476-hydroxy-methoxy-desthio and JAU6476-hydroxy-desthio.
[9] Residues remaining after exhaustive extractions.
[10] Accountability = (Total extractable + Total unextractable)/(TRR from combustion analysis; see TABLE C.2.1) * 100.
[11] Residues of metabolites that are considered tentatively identified are in italics.

C.3. Proposed Metabolic Profile

FIGURE C.3.1. Proposed Metabolic Profile of [phenyl-UL-^{14}C]JAU6476-Desthio (Prothioconazole Metabolite) in Lactating Goat.

The figure below was copied without alteration from MRID 46246201.

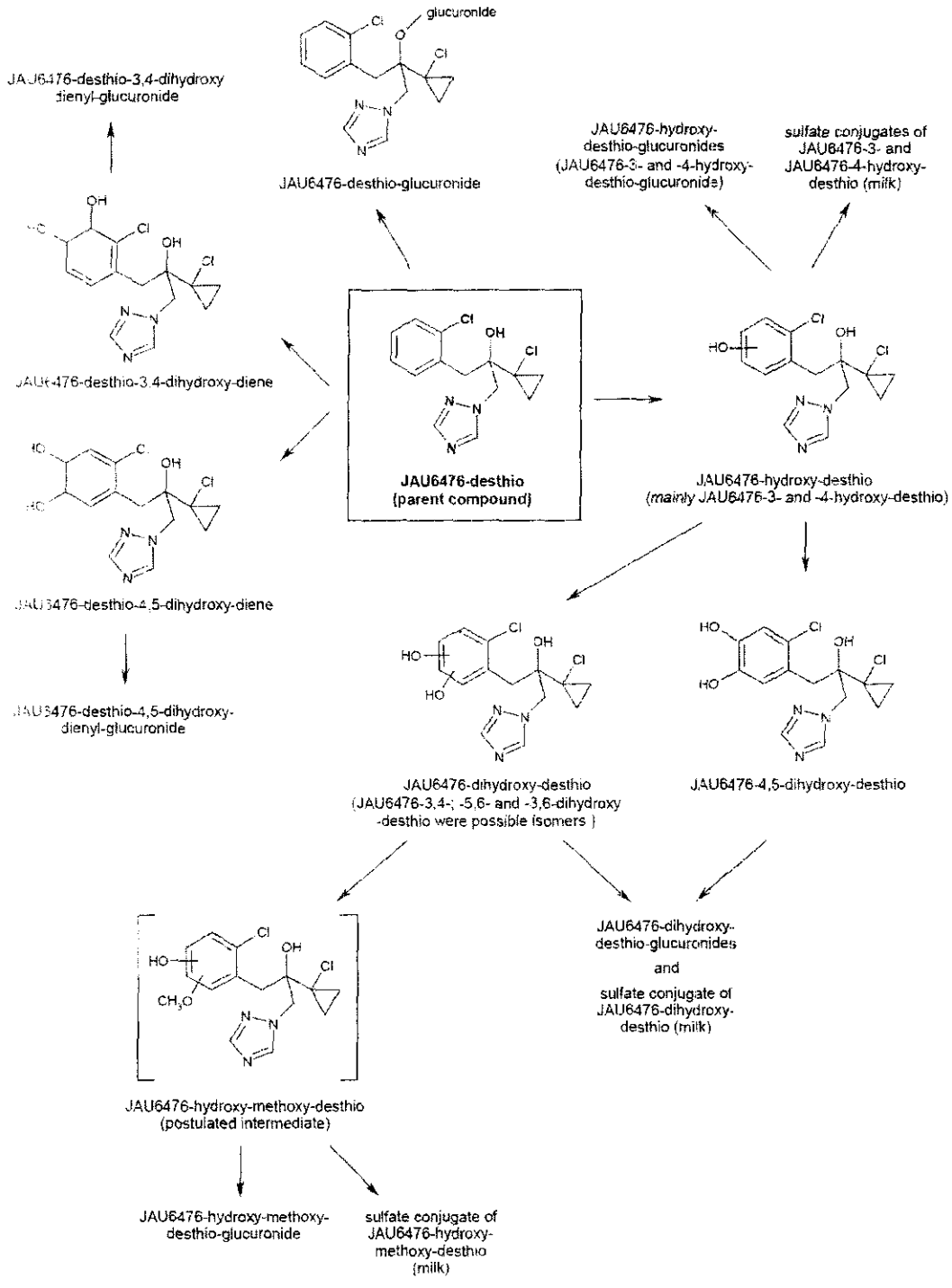
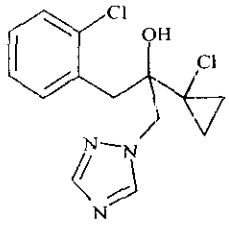
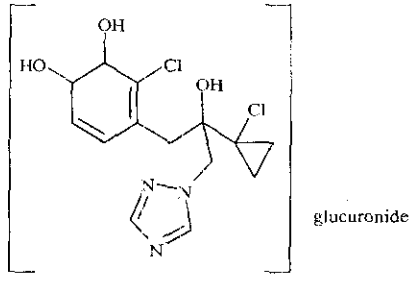
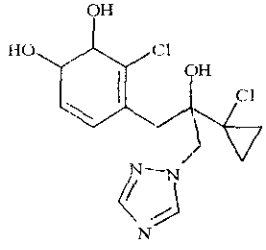
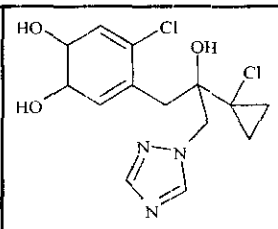
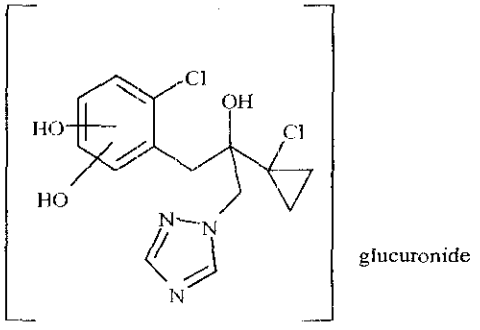
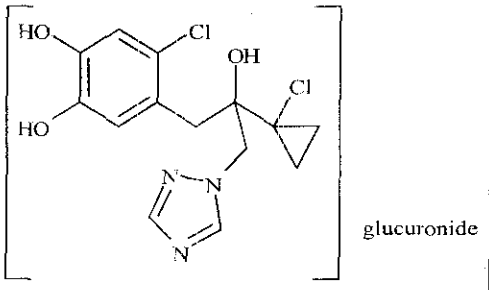
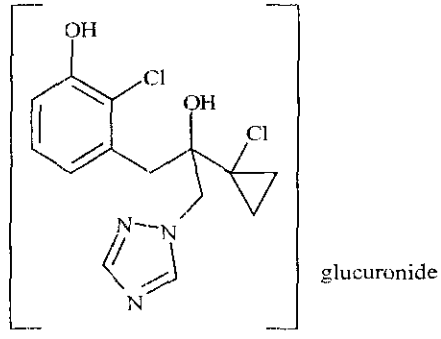
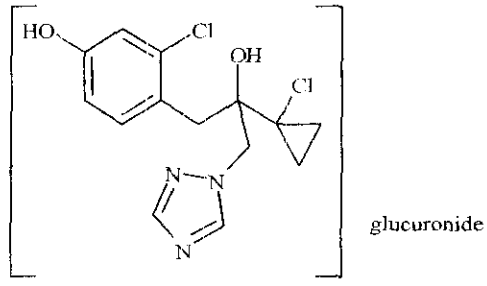
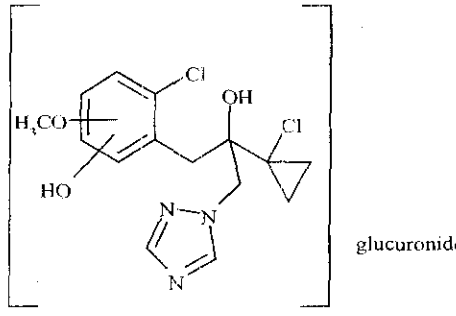
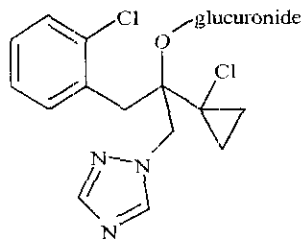
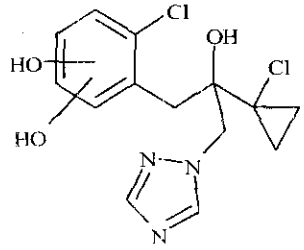
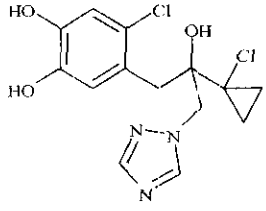
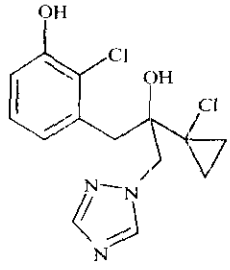
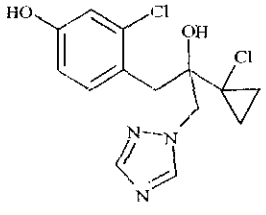
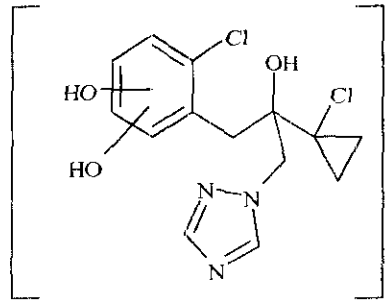
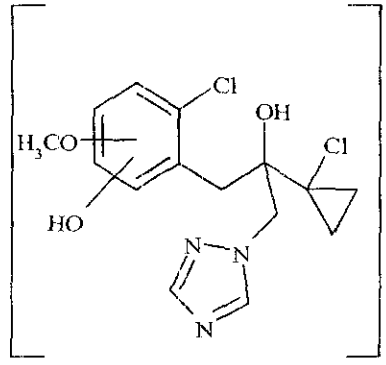
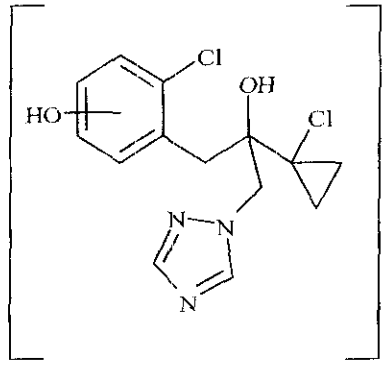

JAU6476-desthio-3,4-dihydroxy dienyl-glucuronide
glucuronide
JAU6476-desthio-glucuronide
JAU6476-hydroxy-desthio-glucuronides (JAU6476-3- and -4-hydroxy-desthio-glucuronide)
sulfate conjugates of JAU6476-3- and JAU6476-4-hydroxy-desthio (milk)
JAU6476-desthio-3,4-dihydroxy-diene
JAU6476-desthio
(parent compound)
JAU6476-hydroxy-desthio
(mainly JAU6476-3- and -4-hydroxy-desthio)
JAU6476-desthio-4,5-dihydroxy-diene
JAU6476-desthio-4,5-dihydroxy-dienyl-glucuronide
JAU6476-dihydroxy-desthio
(JAU6476-3,4-, -5,6- and -3,6-dihydroxy -desthio were possible isomers)
JAU6476-4,5-dihydroxy-desthio
JAU6476-dihydroxy-desthio-glucuronides
and
sulfate conjugate of JAU6476-dihydroxy-desthio (milk)
JAU6476-hydroxy-methoxy-desthio
(postulated intermediate)
JAU6476-hydroxy-methoxy-desthio-glucuronide
sulfate conjugate of JAU6476-hydroxy-methoxy-desthio (milk)

TABLE C.3.1. Identification of Compounds from Metabolism Study		
Common name/code Figure C.3.1 ID No.	Chemical name	Chemical structure
JAU6476-desthio	α-(1-chlorocyclopropyl)-α-[(2-chlorophenyl)methyl]-1H-1,2,4-triazole-1-ethanol	
JAU6476-desthio-3,4-dihydroxy-dienyl-glucuronide		
JAU6476-desthio-3,4-dhydroxy-diene	3-chloro-4-[2-(1-chlorocyclopropyl)-2-hydroxy-3-(1H-1,2,4-triazol-1-yl)propyl]cyclohexa-3,5-diene-1,2-diol [1]	

JAU6476-desthio-4,5-dihydroxy-diene	4-chloro-5-[2-(1-chlorocyclopropyl)-2-hydroxy-3-(1H-1,2,4-triazol-1-yl)propyl]cyclohexa-3,5-diene-1,2-diol [1]	
JAU6476-dihydroxy-desthio-glucuronide		 glucuronide
JAU6476-4,5-dihydroxy-desthio-glucuronide		 glucuronide

JAU6476-3-hydroxy-desthio-glucuronide		
JAU6476-4-hydroxy-desthio-glucuronide		
JAU6476-hydroxy-methoxy-desthio-glucuronide		

JAU6476-desthio-glucuronide		
JAU6476-dihydroxy-desthio		
JAU6476-4,5-dihydroxy-desthio	4-chloro-5-[2-(1-chlorocyclopropyl)-2-hydroxy-3-(1H-1,2,4-triazol-1-yl)propyl]benzene-1,2-diol [1]	
JAU6476-3-hydroxy-desthio	α-(1-chlorocyclopropyl)-α-[(2-chloro-3-hydroxyphenyl)methyl]-1H-1,2,4-triazole-1-ethanol	
JAU6476-4-hydroxy-desthio	α-(1-chlorocyclopropyl)-α-[(2-chloro-4-hydroxyphenyl)methyl]-1H-1,2,4-triazole-1-ethanol	

Sulfate conjugate of JAU6476-dihydroxy-desthio		 sulfate
Sulfate conjugate of JAU6476-hydroxy-methoxy-desthio		 sulfate
Sulfate conjugate of JAU6476-hydroxy-desthio		 sulfate

Chemical name generated using ACD chemical naming software.

D. CONCLUSION

Total radioactive residues (TRR) were 0.074-0.314 ppm in milk, 18.422 ppm in liver, 18.986 ppm in kidney, 0.232-0.277 ppm in muscle, and 0.216-0.240 ppm in fat from a goat dosed orally with [phenyl-UL-^{14}C]JAU6476-desthio, a metabolite of prothioconazole, at 195 ppm in the diet for 3 consecutive days. Residues in milk were generally highest in samples collected 8 hours after dosing, and did not appear to have reached a plateau by the end of the dosing period. A

large portion of the administered dose was excreted, with urine and feces accounting for a total of ~74% of the administered dose.

The majority of the radioactivity (~81-97%) was extracted using methanol (milk) or ACN/water (tissues). Nonextractable residues accounted for ≤0.030 ppm in milk, muscle, and fat, <3% TRR in kidney, and 18.45% TRR (3.398 ppm) in liver. The petitioner normalized the extraction results; however, accountabilities prior to normalization were 89-101%.

Approximately 60-75% TRR were identified (or tentatively identified) in goat matrices. The test substance, JAU6476-desthio, was found to be a major residue in liver and fat, at 31% and 14% TRR, respectively. JAU6476-desthio was not found in milk and was found in kidney and muscle at <8% TRR. JAU6476-desthio-glucuronide was the major metabolite in kidney (24% TRR); this metabolite was also found in milk, muscle, and fat, at <7% TRR. Sulfate conjugates of JAU6476-dihydroxy-desthio, JAU6476-hydroxy-methoxy-desthio, and JAU6476-hydroxy-desthio together accounted for a large portion (44% TRR) of the radioactivity in milk; these conjugates were not detected in goat tissues. One diastereomer of JAU6476-desthio-3,4-dihydroxy-dienyl-glucuronide was a major residue in kidney, muscle, and fat (13-15% TRR), and was a minor residue in milk and liver (<4% TRR); a second diastereomer of this metabolite was found as a minor residue in all goat matrices. JAU6476-4-hydroxy-desthio was a major metabolite in fat (15% TRR); this metabolite was found at lower levels in liver, kidney, and muscle (<9% TRR). Several additional metabolites were identified in goat matrices, each at <8% TRR: JAU6476-desthio-3,4-dihydroxy-diene in all matrices; glucuronides of JAU6476-dihydroxy-desthio, JAU6476-4,5-dihydroxy-desthio, JAU6476-3-hydroxy-desthio, JAU6476-4-hydroxy-desthio, and JAU6476-hydroxy-methoxy-desthio in all matrices; JAU6476-dihydroxy-desthio in milk, liver, muscle, and fat; JAU6476-4,5-dihydroxy-desthio in milk, liver, and muscle; and JAU6476-3-hydroxy-desthio in liver, kidney, and muscle. Unknown metabolites accounted for a significant portion of the radioactivity in liver and kidney; however, HPLC analyses indicated that individual unknowns were ≤5.1% TRR.

Based on the results of the study, the petitioner concluded that JAU6476-desthio is metabolized in goats via several steps: conjugation with glucuronic acid to form JAU6476-desthio-glucuronide; hydroxylation of JAU6476-desthio to form the isomers JAU6476-3-hydroxy-desthio and JAU6476-4-hydroxy-desthio, which was partly followed by conjugation with glucuronic acid; further hydroxylation of the chlorohydroxyphenyl moiety to form JAU6476-3,4- and JAU6476-4,5-dihydroxy-desthio, which was also followed partly by conjugation with glucuronic acid; and oxidation of the chlorophenyl moiety of the isomers of JAU6476-hydroxy-desthio to form JAU6476-desthio-dihydroxy-dienes, which was followed by conjugation with glucuronic acid to some extent. The observation of JAU6476-hydroxy-methoxy-desthio-glucuronide indicated that JAU6476-hydroxy-methoxy-desthio may have formed as an intermediate.

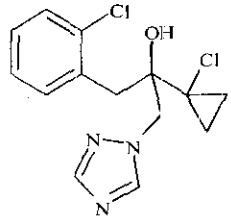
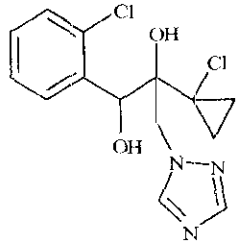
E. REFERENCES

None.

F. DOCUMENT TRACKING

RDI: S.Funk (06/23/06); L Cheng (28/06/2006)
Petition Number: PP#4F6830
DP Barcode: D303508
PC Code: 113961

Template Version September 2003

APPENDIX I. Chemical Names and Structures of Reference Standards Used in Goat Metabolism Study.		
Common name; Company code	Chemical name	Chemical structure
JAU6476-desthio [1] (unlabeled standard also used)	α-(1-Chlorocyclopropyl)-α -[(2-chlorophenyl)methyl]-1H-1,2,4-triazole-1-ethanol	
JAU6476-alpha-hydroxy-desthio [2]	2-(1-chlorocyclopropyl)-1-(2-chlorophenyl)-3-(1H-1,2,4-triazol-1-yl)-1,2-propanediol	

[1] Standard was radiolabeled.
[2] Isolated and identified in a spring wheat metabolism study (refer to the DER for MRID 46246141).

Primary Evaluator	Stephen Funk, Ph.D., Chemist, HED/IO	Date: 26/06/2006
Approved by	Leung Cheng, Team Leader, HED/RAB3	Date: 28/06/2006

In the absence of signatures, this document is considered to be a draft with deliberative material for internal use only.

This DER was originally prepared under contract by Dynamac Corporation (20440 Century Boulevard, Suite 100; Germantown, MD 20874; submitted 06/03/2005). The DER has been reviewed by the Health Effects Division (HED) and revised to reflect current Office of Pesticide Programs (OPP) policies.

STUDY REPORT:

46246214 Heinemann, O., Auer, S. (2001) JAU6476-desthio - Dairy Cattle Feeding Study. Lab Project Number: P 673003007: MR-535/00. Unpublished study prepared by Bayer AG. 262 p.

EXECUTIVE SUMMARY:

Bayer CropScience has submitted a dairy cattle feeding study with prothioconazole-desthio, a metabolite of prothioconazole. Three groups of dairy cattle (3 cows per group) were dosed orally with prothioconazole-desthio at levels equivalent to 5.1, 29.0, and 125 ppm in the feed. The dosing levels correspond to 0.26x, 1.5x, and 6.4x the anticipated dietary burden (see Appendix 1). Cattle were dosed once a day for 29 consecutive days. Cows were milked twice daily, and composited daily samples from Study Days 1, 4, 6, 8, 11, 13, 15, 18, 20, 22, 25, 27, 28, and 29 (mid and high dose groups only) were collected for analysis from each cow. Cattle were sacrificed within 17 hours of the last dose, and samples of composite fat (omental and perirenal), liver, kidneys, and composite muscle (loin, elbow and flank) were collected.

Milk and tissues samples were analyzed using an LC-MS/MS method (Method No. 00655 and its modification 00655/M001). This method determines residues of prothioconazole-desthio, prothioconazole-3-hydroxy-desthio, and prothioconazole-4-hydroxy-desthio and compounds that may be converted to these compounds by acid hydrolysis. The validated LOQ was 0.004 ppm for each analyte in milk and 0.010 ppm for each analyte in tissues; the calculated LODs ranged 0.0001-0.0004 ppm. The method is adequate for data collection based on acceptable concurrent method recovery data.

The maximum combined residues of prothioconazole-desthio, prothioconazole-3-hydroxy-desthio, and prothioconazole-4-hydroxy-desthio in milk and tissues are listed in the table below. Because low residue levels were observed in samples from the mid and high dose groups, milk samples from the low dose group were not analyzed.

Matrix	Maximum Combined Residues of Prothioconazole-Desthio, Prothioconazole-3-Hydroxy-Desthio, and Prothioconazole-4-Hydroxy-Desthio by Feeding Level (ppm)		
	5.1 ppm	29.0 ppm	125 ppm
Milk	--	<0.012	0.021
Fat	<0.030	<0.031	0.145
Kidney	<0.039	0.178	1.10
Liver	<0.053	0.270	1.66
Muscle	<0.030	<0.030	<0.033

Combined residues in kidney and liver were generally found to have a linear relationship with the dosing levels. Residues in milk appeared to reach a plateau within the first week of dosing. A separate experiment evaluating residues in the hexane-soluble portion of whole milk indicated that residues are not likely to accumulate in milk fat.

The maximum storage interval from sample collection to analysis was 20 days for tissues and <30 days for milk. Because all samples were stored frozen and analyzed within 30 days of collection, supporting storage stability data are not needed.

STUDY/WAIVER ACCEPTABILITY/DEFICIENCIES/CLARIFICATIONS:

Under the conditions and parameters used in the study, the feeding study data are tentatively classified as scientifically acceptable, pending submission of Method No. 00655/M001. The acceptability of this study for regulatory purposes is addressed in the forthcoming U.S. EPA Residue Chemistry Summary Document, DP Barcode D303508.

COMPLIANCE:

Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided. No deviations from regulatory requirements were reported which would have an impact on the validity of the study.

A. BACKGROUND INFORMATION

Prothioconazole is a broad-spectrum systemic fungicide intended for the control of ascomycetes, basidiomycetes, and deuteromycetes fungi in barley, canola, the dried shelled and bean crop subgroup, the oilseed crop group, peanuts, rice, and wheat. Prothioconazole is a systemic demethylation inhibitor fungicide (Group 3 fungicide) of the triazolinthione chemical class. The chemical has been submitted for joint EPA and PMRA review by Bayer CropScience. The crops proposed for joint review include barley, the dried shelled and bean subgroup, the oilseed crop group, and wheat. Uses on peanuts and rice are being proposed for the U.S. only.

TABLE A.1. Test Compound Nomenclature.

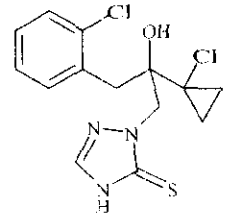
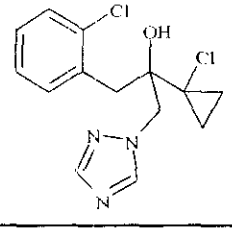
Chemical structure	
Common name	Prothioconazole
Company experimental name	JAU6476
IUPAC name	(*RS*)-2-[2-(1-chlorocyclopropyl)-3-(2-chlorophenyl)-2-hydroxypropyl]-2,4-dihydro-1,2,4-triazole-3-thione
CAS name	2-[2-(1-chlorocyclopropyl)-3-(2-chlorophenyl)-2-hydroxypropyl]-1,2-dihydro-3H-1,2,4-triazole-3-thione
CAS registry number	178928-70-6
PC Code	113961
End-use product (EP)	Proline® 480 SC (4 lb/gal suspension concentrate)
Chemical structure	
Common name	Prothioconazole-desthio
Company experimental name	JAU6476-desthio
IUPAC name	2-(1-chlorocyclopropyl)-1-(2-chlorophenyl)-3-(1H-1,2,4-triazol-1-yl)propan-2-ol
CAS name	α-(1-chlorocyclopropyl)-α -(2-chlorophenyl)methyl-1H-1,2,4-triazole-1-ethanol
CAS registry number	120983-64-4
PC Code	613962
End-use product (EP)	Not applicable

TABLE A.2. Physicochemical Properties of the Technical Grade Test Compound.		
Parameter	Value	Reference
Prothioconazole		
Melting point/range	139.1 to 144° C	MRID 46246003
pH	5.8 (1% solution)	MRID 46246003
Density	1.36 g/mL at 20° C	MRID 46246003
Water solubility	mg/L (20° C) pH 4 5 pH 8 300 pH 9 2000	MRID 46246003
Solvent solubility	g/L at 20° C) Acetone >250 Acetonitrile 69 Dichloromethane 88 Dimethylsulfoxide 126 Ethyl acetate >250 n-Heptane <0.1 1-Octanol 58 Polyethylene glycol >250 2-Propanol 87 Xylene 8	MRID 46246003
Vapor pressure	<<4 x 10^{-7} Pa at 20 or 25° C (calculated from determinations at 70° C)	MRID 46246003
Dissociation constant, pK_a	6.9 (calculated from K_{OW})	MRID 46246003
Octanol/water partition coefficient, Log(K_{OW})	20° C unbuffered water 4.05 pH 4 4.16 pH 7 3.82 pH 9 2.00	MRID 46246003
UV/visible absorption spectrum	Peak maxima at 257 nm	MRID 46246003
Prothioconazole-desthio		
Melting point	108.5° C	MRID 46477704
pH	Not provided	
Density	Not provided	
Water solubility	g/L (20° C) unbuffered, pH 5.5 0.051	MRID 46477704
Solvent solubility	g/L at 20° C Hexane 3.0 Toluene 84.0 Dichloromethane >200.0 2-Propanol 35.0 Acetone 100.0 Acetonitrile 43.0	MRID 46477704

TABLE A.2. Physicochemical Properties of the Technical Grade Test Compound.		
Parameter	Value	Reference
Vapor pressure	2.7×10^{-7} Pa at 20° C	MRID 46477704
Dissociation constant, pK_a	Not provided	
Octanol/water partition coefficient, $Log(K_{OW})$	Not provided	
UV/visible absorption spectrum	Not provided	

B. EXPERIMENTAL DESIGN

The in-life phase of the feeding study was conducted at Bayer AG, Animal Health Research Division (Leverkusen, Germany) from 2/19/01 to 3/30/01. Three groups of dairy cattle (3 cows per group) were dosed orally with prothioconazole-desthio at levels equivalent to 5.1, 29.0, and 125 ppm in the feed. Cattle were dosed once a day for 29 consecutive days.

Cows were milked twice daily, and composited daily samples from Study Days 1, 4, 6, 8, 11, 13, 15, 18, 20, 22, 25, 27, 28, and 29 (mid and high dose groups only) were collected for analysis from each cow. Cattle were sacrificed within 17 hours of the last dose, and samples of composite fat (omental and perirenal), liver, kidneys, and composite muscle (loin, elbow and flank) were collected.

B.1. Livestock

TABLE B.1.1. Description of Livestock Used in the Feeding Study.					
Species	Breed	Age (years)	Weight at study initiation (kg)	Health status	Description of housing/holding area
Dairy Cattle (*Bos taurus*)	Holstein Friesian	2.5-4	469-652	Cattle were free from any injury or illness that would preclude use in the study.	Cattle were housed individually in stalls indoors in a barn, with average temperatures of 16-17.5 EC, relative humidity at 60-75%, with 14 hours of light and 10 hours of darkness.

TABLE B.1.2. Dietary Regime.					
Composition of Diet	Treatment group	Average feed consumption (kg/cow/day) [2]	Water	Acclimation period	Predosing
Cobs mixture[1] twice daily (18 kg total); mineralized dairy cattle concentrate three times daily (6 kg total)	Low dose	17.3	Tap water, *ad libitum*	11 days	None
	Mid dose	18.9			
	High dose	18.1			

[1] The cobs mixture consisted of approximately equal amounts of maize cobs (pellets), grass cobs, and plant silage cobs.
[2] Feed consumption is expressed on a dry weight basis.

TABLE B.1.3 Dosing Regime. [1]					
Treatment group	Treatment Type	Level of administered dose (mg/cow/day)	Residue intake in diet (ppm)	Vehicle	Timing/Duration
Low dose	Oral	88	5.1	Gel capsule administered via balling gun	Once daily for 28 days
Mid dose		550	29.0		
High dose		2246	125		

[1] The values for administered dose and residue intake in diet reflect the average for the group over the dosing period.

TABLE B.1.4 Sample Collection.						
Treatment group	Milk collected	Average daily milk production during treatment period (kg/cow)	Average daily milk production during acclimation period (kg/cow)	Urine, feces and cage wash	Interval from last dose to sacrifice	Tissues collected and analyzed
Low dose	Twice daily	19.3-21.6	20.9-22.8	Not collected	Within 17 hours	Liver, kidney, muscle, and fat
Mid dose		19.4-23.8	18.8-23.6			
High dose		17.4-24.9	16.9-22.4			

B.2. Sample Handling and Preparation

Milk samples were placed in frozen storage immediately after collection. Tissue samples were placed in cold storage (4°C) ,then chopped into small pieces, frozen, homogenized and placed back in frozen storage(≤ 18° C) until analysis. Milk samples were thawed prior to homogenization.

B.3. Analytical Methodology

Samples of milk and tissues were analyzed using an LC-MS/MS method, Method No. 00655 and its modification 00655/M001 (MRID 46477704). This method determines residues of prothioconazole-desthio, prothioconazole-3-hydroxy-desthio, and prothioconazole-4-hydroxy-desthio and compounds that may be converted to these compounds by acid hydrolysis. The petitioner did not submit a complete description of Method No. 00655/M001, which is a method for milk only, or describe how the method differs from Method No. 00655 (Method No. 00655 does include instructions for the analysis of milk); based on the limited description of the extraction procedures included in the submission, Method No. 00655/M001 does not appear to be significantly different from Method No. 00655.

Briefly, samples of milk were mixed with water and then hydrolyzed using 5 N HCl at reflux for 2 hours. Samples of liver, kidney, and muscle were extracted with acetonitrile (ACN) and water and the extract was hydrolyzed as for milk samples. Fat samples were extracted twice with ACN/water and hexane and the ACN/water extract, after partitioning with n-hexane, was hydrolyzed as for milk samples. The hydrolysate was neutralized and cleaned up on a silica gel cartridge, using cyclohexane/ethyl acetate to elute residues. The eluate was evaporated to dryness, redissolved in ACN/water, and analyzed by LC-MS/MS. Samples were analyzed for residues of prothioconazole-desthio, prothioconazole-3-hydroxy-desthio, and prothioconazole-4-hydroxy-desthio, and all results were reported in prothioconazole-desthio equivalents. The validated LOQ was 0.004 ppm for each analyte in milk and 0.010 ppm for each analyte in tissues. The calculated LODs were 0.0001 ppm for each analyte in milk and fat, for prothioconazole-3-hydroxy-desthio in muscle and kidney, and for prothioconazole-4-hydroxy-desthio in muscle; 0.0002 ppm for prothioconazole-3-hydroxy-desthio in liver and for prothioconazole-4-hydroxy-desthio in liver and kidney; 0.0003 ppm for prothioconazole-desthio in liver; and 0.0004 ppm for prothioconazole-desthio in muscle and kidney.

C. RESULTS AND DISCUSSION

Three groups of dairy cattle (3 cows per group) were dosed orally with prothioconazole-desthio at levels equivalent to 5.1, 29.0, and 125 ppm in the feed. The dosing levels correspond 0.26x, 1.5x, and 6.4x the anticipated dietary burden (see Appendix 1). Cattle were dosed once a day for 29 consecutive days. Cows were milked twice daily, and composited daily samples from Study Days 1, 4, 6, 8, 11, 13, 15, 18, 20, 22, 25, 27, 28, and 29 (mid and high dose groups only) were collected for analysis from each cow. Cattle were sacrificed within 17 hours of the last dose, and samples of composite fat (omental and perirenal), liver, kidneys, and composite muscle (loin, elbow and flank) were collected.

Concurrent method recovery data are presented in Table C.1. Milk and tissues samples were analyzed using an LC-MS/MS method (Method No. 00655 and its modification 00655/M001) which determines residues of prothioconazole-desthio, prothioconazole-3-hydroxy-desthio, and

prothioconazole-4-hydroxy-desthio and compounds that may be converted to these compounds by acid hydrolysis. The validated LOQ was 0.004 ppm for each analyte in milk and 0.010 ppm for each analyte in tissues; the calculated LODs ranged 0.0001-0.0004 ppm. The method is adequate for data collection based on acceptable concurrent method recovery data. Concurrent recoveries from milk and cow tissues, fortified with each analyte at 0.004-0.1 ppm for milk and 0.01 and 0.10 ppm for tissues, were within the acceptable 70-120% range for all matrices. Apparent residues were <LOQ in all samples of cattle matrices from undosed cattle. Adequate sample calculations and chromatograms were provided.

Sample storage conditions and intervals are reported in Table C.2. All tissue samples were analyzed within 20 days of collection. The storage intervals for milk were reported to be <30 days; actual storage intervals were not reported. Supporting storage stability data for the feeding study are not required as all samples were stored frozen and analyzed within 30 days of sample collection.

The results of the feeding study are presented in Table C.3 and a summary of the combined residues of prothioconazole-desthio, prothioconazole-3-hydroxy-desthio, and prothioconazole-4-hydroxy-desthio in milk and cattle tissues is presented in Table C.4. In milk samples, residues of each analyte were below the LOQ (<0.004 ppm) in all samples from the 29.0-ppm dose groups, with the exception of one milk sample from Day 1, which bore quantifiable residues of prothioconazole-desthio at 0.0043 ppm. Residues of prothioconazole-desthio, prothioconazole-3-hydroxy-desthio, and prothioconazole-4-hydroxy-desthio ranged <0.004-0.0068 ppm, <0.004-0.0115 ppm, and <0.004-0.0043 ppm, respectively, in milk samples from the 125-ppm dose group over the course of the study; combined residues were <0.012-0.021 ppm. Residues in milk appeared to reach a plateau within the first week of dosing; a graph of combined residues in milk samples from the 125-ppm dose group over the course of the study is presented in Figure C.1. Because residue levels were below the LOQ in virtually all samples from the 29.0-ppm dosing group, no milk samples from the 5.1-ppm dose group were analyzed.

In liver samples, residues of prothioconazole-desthio were 0.011-0.030 ppm, 0.102-0.178 ppm, and 0.414-1.193 ppm in samples from the 5.1-, 29.0-, and 125-ppm dose groups, respectively. Residues of prothioconazole-3-hydroxy-desthio were <0.01-0.013 ppm, 0.039-0.055 ppm, and 0.120-0.300 ppm in samples from the 5.1-, 29.0-, and 125-ppm dose groups, respectively. Residues of prothioconazole-4-hydroxy-desthio were <0.01 ppm, 0.024-0.037 ppm, and 0.071-0.171 ppm in samples from the 5.1-, 29.0-, and 125-ppm dose groups, respectively. Combined residues were <0.031-<0.053 ppm, 0.184-0.270 ppm, and 0.617-1.66 ppm, respectively, for each dose group.

In kidney samples, residues of prothioconazole-desthio were <0.01 ppm, 0.016-0.033 ppm, and 0.066-0.237 ppm in samples from the 5.1-, 29.0-, and 125-ppm dose groups, respectively. Residues of prothioconazole-3-hydroxy-desthio were <0.01 ppm, 0.048-0.064 ppm, and 0.186-0.477 ppm in samples from the 5.1-, 29.0-, and 125-ppm dose groups, respectively. Residues of

prothioconazole-4-hydroxy-desthio were <0.01-0.019 ppm, 0.051-0.085 ppm, and 0.180-0.383 ppm in samples from the 5.1-, 29.0-, and 125-ppm dose groups, respectively. Combined residues were <0.030-<0.039 ppm, 0.120-0.178 ppm, and 0.432-1.10 ppm, respectively, for each dose group.

In muscle samples, residues of each analyte were below the LOQ (<0.01 ppm) in all samples from all three dose groups, with the exception of one sample from the high dose group which bore quantifiable residues of prothioconazole-3-hydroxy-desthio at 0.013 ppm.

In fat samples, residues of each analyte were below the LOQ (<0.01 ppm) in all samples from the 5.1- and 29.0-ppm dose groups, with the exception of one sample from the 29.0-ppm dose group which bore quantifiable residues of prothioconazole-desthio at 0.011 ppm. Residues of prothioconazole-desthio, prothioconazole-3-hydroxy-desthio, and prothioconazole-4-hydroxy-desthio were 0.024-0.091 ppm, <0.01-0.030 ppm, and <0.01-0.024 ppm, respectively, in samples from the 125-ppm dose group; combined residues were <0.044-0.145 ppm.

Combined residues in kidney and liver were generally found to have a linear relationship with the dosing levels. A graph of the linear regression of combined residues over feeding level in kidney and liver is presented in Figure C.2. Because residue levels in fat and muscle were low in the 5.1- and 29.0-ppm dose groups, graphs of the linear regression of residues over feeding level were not prepared.

To evaluate the potential for accumulation of residues in milk fat, the petitioner subjected a sample of Day 29 whole milk from the high dose group to liquid/liquid partitioning with n-hexane. The resulting n-hexane fraction was evaporated to dryness, the residue was dissolved in water, and the mixture was then hydrolyzed and analyzed according to procedures of Method No. 00655/M001. The analysis indicated that prothioconazole-desthio was found preferentially (5:1) in the n-hexane fraction (and therefore would potentially be found in milk fat). Prothioconazole-3-hydroxy-desthio and prothioconazole-4-hydroxy-desthio remained in the aqueous phase. The total residues, comprised mainly of the prothioconazole-3-hydroxy-desthio and prothioconazole-4-hydroxy-desthio metabolites, remained preferentially in the aqueous phase; total residues were 0.019 ppm with 0.004 ppm found in the hexane phase.

TABLE C.1 *Concurrent* Recoveries from Various Cattle Matrices using an LC-MS/MS Method (Bayer Method Nos. 00655 and 00655/M001).

Matrix	Analyte	Spiking Level (ppm)	Recoveries Obtained	Recoveries (%)		
				n	Mean	SD
Milk	Prothioconazole-desthio	0.004	93, 96, 96, 97, 97, 100	6	95	4
		0.01	87, 89, 92, 93, 93, 96, 98, 102, 103	9		
		0.04	87, 98, 98, 100	4		
		0.10	86, 91, 92, 92, 93, 94, 95, 95, 97, 97	10		
	Prothioconazole-4-hydroxy-desthio	0.004	91, 96, 96, 98, 98, 101	6	94	4
		0.01	89, 89, 89, 90, 91, 93, 96, 96, 96, 96	10		
		0.04	88, 100, 101, 102	4		
		0.10	89, 91, 91, 92, 94, 95, 95, 95, 96, 97	10		
	Prothioconazole-3-hydroxy-desthio	0.004	99, 99, 100, 100, 102, 104	6	96	4
		0.01	89, 91, 91, 91, 93, 95, 96, 96, 97, 97	10		
		0.04	89, 98, 100, 100	4		
		0.10	89, 91, 93, 94, 95, 95, 96, 96, 97, 99	10		
Liver	Prothioconazole-desthio	0.01	96	1	96	--
		0.10	95	1		
	Prothioconazole-4-hydroxy-desthio	0.01	90	1	92	--
		0.10	93	1		
	Prothioconazole-3-hydroxy-desthio	0.01	90	1	94	--
		0.10	98	1		
Kidney	Prothioconazole-desthio	0.01	98	1	95	--
		0.10	91	1		
	Prothioconazole-4-hydroxy-desthio	0.01	98	1	96	--
		0.10	93	1		
	Prothioconazole-3-hydroxy-desthio	0.01	98	1	98	--
		0.10	98	1		
Muscle	Prothioconazole-desthio	0.01	100	1	99	--
		0.10	98	1		
	Prothioconazole-4-hydroxy-desthio	0.01	95	1	97	--
		0.10	98	1		

	Prothioconazole-3-hydroxy-desthio	0.01	95	1	97	--
		0.10	98	1		
Fat	Prothioconazole-desthio	0.01	83	1	83	--
		0.10	83	1		
	Prothioconazole-4-hydroxy-desthio	0.01	93	1	94	--
		0.10	95	1		
	Prothioconazole-3-hydroxy-desthio	0.01	92	1	93	--
		0.10	94	1		

TABLE C.2. Summary of Storage Conditions

Matrix	Storage Temp. (°C)	Actual Storage Duration (days) [1]	Limit of Demonstrated Storage Stability (days)
Milk	# -18	<30	Not provided
Liver		10-19	Not provided
Kidney		11-19	Not provided
Muscle		18-20	Not provided
Fat		12-20	Not provided

[1] Extracts were stored for 1-8 days prior to analysis. Storage intervals for milk were reported to be <30 days; actual storage intervals were not reported.

TABLE C.3.1. Residue Data for Whole Milk from Cattle Feeding Study with Prothioconazole-Desthio.

Collection Time (dose day)	Average Feeding Level (ppm)	Residues (ppm) [1]			
		Prothioconazole-3-hydroxy-desthio	Prothioconazole-4-hydroxy-desthio	Prothioconazole-desthio	Combined [2]
1	29.0	ND, ND, ND	ND, ND, ND	(0.0029), 0.0043, (0.0009)	<0.012, <0.012, <0.012
4	29.0	(0.0008, 0.0006, 0.0007)	(0.0004, 0.0003, 0.0003)	(0.0009, 0.0009, 0.0005)	<0.012, <0.012, <0.012
6	29.0	(0.0010, 0.0006, 0.0008)	(0.0005, 0.0003, 0.0004)	(0.0010, 0.0009, 0.0008)	<0.012, <0.012, <0.012
8	29.0	(0.0011, 0.0007, 0.0008)	(0.0005, 0.0004, 0.0005)	(0.0006, 0.0003, 0.0003)	<0.012, <0.012, <0.012
11	29.0	(0.0006, 0.0008, 0.0008)	(0.0004, 0.0004, 0.0004)	(0.0023, 0.0006, 0.0023)	<0.012, <0.012, <0.012
13	29.0	(0.0006, 0.0006, 0.0008)	(0.0003, 0.0003, 0.0004)	(0.0014, 0.0007, 0.0004)	<0.012, <0.012, <0.012
15	29.0	(0.0007, 0.0007, 0.0010)	(0.0004, 0.0004, 0.0005)	(0.0004, 0.0024, 0.0006)	<0.012, <0.012, <0.012
18	29.0	(0.0007, 0.0007, 0.0008)	(0.0003, 0.0003, 0.0004)	(0.0003), ND, (0.0003)	<0.012, <0.012, <0.012

TABLE C.3.1. Residue Data for Whole Milk from Cattle Feeding Study *with Prothioconazole-Desthio.*

Collection Time (dose day)	Average Feeding Level (ppm)	Residues (ppm) [1]			
		Prothioconazole-3-hydroxy-desthio	Prothioconazole-4-hydroxy-desthio	Prothioconazole-desthio	Combined [2]
20	29.0	(0.0007, 0.0007, 0.0008)	(0.0004, 0.0002, 0.0005)	(0.0004, 0.0003, 0.0003)	<0.012, <0.012, <0.012
22	29.0	(0.0007, 0.0008, 0.0009)	(0.0004, 0.0004, 0.0005)	(0.0003, 0.0003, 0.0003)	<0.012, <0.012, <0.012
25	29.0	(0.0009, 0.0011, 0.0010)	(0.0004, 0.0005, 0.0005)	(0.0003), ND, (0.0003)	<0.012, <0.012, <0.012
27	29.0	(0.0007, 0.0012, 0.0008)	(0.0003, 0.0006, 0.0005)	(0.0004), ND, ND	<0.012, <0.012, <0.012
28	29.0	(0.0007, 0.0009, 0.0010)	(0.0004, 0.0005, 0.0006)	(0.0003, 0.0003, 0.0003)	<0.012, <0.012, <0.012
29	29.0	(0.0010, 0.0008, 0.0010)	(0.0004, 0.0004, 0.0006)	(0.0004, 0.0011, 0.0003)	<0.012, <0.012, <0.012
1	125	ND, ND, ND	ND, ND, ND	(0.0004), ND, ND	<0.012, <0.012, <0.012
4	125	0.0089, (0.0037, 0.0038)	(0.0034, 0.0017, 0.0017)	0.0056, (0.0014, 0.0013)	<0.019, <0.012, <0.012
6	125	0.0086, (0.0037, 0.0039)	(0.0034, 0.0018, 0.0017)	0.0052, (0.0014, 0.0018)	<0.018, <0.012, <0.012
8	125	0.0104, (0.0036, 0.0038)	0.0041, (0.0018, 0.0017)	0.0068, (0.0018, 0.0015)	0.021, <0.012, <0.012
11	125	0.0077, (0.0037, 0.0034)	(0.0031, 0.0019, 0.0015)	(0.0032, 0.0015, 0.0014)	<0.016, <0.012, <0.012
13	125	0.0083, 0.0040, (0 0038)	(0.0034, 0.0020, 0.0018)	(0.0035, 0.0014, 0.0013)	<0.016, <0.012, <0.012
15	125	0.0078, 0.0040, 0.0044	(0.0030, 0.0020, 0.0020)	(0.0034, 0.0018, 0.0035)	<0.016, <0.012, <0.012
18	125	0.0082, (0.0036), 0.0048	(0.0033, 0.0017, 0.0021)	(0.0035, 0.0010, 0.0014)	<0.016, <0.012, <0.013
20	125	0.0098, (0.0039, 0.0036)	(0.0039, 0.0018, 0.0017)	(0.0038, 0 0012, 0.0010)	<0.018, <0.012, <0.012
22	125	0.0082, (0.0038, 0.0033)	(0.0031, 0.0017, 0.0016)	(0.0022, 0.0009, 0.0009)	<0.016, <0.012, <0.012
25	125	0.0079, 0.0041, (0.0037)	(0.0033, 0.0019, 0.0018)	(0.0021, 0.0010, 0.0010)	<0.016, <0.012, <0.012
27	125	0.0081, (0.0036), 0.0043	(0.0032, 0.0016, 0.0019)	(0.0027, 0.0009, 0.0012)	<0.016, <0.012, <0.012

TABLE C.3.1. Residue Data for Whole Milk from Cattle Feeding Study with Prothioconazole-Desthio.

Collection Time (dose day)	Average Feeding Level (ppm)	Residues (ppm) [1]			
		Prothioconazole-3-hydroxy-desthio	Prothioconazole-4-hydroxy-desthio	Prothioconazole-desthio	Combined [2]
28	125	0.0080, (0.0038), 0.0040	(0.0032, 0.0018, 0.0019)	(0.0026, 0.0011, 0.0010)	<0.016, <0.012, <0.012
29	25	0.0115, 0.0047, 0.0048	0.0043, (0.0022, 0.0025)	(0.0038, 0.0014, 0.0012)	<0.020, <0.013, <0.013

[1] Residues of each analyte are expressed as prothioconazole-desthio equivalents. The LOQ for each analyte was 0.004 ppm; the LOD for each analyte was 0.0001 ppm. ND = Not detected. Residues reported below the LOQ but above the LOD are presented in parentheses.

[2] Total combined residues of prothioconazole-desthio, prothioconazole-3-hydroxy-desthio, and prothioconazole-4-hydroxy-desthio. The LOQ value (0.004 ppm) was used for residues reported as ND or below the LOQ when calculating combined residues.

TABLE C.3.2. Tissue Residue Data from Cattle Feeding Study with Prothioconazole-Desthio.

Matrix	Collection Time (dose day)	Average Feeding Level (ppm)	Residues (ppm) [1]			
			Prothioconazole-3-hydroxy-desthio	Prothioconazole-4-hydroxy-desthio	Prothioconazole-desthio	Combined [2]
Liver	29	5.1	(0.010), 0.013, (0.005)	(0.005, 0.010, 0.002)	0.021, 0.030, 0.011	<0.041, <0.053, <0.031
		29.0	0.039, 0.055, 0.051	0.024, 0.037, 0.031	0.155, 0.178, 0.102	0.218, 0.270, 0.184
		125	0.300, 0.120, 0.132	0.171, 0.083, 0.071	1.193, 0.434, 0.414	1.66, 0.637, 0.617
Kidney	29	5.1	(0.007, 0.009, 0.004)	0.011, 0.019, (0.005)	(0.005, 0.008, 0.002)	<0.031, <0.039, <0.030
		29.0	0.054, 0.064, 0.048	0.051, 0.085, 0.056	0.033, 0.029, 0.016	0.138, 0.178, 0.120
		125	0.477, 0.186, 0.186	0.383, 0.191, 0.180	0.237, 0.090, 0.066	1.10, 0.467, 0.432
Muscle	29	5.1	(0.0002, 0.0002, 0.0001)	(0.0002, 0.0002, 0.0001)	(0.0004), ND, ND	<0.030, <0.030, <0.030
		29.0	(0.001, 0.002, 0.001)	(0.001, 0.001, 0.001)	(0.001, 0.001, 0.001)	<0.030, <0.030, <0.030
		125	0.013, (0.004, 0.004)	(0.007, 0.004, 0.003)	(0.007, 0.002, 0.003)	<0.033, <0.030, <0.030
Fat	29	5.1	(0.001, 0.0002, 0.0002)	(0.001, 0.0002, 0.0002)	(0.001, 0.001, 0.001)	<0.030, <0.030, <0.030
		29.0	(0.003, 0.003, 0.001)	(0.003, 0.004, 0.001)	0.011, (0.008, 0.005)	<0.031, <0.030, <0.030
		125	0.030, (0.005, 0.010)	0.024, (0.005, 0.010)	0.091, 0.024, 0.035	0.145, <0.044, <0.055

[1] Residues of each analyte are expressed as prothioconazole-desthio equivalents. The LOQ for each analyte was 0.01 ppm. The LODs were: 0.0001 ppm for each analyte in fat, for prothioconazole-3-hydroxy-desthio in muscle and kidney, and for prothioconazole-4-hydroxy-desthio in muscle; 0.0002 ppm for prothioconazole-3-hydroxy-desthio in liver and for prothioconazole-4-hydroxy-desthio in liver and kidney; 0.0003 ppm for prothioconazole-desthio in liver; and 0.0004 ppm for prothioconazole-desthio in muscle and kidney. ND = Not detected. Residues reported below the LOQ but above the LOD are presented in parentheses.
[2] Total combined residues of prothioconazole-desthio, prothioconazole-3-hydroxy-desthio, and prothioconazole-4-hydroxy-desthio. The LOQ value (0.01 ppm) was used for residues reported as ND or below the LOQ when calculating combined residues.

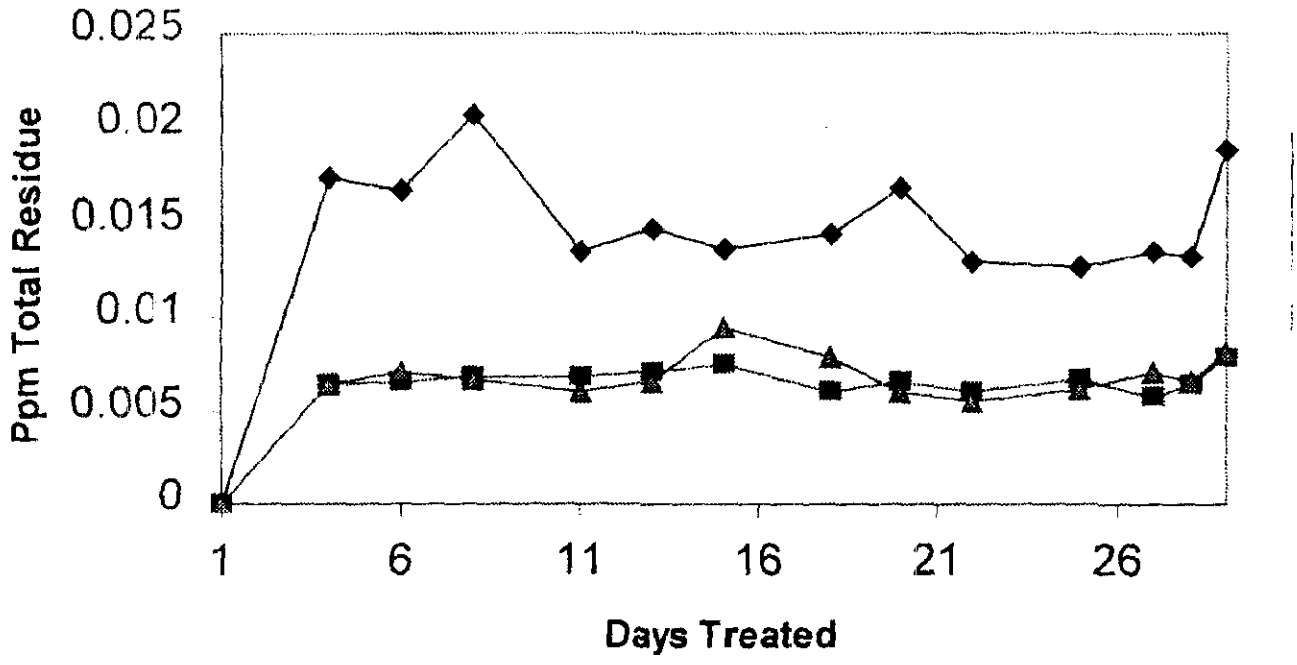
TABLE C.4. Summary of Milk and Tissue Residue Data from Cattle Feeding Study with Prothioconazole-Desthio.

Matrix	Feeding Level (ppm)	Combined Prothioconazole-Desthio Residue Levels (ppm) [1]					
		n	Min.	Max.	Median (STMdR[2])	Mean (STMR[3])	Std. Dev.
Whole Milk	29.0	42	<0.012	<0.012	0.006	0.006	<0.001
	125	42	<0.012	0.021	0.006	0.009	0.004
Liver	5.1	3	<0.031	<0.053	0.031	0.033	0.014
	29.0	3	0.184	0.270	0.218	0.224	0.043
	125	3	0.617	1.66	0.637	0.973	0.599
Kidney	5.1	3	<0.030	<0.039	0.021	0.022	0.007
	29.0	3	0.120	0.178	0.138	0.145	0.030
	125	3	0.432	1.10	0.467	0.666	0.374
Muscle	5.1	3	<0.030	<0.030	0.015	0.015	0
	29.0	3	<0.030	<0.030	0.015	0.015	0
	125	3	<0.030	<0.033	0.015	0.018	0.005
Fat	5.1	3	<0.030	<0.030	0.015	0.015	0
	29.0	3	<0.030	<0.031	0.015	0.017	0.003
	125	3	0.044	0.145	0.045	0.075	0.061

[1] For the calculation of minimum and maximum values, the LOQ value for each analyte (0.004 ppm for milk and 0.01 ppm for tissues) was used for residues reported as ND or <LOQ in Tables C.3.1 and C.3.2. For calculation of the median, mean, and standard deviation, ½ LOQ was used for residues reported as ND or below the LOQ.
[2] STMdR = Supervised Trial Median Residue.
[3] STMR = Supervised Trial Mean Residue.

FIGURE C.1. Combined Residues in Whole Milk from the Highest Dosing Level as a Function of Time.

The figure below was copied without alteration from MRID 46246214.



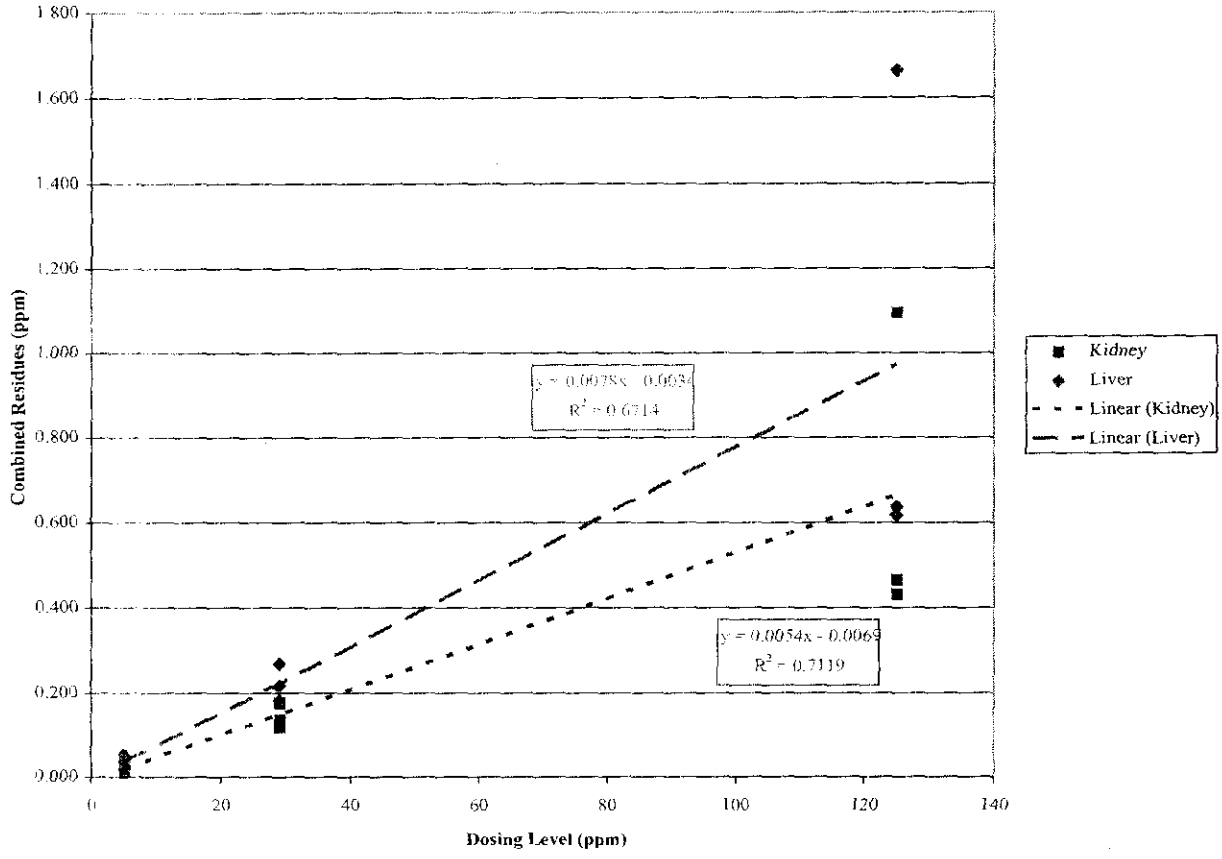


FIGURE C.2. Linear Regression of Combined Residues on Feeding Level in Kidney and Liver

D. CONCLUSION

The submitted dairy cattle feeding study is adequate to demonstrate the magnitude of residues of prothioconazole-desthio, its metabolites prothioconazole-desthio-3-hydroxy, and prothioconazole-desthio-4-hydroxy, and its metabolites hydrolyzable to these compounds in cattle commodities. The study indicates that there is potential for transfer of residues to milk and tissues except muscle. The feeding study reflects dietary levels of prothioconazole-desthio at 5.1, 29.0, and 125 ppm. Residues of prothioconazole-desthio, prothioconazole-desthio-3-hydroxy, and prothioconazole-desthio-4-hydroxy were each <LOQ in milk samples from dairy cows dosed with prothioconazole-desthio at 29.0 ppm in the diet, in fat from dairy cows dosed at 5.1 ppm, and in muscle from dairy cows dosed at 5.1 and 29.0 ppm. Quantifiable residues of prothioconazole-desthio and metabolites were observed in kidney and liver samples at all three dose levels, and were proportional to the dosing level. An acceptable data collection method was used for quantitation of residues in milk and tissues.

E. REFERENCES

None.

F. DOCUMENT TRACKING

RDI: S.Funk (23/06/2006); L. Cheng (28/06/2006).
Petition Number: PP#4F6830
DP Barcode: D303508
PC Code: 113961

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Appendix 1. Livestock Dietary Burden Calculations.

Note: Dietary burden calculations based on highest combined residues of prothioconazole and prothioconazole-desthio from field trials.

				Maximum % of Diet				% of Diet Used				Dietary Burden, ppm			
Crop	Commodity	Residue	%DM	Beef	Dairy	Poultry	Swine	Beef	Dairy	Poultry	Swine	Beef	Dairy	Poultry	Swine
Grain	Aspirated grain fractions	11.025	85	20	20	--	20	20	20	--	20	2.59	2.59	0.00	2.21
Wheat	forage	6.987	25	25	60	--	--	25	60	--	--	6.99	16.77	0.00	0.00
Barley	Hay	6.59	88	25	60	--	--	25	20	--	--	1.87	1.50	0.00	0.00
Peanut	Hay	4.458	85	25	50	--	--	25	0	--	--	1.31	0.00	0.00	0.00
Wheat	Hay	3.571	88	25	60	--	--	5	0	--	--	0.20	0.00	0.00	0.00
Wheat	Straw	1.96	88	10	10	--	--	0	0	--	--	0.00	0.00	0.00	0.00
Barley	Straw	1.871	89	10	10	--	--	0	0	--	--	0.00	0.00	0.00	0.00
Rice	Straw	1.277	90	10	10	--	--	0	0	--	--	0.00	0.00	0.00	0.00
Rice	Hulls	0.8404	90	10	10	15	--	0	0	15	--	0.00	0.00	0.13	0.00
Cowpea	Seed	0.684	88	20	20	10	50	0	0	10	50	0.00	0.00	0.07	0.34
Pea, field	Seed	0.684	90	20	20	20	20	0	0	20	20	0.00	0.00	0.14	0.14
Rice	Bran	0.222	90	15	15	25	15	0	0	25	10	0.00	0.00	0.06	0.02
Rice	Grain	0.222	88	40	40	60	65	0	0	30	0	0.00	0.00	0.07	0.00
Barley	Grain	0.158	88	50	40	75	80	0	0	0	0	0.00	0.00	0.00	0.00
Peanut	Meal	0.158	85	15	15	25	15	0	0	0	0	0.00	0.00	0.00	0.00
Wheat	Milled byproducts	0.108	88	40	50	50	50	0	0	0	0	0.00	0.00	0.00	0.00
Canola	Meal	0.097	88	15	15	15	15	0	0	0	0	0.00	0.00	0.00	0.00
Wheat	Grain	0.061	89	50	40	80	80	0	0	0	0	0.00	0.00	0.00	0.00
							Total	100	100	100	100	12.97	20.86	0.45	2.71

Primary Evaluators

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In the absence of signatures, this document is considered to be a draft with deliberative material for internal use only.

This DER was originally prepared under contract by Dynamac Corporation (20440 Century Boulevard, Suite 100; Germantown, MD 20874; submitted 06/03/2005). The DER has been reviewed by the Health Effects Division (HED) and Health Canada's Pest Management Regulatory Agency (PMRA). The DER has been revised to reflect current Office of Pesticide Programs (OPP) policies and Directive 98-02.

STUDY REPORT:

46246213 Duah, F. (2004) A 28-Day Feeding Study with JAU6476 in Dairy Cattle. Lab Project Numbers: J6060401, 200715. Unpublished study prepared by Bayer CropScience and Genesis Midwest Laboratories. 498 p.

EXECUTIVE SUMMARY:

Prothioconazole was administered orally (via gelatin capsules) to three groups of dairy cattle (3 cows per group) once daily for 29 consecutive days. Dosing was made at levels equivalent to 9.9, 29.5, and 98.4 ppm in the feed. The dosing levels correspond to 0.5-fold, 1.4-fold, and 4.7-fold the anticipated dietary burden. Milk and tissue samples were analyzed using the proposed enforcement method for animal commodities, which was an LC-MS/MS method (Bayer Report No. 200537) This method determined residues of prothioconazole, prothioconazole-desthio, and prothioconazole-4-hydroxy, plus any metabolites hydrolyzable to these compounds. Tissue samples (except fat) were extracted with acetonitrile (ACN)/water and aqueous L-cysteine HCl. Fat samples were first extracted with n-hexane, then ACN, L-cysteine HCl and acetone. All

samples (including milk and cream) were hydrolysed with aqueous HCl, then partitioned with methylene chloride and acetone before LC-MS/MS analysis. The method was adequate for data collection based on acceptable concurrent method recovery data. The storage intervals for all matrices except fat were reported to be <30 days, therefore no storage stability data is needed for these matrices. Fat samples from the lowest feeding level (0.5-fold) were stored frozen for up to 86 days prior to analysis. For the 1.4-fold and 4.7-fold feeding levels, samples were stored for 43 and 37 days, respectively. A supporting storage stability study indicated that residues of prothioconazole, prothioconazole-desthio, and prothioconazole-4-hydroxy were stable for 27 days (<30% decline). After 89 days in storage, residues of prothioconazole-4-hydroxy showed a 33% decline. The 1.4-fold dose group (29.5 ppm) was closest to the anticipated dietary burden (21 ppm). Confirmatory data will be generated to confirm the stability of the prothioconazole-4-hydroxy in fat for a duration of 45 days.

The combined residues of prothioconazole, prothioconazole-desthio and prothioconazole-4-hydroxy in milk and tissues ranged from 0.065 to 0.150 ppm at the 9.9 ppm feeding level; 0.015 to 0.303 ppm at the 29.5 ppm feeding level; and 0.015 to 1.156 ppm at the 98.4 ppm feeding level. Because low residue levels were observed in milk and muscle samples from both the mid and high dose groups, samples from the low dose groups were not analyzed.

Combined residues in kidney and liver were generally found to have a linear relationship with the dosing levels. Quantifiable residues (of prothioconazole) were observed in only two samples of milk from the highest dosing level. However, detectable residues of prothioconazole were observed in several samples. Based on these residues, it appeared that residues had reached a plateau by within the first week of dosing.

STUDY/WAIVER ACCEPTABILITY/DEFICIENCIES/CLARIFICATIONS:

The feeding study data are classified as scientifically acceptable. The acceptability of this study for regulatory purposes is addressed in the forthcoming U.S. EPA Residue Chemistry Summary Document [DP Barcode D303508], and in Canada's Regulatory Decision Document.

COMPLIANCE:
Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided. No deviations from regulatory requirements were reported which would have an impact on the validity of the study.

A. BACKGROUND INFORMATION

Prothioconazole is a broad-spectrum systemic fungicide intended for the control of many diseases in different crops such as cereals, oilseed rape or peanuts. The biochemical mode of action is the inhibition of the demethylation of lanosterol or 24-methylene-dihydrolanosterol, which are precursors of sterols in fungi. The chemical has been submitted for joint EPA and PMRA review by Bayer CropScience. The crops proposed for joint review include barley, the

dried shell and bean subgroup, the oilseed crop group, and wheat. Uses on peanuts and rice are being proposed for the U.S. only.

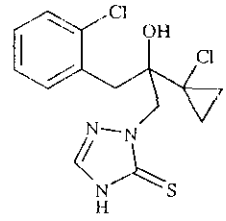
TABLE A.1. Test Compound Nomenclature.	
Chemical structure	
Common name	Prothioconazole (ISO accepted)
Company experimental name	JAU6476
IUPAC name	2-[(2*RS*)-2-(1-chlorocyclopropyl)-3-(2-chlorophenyl)-2-hydroxypropyl]-2*H*-1,2,4-triazole-3(*4H*)-*thione*
CAS name	2-[2-(1-chlorocyclopropyl)-3-(2-chlorophenyl)-2-hydroxypropyl]-1,2-dihydro-3*H*-1,2,4-triazole-3-thione
CAS registry number	178928-70-6
PC Code	113961
End-use product (EP)	Proline® 480 SC (4 lb/gal suspension concentrate), EPA File Symbol 264-IEL

TABLE A.2. Physicochemical Properties of the Technical Grade Test Compound.			
Parameter	Value		References
Melting range	139.1 to 144.5°C		MRID 46246003/CES
pH	5.8 (1% solution)		MRID 46246003/CES
Density at 20°C	1.36 g/mL		MRID 46246003/CES
Water solubility at 20°C	pH 4 8 9	mg/L 5 300 2000	MRID 46246003/CES
Solvent solubility at 20°C	Solvent Acetone Acetonitrile Dichloromethane Dimethysulfoxide Ethyl acetate n-Heptane 1-Octanol Polyethylene glycol 2-Propanol Xylene	g/L >250 69 88 126 >250 <0.1 58 >250 87 8	MRID 46246003/CES
Vapor pressure at 20 or 25°C	$<4 \times 10^{-7}$ Pa (calculated from determinations at 70°C)		MRID 46246003/CES
Dissociation constant, pK_a	6.9 (calculated from K_{OW})		MRID 46246003/CES
Octanol/water partition coefficient, at 20°C	pH unbuffered water 4 7 9	$Log(K_{OW})$ 4.05 4.16 3.82 2.00	MRID 46246003/CES
UV/visible absorption spectrum	Peak maxima at 257 nm. No absorption at > 300 nm.		MRID 46246003/CES

CES - Chemistry Evaluation Section of PMRA

B. EXPERIMENTAL DESIGN

The in-life phase of the feeding study was conducted at Genesis Midwest Laboratories (Neillsville, WI) from 1/15/03 to 2/26/03.

B.1. Livestock

TABLE B.1.1. Description of Livestock Used in the Feeding Study.					
Species	Breed	Age (years)	Weight at study initiation (kg)	Health status	Description of housing/holding area
Dairy Cattle (*Bos taurus*)	Holstein	3-7	463-647	Cattle were free from any injury or illness that would preclude use in the study.	Cattle were housed individually in stalls (4 ft X 7 ft) indoors in a barn for acclimation and study periods, with temperatures between 10-18 °C, and relative humidity between 54-84%.

TABLE B.1.2. Dietary Regime.

Composition of Diet	Treatment group	Average feed consumption (kg/cow/day) [1]	Water	Acclimation period	Predosing
Twice daily: dairy ration, 4 kg, and alfalfa hay cubes, 8 kg; Once daily: baled hay, 2 kg	Low dose	21.6	Tap water, *ad libitum*	14 days	None
	Mid dose	21.7			
	High Dose	22.5			

[1] Feed consumption is expressed on a dry weight basis.

TABLE B.1.3. Dosing Regime. [1]

Treatment group	Treatment Type	Level of administered dose (mg/cow/day)	Residue intake in diet (ppm)	Vehicle	Timing/Duration
Control	Oral	0	0	Gelatin capsule administered via balling gun	Once daily for 29 days
Low dose		213.8	9.9		
Mid dose		639.9	29.5		
High dose		2215.7	98.4		

[1] The values for administered dose and residue intake in diet reflect the averages for the group over the study period.

TABLE B.1.4 Sample Collection.

Treatment group	Milk collected	Amount of milk produced during treatment period (kg/cow)	Amount of milk produced during acclimation period (kg/cow)	Urine, feces and cage wash collected	Interval from last dose to sacrifice (days)	Tissues collected and analyzed
Low dose	Twice daily (AM and PM) on study days -1, 0, 4, 8, 12, 16, 18, 20, 22, 24, 26, and 28. Day 26 milk samples from the high dose group were separated into cream and skim milk.	15.8-24.3	14.7-23.0	Not collected	Within 23 hours	Liver (each lobe), kidney (both), *muscle (composite* loin, round and flank), and fat (omental, renal, *and subcutaneous)*
Mid dose		17.9-21.5	17.0-20.4			
High dose		16.0-22.2	15.5-18.5			

B.2. Sample Handling and Preparation

After collection, tissue samples were weighed, cubed and frozen. The tissue samples were homogenized in the presence of dry ice, then frozen again. Milk, cream and skim milk samples were prepared with a commercial centrifugal cream separator. Milk (whole, skim, and cream) and tissue samples were placed in frozen storage after collection at GML. All samples were stored frozen for up to 8 days, then shipped on dry ice via FedEx to Bayer CropScience (Stilwell, KS), where samples were placed in storage at < -15 °C until preparation for analysis. Milk samples were thawed prior to homogenization.

B.3. Analytical Methodology

Milk and tissue samples were analyzed using the proposed enforcement method for animal commodities, which was an LC-MS/MS method (Bayer Report No. 200537). This method determined residues of prothioconazole, prothioconazole-desthio, and prothioconazole-4-hydroxy, plus any metabolites hydrolyzable to these compounds.

Briefly, samples of liver, kidney, and muscle were extracted with acetonitrile (ACN) and 25% aqueous L-cysteine HCl; internal standard solution was added to the extract. Internal standard solution consisted 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 were extracted with n-hexane and then with a mixture of ACN, 25% aqueous L-cysteine HCl, and acetone; the combined extracts were allowed to separate and internal standard solution was added to the aqueous phase. Samples of milk and cream were mixed with internal standard solution. For all matrices, the extract/sample was hydrolyzed using aqueous HCl and the hydrolysate was partitioned with methylene chloride and acetone. The organic phase was concentrated to aqueous, mixed with ACN and water, and analyzed by LC-MS/MS. Samples were analyzed for residues of prothioconazole, prothioconazole-desthio, and prothioconazole-4-hydroxy, and all results were reported in prothioconazole equivalents.

The LOQ was defined as the lowest spiking level at which satisfactory recovery of an analyte is obtained. The validated LOQs were 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 LOD was defined as the lowest concentration of an analyte that is determined to be statistically different from the blank. The calculated LODs for prothioconazole were 0.001 ppm in milk, skim milk, and liver, 0.002 ppm in muscle, 0.003 ppm in cream and kidney, and 0.012 ppm in fat. The LODs for prothioconazole-desthio were 0.001 ppm in milk and muscle, 0.002 ppm in skim milk, 0.003 ppm in kidney and liver, 0.004 ppm in cream, and 0.005 ppm in fat. The LODs for prothioconazole-4-hydroxy were 0.001 ppm in liver and muscle, 0.002 ppm in skim milk, 0.003 ppm in milk and kidney, 0.004 ppm in cream, and 0.008 ppm in fat.

C. RESULTS AND DISCUSSION

The storage intervals for all matrices except fat were less than 30 days. For fat, the 9.9 ppm treatment group samples (0.5-fold the anticipated dietary burden) were stored for 86 days (from collection to analysis), the 29.5 ppm treatment group (1.4-fold the anticipated dietary burden) was stored for 43 days, and the 98.4 ppm treatment group (4.7-fold the anticipated dietary burden) was stored for 37 days. To support the storage conditions and intervals for the fat samples, a storage stability study was conducted. Control samples of fat were separately spiked with prothioconazole, prothioconazole-desthio, and prothioconazole-4-hydroxy at 1.00 ppm and stored frozen. Samples were analyzed after storage intervals of 27 and 89 days. Sample storage conditions and intervals are summarized in TABLE C.2.1. The results of the storage stability study are presented in TABLE C.2.2 and the concurrent method recovery data that were generated in conjunction with the storage stability study are presented in TABLE C.1.2.

Concurrent recoveries were acceptable at the 27 day storage interval for all analytes (76-98%), while the recoveries at the 89 day storage interval were low for prothioconazole and prothioconazole-4-hydroxy (33-44%). Although the actual residues of prothioconazole and prothioconazole-4-hydroxy were low, correcting for the similary low concurrent recoveries indicated stability of the stored samples. The data indicated that residues of prothioconazole and prothioconazole-desthio were stable in cattle fat up to 89 days of frozen storage. Residues of prothioconazole-4-hydroxy were stable at 27 days of frozen storage, and reasonably stable at 89 days (~67% corrected recoveries). Method performance for prothioconazole-4-hydroxy was poor for both the concurrent and stored samples from the 3-month storage interval. The applicant stated that some slight modifications to the method were made in an attempt to improve performance. However, recoveries did not improve significantly. If the concurrent recoveries from the storage stability study are taken into account, the samples that had the lowest recoveries (the ~3 month storage interval) represent the 9.9 ppm dose group (0.5-fold the anticipated dietary burden). The dose group that reflected the anticipated dietary burden most closely was the 29.5 ppm treatment group (1.4-fold the anticipated dietary burden), where the samples were only stored for 43 days. Confirmatory data will be generated by the applicant (and provided to both agencies) to demonstrate the stability of prothioconazole and prothioconazole-4-hydroxy residues in fat for a duration of 45 days. At this time, the apparent loss of prothioconazole-4-hydroxy will not be corrected for, since the TRRs and absolute values of the metabolite in fat from the goat metabolism study (both radiolabels) were low (3.6-8.3% of the TRRs; 0.006-0.014 ppm).

The analytical method used is adequate for data collection based on acceptable concurrent method recovery data. Concurrent recoveries from milk and cow tissues, spiked with each analyte at 0.005 ppm (LOQ) and 0.010 ppm for milk; 0.010 ppm (LOQ) for skim milk, cream, and muscle; 0.010 ppm (LOQ) and 0.60 ppm for liver; 0.010 ppm (LOQ), 0.050 ppm, and 0.80 ppm for kidney; and 0.050 ppm (LOQ) and 0.080 ppm for fat, were generally within the acceptable 70-120% range (SD<20%) for all matrices. Concurrent method recovery data are presented in TABLE C.1.1. Both concurrent and stored sample recoveries were corrected for interference (in the controls) before percent recoveries were calculated. Apparent residues were <LOQ in all control samples. Adequate sample calculations and chromatograms were provided..

The results of the feeding study are reported in TABLE C.3 and a summary of the combined residues of prothioconazole, prothioconazole-desthio, and prothioconazole-4-hydroxy in milk and cattle tissues is presented in TABLE C.4. In milk samples, residues of each analyte were below the LOQ (<0.005 ppm) in all samples from the 29.5- and 98.4-ppm dose groups, with the exception of two milk samples from Day 24, which bore quantifiable residues of prothioconazole at 0.006 ppm. Detectable residues of prothioconazole were observed in several samples and based on these residues, it appeared that prothioconazole residues in milk had reached a plateau within the first week of dosing. Residues of each analyte were below the LOQ (<0.01 ppm) in the skim milk and cream samples from Day 26 from the high dose group. Because residue levels were below the LOQ in all samples from the 29.5-ppm dosing group, no milk samples from the 9.9-ppm dose group were analyzed.

In fat samples, residues of each analyte were below the LOQ (<0.05 ppm) in all samples from the 9.9-, 29.5-, and 98.4-ppm dose group, with the exception of one sample from the high dose group which bore quantifiable prothioconazole residues at 0.062 ppm.

In kidney samples, residues of prothioconazole were 0.039-0.062 ppm, 0.100-0.176 ppm, and 0.394-0.790 ppm in samples from the 9.9-, 29.5-, and 98.4-ppm dose groups, respectively. Residues of prothioconazole-desthio were below the LOQ (0.01 ppm) in all samples from all three dose groups, with the exception of two samples from the high dose group (each at 0.011 ppm). Residues of prothioconazole-4-hydroxy were 0.013-0.017 ppm, 0.037-0.063 ppm, and 0.172-0.356 ppm in samples from the 9.9-, 29.5-, and 98.4-ppm dose groups, respectively. For the same respective dose groups, combined residues were 0.065-0.089 ppm, 0.147-0.249 ppm, and 0.580-1.156 ppm.

In liver samples, residues of prothioconazole were 0.028-0.063 ppm, 0.089-0.120 ppm, and 0.256-0.467 ppm in samples from the 9.9-, 29.5-, and 98.4-ppm dose groups, respectively. Residues of prothioconazole-desthio were below the LOQ (0.01 ppm), <0.01-0.011 ppm, and 0.020-0.030 ppm in samples from the 9.9-, 29.5-, and 98.4-ppm dose groups, respectively. Residues of prothioconazole-4-hydroxy were 0.043-0.054 ppm, 0.137-0.181 ppm, and 0.359-0.518 ppm in samples from the 9.9-, 29.5-, and 98.4-ppm dose groups, respectively. For the same respective dose groups, combined residues were 0.081-0.127 ppm, 0.237-0.303 ppm, and 0.636-1.005 ppm.

In muscle samples, residues of each analyte were below the LOQ (<0.01 ppm) in all samples from the 29.5 and 98.4 ppm dose groups. Because residue levels were below the LOQ in all samples from the 29.5 ppm dosing group, no muscle samples from the 9.9-ppm dose group were analyzed.

Combined residues in kidney and liver were generally found to have a linear relationship with the dosing levels. A graph of the linear regression of combined residues over feeding level in kidney and liver is presented in Figure C.1.

TABLE C.1.1. Concurrent Method Recoveries from Various Cattle Matrices using an LS-MS/MS Method (Bayer Report No. 200537).[1]

Matrix	Analyte	Spiking Level (ppm)	Recoveries Obtained (%)	Recovery (%)		
				Mean	SD	CV
Milk	Prothioconazole	0.0050	101, 102, 103, 103, 104, 104, 104, 104, 104, 106, 106, 106,107, 111, 115	105	4	3
		0.010	99, 101, 104	102	3	2
	Prothioconazole-desthio	0.0050	91, 100, 101, 101, 101, 104, 104, 105, 107, 109, 109, 110, 110, 110, 116	105	6	6
		0.010	102, 106, 108	105	3	3
	Prothioconazole-4-hydroxy [2]	0.0050	69, 72, 76, 80, 80, 85, 87, 95, 99, 102, 105, 111, 113, 116, 117	94	17	18
		0.010	71, 77, 93	80	11	14

TABLE C.1.1. Concurrent Method Recoveries from Various Cattle Matrices using an LS-MS/MS Method (Bayer Report No. 200537).[1]

Matrix	Analyte	Spiking Level (ppm)	Recoveries Obtained (%)	Recovery (%)		
				Mean	SD	CV
Skim milk	Prothioconazole	0.010	100, 103, 103	102	2	2
	Prothioconazole-desthio	0.010	97, 100, 100	99	2	2
	Prothioconazole-4-hydroxy [2]	0.010	83, 86, 89	86	3	3
Cream	Prothioconazole	0.010	98, 103, 104	102	3	3
	Prothioconazole-desthio	0.010	103, 106, 112	107	5	4
	Prothioconazole-4-hydroxy [2]	0.010	63, 65, 72	67	5	7
Muscle	Prothioconazole	0.010	91, 91, 92, 92	92	1	1
	Prothioconazole-desthio	0.010	100, 103, 104, 106	103	3	2
	Prothioconazole-4-hydroxy	0.010	98, 99, 99, 101	99	1	1
Liver	Prothioconazole	0.010	96, 98, 99, 101	99	2	2
		0.60	89, 91, 92	91	2	2
	Prothioconazole-desthio	0.010	105, 115, 116, 117	113	6	5
		0.60	104, 109, 113	109	5	4
	Prothioconazole-4-hydroxy	0.010	101, 102, 104, 104	103	2	1
		0.60	93, 95, 99	96	3	3
Kidney	Prothioconazole	0.010	77, 78, 84	80	4	5
		0.050	96, 101, 106, 109	103	6	6
		0.80	81, 83, 87	84	3	4
	Prothioconazole-desthio	0.010	106, 111, 112	110	3	3
		0.050	92, 101, 102, 106	100	6	6
		0.80	104, 104, 107	105	2	2
	Prothioconazole-4-hydroxy	0.010	93, 94, 99	95	3	3
		0.050	102, 106, 111, 112	108	5	4
		0.80	97, 101, 101	99	2	2
Fat	Prothioconazole	0.050	83, 86, 87, 93	87	4	5
		0.080	91, 92, 93	92	1	1
	Prothioconazole-desthio	0.050	91, 92, 93, 100	94	4	4
		0.080	102, 104, 104	103	1	1
	Prothioconazole-4-hydroxy	0.050	78, 79, 79, 86	81	4	5
		0.080	84, 85, 86	85	1	1

[1] Recoveries were corrected for any apparent residues in controls.

[2] Residues of prothioconazole-4-hydroxy were quantitated using external standards only for milk, skim milk, and cream. An internal standard for prothioconazole-4-hydroxy was not available at the time the validation was conducted.

TABLE C.1.2. Concurrent Recoveries of Prothioconazole, Prothioconazole-Desthio, and Prothioconazole-4-Hydroxy from Cattle Fat for Storage Stability Study.

Analyte	Spike level (ppm)	Storage interval (days)	Recoveries (%)	Mean
Prothioconazole	1.00	27	76, 77	77
Prothioconazole	1.00	89	34, 44	39
Prothioconazole-desthio	1.00	27	97, 98	98
Prothioconazole-desthio	1.00	89	88, 90	89
Prothioconazole-4-hydroxy	1.00	27	84, 84	84
Prothioconazole-4-hydroxy	1.00	89	33, 41	37

TABLE C.2.1. Summary of Storage Conditions.

Matrix	Storage Temp. (°C)	Treatment Level (ppm)	Actual Storage Duration (days) [1]	Limit of Demonstrated Storage Stability (days)
Milk	< -15	9.9, 29.5 and 98.4	24-27	Not necessary as samples were stored for <30 days.
Skim milk			14	
Cream			18	
Liver			22-24	
Kidney			25-27	
Muscle			20-29	
Fat		9.9	86	Concurrent stability data indicate that residues of prothioconazole and prothioconazole-desthio are stable for up to 89 days. Residues of prothioconazole-4-hydroxy are stable in fat for 27 days, and reasonably stable in fat for 89 days.
		29.5	43	
		98.4	37	

[1] Extracts were stored for 0-6 days prior to analysis, with the exception of fat samples from the mid-dose group which were stored 32 days prior to analysis.

TABLE C.2.2. Stability of Prothioconazole Residues in Cattle Fat Following Storage at <-15°C.

Analyte	Spike level (ppm)	Storage interval (days)	Recovered residues in stored samples (ppm)	Corrected % recovery [1]
Prothioconazole	1.00	0	0.654, 0.673, 0.690	--
		27	0.616, 0.712, 0.721	80, 93, 93
		89	0.366, 0.370, 0.393	87, 87, 93
Prothioconazole-desthio	1.00	0	0.923, 0.940, 0.948	--
		27	0.926, 0.968, 1.008	95, 100, 104
		89	0.934, 0.938, 0.961	105, 106, 108
Prothioconazole-4-hydroxy	1.00	0	0.616, 0.642, 0.676	--
		27	0.597, 0.628, 0.639	71, 75, 76
		89	0.245, 0.248 , 0.253	66, 67, 68

[1] Corrected for concurrent method recoveries.

TABLE C.3.1. Residue Data for Whole Milk, Skim Milk, and Cream from Cattle Feeding Study with Prothioconazole.

Collection Time (dose day)	Average Feeding Level (ppm)	Matrix	Residues (ppm) [1]			
			Prothioconazole	Prothioconazole-desthio	Prothioconazole-4-hydroxy	Combined [2]
0 (predose)	29.5	Whole Milk	ND, ND, ND	ND, ND, ND	ND, ND, ND	0.015, 0.015, 0.015
4	29.5	Whole Milk	(0.001), ND, (0.002)	ND, ND, ND	ND, ND, ND	0.015, 0.015, 0.015
10	29.5	Whole Milk	(0.001), ND, (0.002)	ND, ND, ND	ND, ND, ND	0.015, 0.015, 0.015
12	29.5	Whole Milk	(0.001, 0.001, 0.002)	ND, ND, ND	ND, ND, ND	0.015, 0.015, 0.015
16	29.5	Whole Milk	(0.001), ND, (0.002)	ND, ND, ND	ND, ND, ND	0.015, 0.015, 0.015
18	29.5	Whole Milk	(0.001), ND, (0.002)	ND, ND, ND	ND, ND, ND	0.015, 0.015, 0.015
20	29.5	Whole Milk	ND, ND, (0.002)	ND, ND, ND	ND, ND, ND	0.015, 0.015, 0.015
22	29.5	Whole Milk	(0.001, 0.001, 0.001)	ND, ND, ND	ND, ND, ND	0.015, 0.015, 0.015
24	29.5	Whole Milk	(0.001, 0.001, 0.003)	ND, ND, ND	ND, ND, ND	0.015, 0.015, 0.015
26	29.5	Whole Milk	(0.001, 0.002, 0.002)	ND, ND, ND	ND, ND, ND	0.015, 0.015, 0.015
28	29.5	Whole Milk	(0.001), ND, (0.002)	ND, ND, ND	ND, ND, ND	0.015, 0.015, 0.015
0 (predose)	98.4	Whole Milk	ND, ND, ND	ND, ND, ND	ND, ND, ND	0.015, 0.015, 0.015
4	98.4	Whole Milk	0.005, (0.003, 0.004)	ND, ND, ND	ND, ND, ND	0.015, 0.015, 0.015
10	98.4	Whole Milk	(0.004, 0.003, 0.004)	ND, ND, ND	ND, ND, ND	0.015, 0.015, 0.015
12	98.4	Whole Milk	(0.005, 0.004, 0.004)	ND, ND, ND	ND, ND, ND	0.015, 0.015, 0.015
16	98.4	Whole Milk	(0.005, 0.003, 0.004)	ND, ND, ND	ND, ND, ND	0.015, 0.015, 0.015
18	98.4	Whole Milk	(0.005, 0.003, 0.003)	ND, ND, ND	ND, ND, ND	0.015, 0.015, 0.015
20	98.4	Whole Milk	(0.004, 0.003, 0.003)	ND, ND, ND	ND, ND, ND	0.015, 0.015, 0.015
22	98.4	Whole Milk	(0.004, 0.003, 0.004)	ND, ND, ND	ND, ND, ND	0.015, 0.015, 0.015
24	98.4	Whole Milk	0.005, 0.006, 0.006	ND, ND, ND	ND, ND, ND	0.015, 0.016, 0.016
26	98.4	Whole Milk	0.005, (0.003, 0.005)	ND, ND, ND	ND, ND, ND	0.015, 0.015, 0.015
26	98.4	Skim Milk	(0.004, 0.004, 0.003)	ND, ND, ND	ND, ND, ND	0.030, 0.030, 0.030

TABLE C.3.1. Residue Data for Whole Milk, Skim Milk, and Cream from Cattle Feeding Study with Prothioconazole.

Collection Time (dose day)	Average Feeding Level (ppm)	Matrix	Residues (ppm) [1]			
			Prothioconazole	Prothioconazole-desthio	Prothioconazole-4-hydroxy	Combined [2]
26	98.4	Cream	(0.005, 0.004)	ND, ND	ND, ND	0.030, 0.030
28	98.4	Whole Milk	(0.005, 0.003, 0.004)	ND, ND, ND	ND, ND, ND	0.015, 0.015, 0.015

[1] Residues of each analyte are expressed as prothioconazole equivalents. The LOQ for each analyte was 0.005 ppm for milk and 0.01 ppm for skim milk and cream; the LODs for prothioconazole were 0.001 ppm for milk and skim milk, and 0.003 ppm for cream and kidney, the LODs for prothioconazole-desthio were 0.001 ppm for milk, 0.002 ppm for skim milk, and 0.004 ppm for cream, and the LODs for prothioconazole-4-hydroxy were 0.002 ppm for skim milk, 0.003 ppm for milk, and 0.004 ppm for cream. ND = Not detected. Residues reported below the LOQ but above the LOD are presented in parentheses.
[2] Total combined residues of prothioconazole, prothioconazole-desthio, and prothioconazole-4-hydroxy. The LOQ value (0.005 or 0.01 ppm) was used for residues reported as ND or below the LOQ when calculating combined residues.

TABLE C.3.2. Residue Data for Tissues from Cattle Feeding Study with Prothioconazole.

Matrix	Collection (dose days)	Average Feeding Level (ppm)	Residues (ppm) [1]			
			Prothioconazole	Prothioconazole-desthio	Prothioconazole-4-hydroxy	Combined [2]
Fat	29	9.9	ND, ND, ND	ND, ND, ND	ND, ND, ND	0.150, 0.150, 0.150
		29.5	ND, ND, (0.019)	ND, ND, ND	ND, ND, ND	0.150, 0.150, 0.150
		98.4	ND, ND, 0.062	(0.008, 0.006, 0.006)	ND, ND, (0.022)	0.150, 0.150, 0.162
Kidney	29	9.9	0.058, 0.062, 0.039	(0.003), ND, ND	0.013, 0.017, 0.016	0.081, 0.089, 0.065
		29.5	0.167, 0.100, 0.176	(0.005, 0.004, 0.004)	0.062, 0.037, 0.063	0.239, 0.147, 0.249
		98.4	0.470, 0.394, 0.790	0.011, 0.011, (0.009)	0.172, 0.175, 0.356	0.653, 0.580, 1.156
Liver	29	9.9	0.028, 0.048, 0.063	ND, (0.007, 0.006)	0.043, 0.045, 0.054	0.081, 0.103, 0.127
		29.5	0.120, 0.089, 0.112	(0.008), 0.011, (0.010)	0.170, 0.137, 0.181	0.300, 0.237, 0.303
		98.4	0.256, 0.294, 0.467	0.021, 0.030, 0.020	0.359, 0.424, 0.518	0.636, 0.748, 1.005
Muscle	29	29.5	ND, (0.003, 0.002)	(0.001), ND, ND	ND, ND, (0.001)	0.030, 0.030, 0.030
		98.4	(0.005, 0.005, 0.007)	(0.001), ND, ND	(0.001, 0.003, 0.002)	0.030, 0.030, 0.030

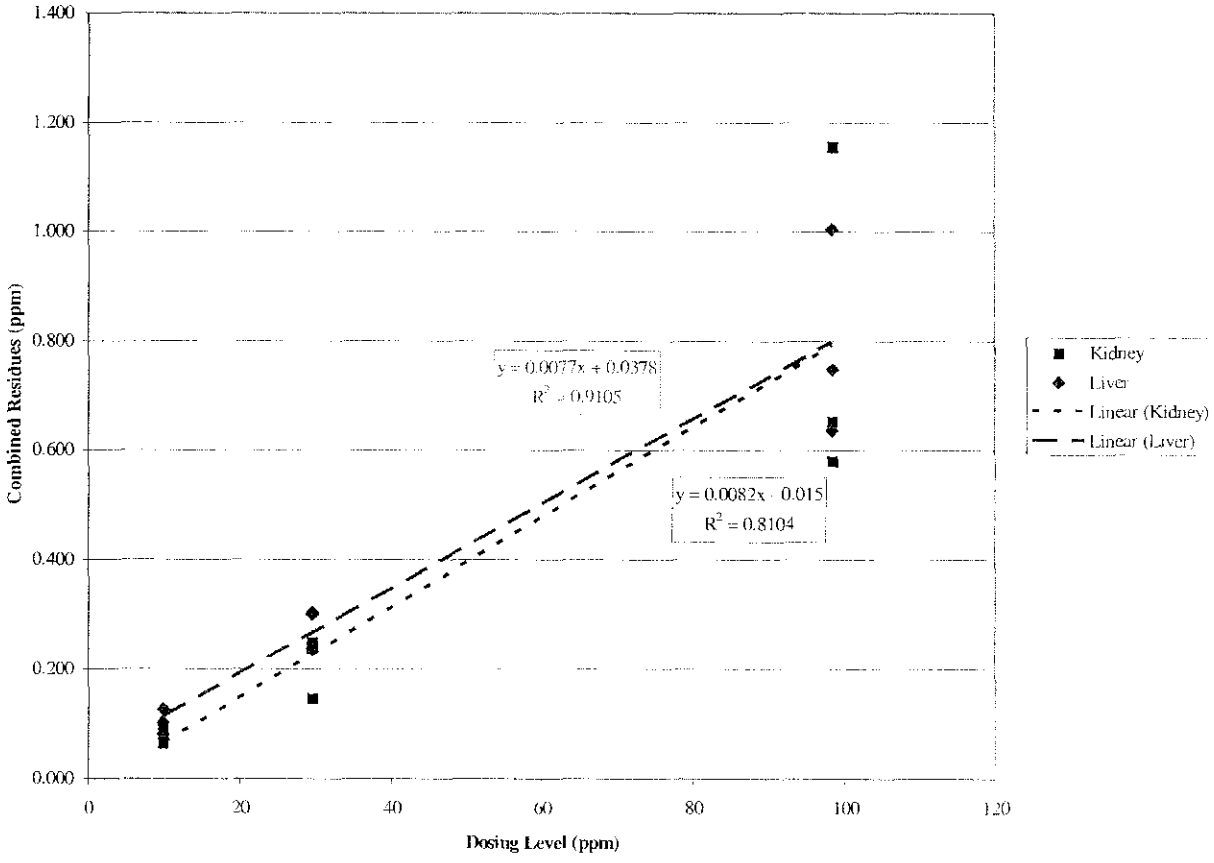
[1] Residues of each analyte are expressed as prothioconazole equivalents, and are listed respectively. The LOQ was 0.01 ppm for each analyte in all matrices except fat, and was 0.05 ppm for each analyte in fat; the LODs for prothioconazole were 0.001 ppm for liver, 0.002 ppm for muscle, 0.003 ppm for kidney, and 0.012 ppm for fat, the LODs for prothioconazole-desthio were 0.001 ppm for muscle, 0.003 ppm for kidney and liver, and 0.005 ppm for fat, and the LODs for prothioconazole-4-hydroxy were 0.001 ppm for liver and muscle, 0.003 ppm for kidney, and 0.008 ppm for fat. ND = Not detected. Residues reported below the LOQ but above the LOD are presented in parentheses.
[2] Total combined residues of prothioconazole, prothioconazole-desthio, and prothioconazole-4-hydroxy. The LOQ value (0.01 or 0.05 ppm) was used for residues reported as ND or below the LOQ when calculating combined residues.

TABLE C.4. Summary of Milk and Tissue Residue Data from Cattle Feeding Study with Prothioconazole.

Matrix	Feeding Level (ppm)	Combined Prothioconazole Residue Levels (ppm) [1]					
		n	Min.	Max.	Median (STMdR[2])	Mean (STMR[3])	Std. Dev.
Whole Milk	29.5	30	0.015	0.015	0.0075	0.0075	0
	98.4	30	0.015	0.016	0.0075	0.0077	<0.001
Fat	9.9	3	0.150	0.150	0.075	0.075	0
	29.5	3	0.150	0.150	0.075	0.075	0
	98.4	3	0.150	0.162	0.075	0.087	0.021
Kidney	9.9	3	0.065	0.089	0.076	0.073	0.012
	29.5	3	0.147	0.249	0.234	0.207	0.056
	98.4	3	0.580	1.156	0.653	0.795	0.311
Liver	9.9	3	0.081	0.127	0.098	0.099	0.023
	29.5	3	0.237	0.303	0.295	0.277	0.034
	98.4	3	0.636	1.005	0.748	0.796	0.189
Muscle	29.5	3	0.030	0.030	0.015	0.015	0
	98.4	3	0.030	0.030	0.015	0.015	0

[1] For the calculation of minimum and maximum values, the LOQ value for each analyte (0.005 ppm for milk, 0.010 ppm for kidney, liver, and muscle, and 0.050 ppm for fat) was used for residues reported as ND or <LOQ in TABLES C.3.1 and C.3.2. For calculation of the median, mean, and standard deviation, ½ LOQ was used for residues reported as ND or below the LOQ.
[2] STMdR = Supervised Trial Median Residue.
[3] STMR = Supervised Trial Mean Residue.

FIGURE C.1. Linear Regression of Combined Residues on Feeding Level in Kidney and Liver.



D. CONCLUSION

The submitted dairy cattle feeding study is adequate to demonstrate the magnitude of residues of prothioconazole, the metabolites prothioconazole-desthio and prothioconazole-4-hydroxy, and the metabolites hydrolyzable to these compounds in cattle commodities. The study indicated that there is potential for transfer of residues to milk and tissues except muscle. The feeding study reflected dietary levels of prothioconazole at 9.9, 29.5, and 98.4 ppm. Residues of prothioconazole, prothioconazole-desthio, and prothioconazole-4-hydroxy were each ≤LOQ in muscle from dairy cows dosed with prothioconazole at up to 98.4 ppm in the diet and in milk and fat from dairy cows dosed at 29.5 ppm. Quantifiable residues of prothioconazole were observed in two samples of milk from the 98.4-ppm dosing level. Maximum combined residues of prothioconazole, prothioconazole-desthio, and prothioconazole-4-hydroxy were 0.249 ppm in kidney and 0.303 ppm in liver from cows treated at 29.5 ppm; and 0.162 ppm in fat, 1.156 ppm in kidney, and 1.005 ppm in liver from cows treated at 98.4 ppm. Residues in liver and kidney

were proportional to the dosing level. An acceptable method was used for quantitation of residues in milk and tissues.

E. REFERENCES

None.

F. DOCUMENT TRACKING

RDI:.Suzan Mathew (PMRA) 23/01/2006; Henri Bietlot (PMRA) 24/01/2006; Stephen Funk (IO) 13/03/2006; Leung Cheng (RAB3).
Petition Number: PP#4F6830
DP Barcode: D303508
PC Code: 113961

Template Version September 2003

Appendix 1. Livestock Dietary Burden Calculations.

				Maximum % of Diet				% of Diet Used				Dietary Burden, ppm			
Crop	Commodity	Residue	%DM	Beef	Dairy	Poultry	Swine	Beef	Dairy	Poultry	Swine	Beef	Dairy	Poultry	Swine
Grain	Aspirated grain fractions	11.025	85	20	20	--	20	20	20	--	20	2.59	2.59	0.00	2.21
Wheat	forage	6.987	25	25	60	--	--	25	60	--	--	6.99	16.77	0.00	0.00
Barley	Hay	6.59	88	25	60	--	--	25	20	--	--	1.87	1.50	0.00	0.00
Peanut	Hay	4.458	85	25	50	--	--	25	0	--	--	1.31	0.00	0.00	0.00
Wheat	Hay	3.571	88	25	60	--	--	5	0	--	--	0.20	0.00	0.00	0.00
Wheat	Straw	1.96	88	10	10	--	--	0	0	--	--	0.00	0.00	0.00	0.00
Barley	Straw	1.871	89	10	10	--	--	0	0	--	--	0.00	0.00	0.00	0.00
Rice	Straw	1.277	90	10	10	--	--	0	0	--	--	0.00	0.00	0.00	0.00
Rice	Hulls	0.8404	90	10	10	15	--	0	0	15	--	0.00	0.00	0.13	0.00
Cowpea	Seed	0.684	88	20	20	10	50	0	0	10	50	0.00	0.00	0.07	0.34
Pea, *field*	Seed	0.684	90	20	20	20	20	0	0	20	20	0.00	0.00	0.14	0.14
Rice	Bran	0.222	90	15	15	25	15	0	0	25	10	0.00	0.00	0.06	0.02
Rice	Grain	0.222	88	40	40	60	65	0	0	30	0	0.00	0.00	0.07	0.00
Barley	Grain	0.158	88	50	40	75	80	0	0	0	0	0.00	0.00	0.00	0.00
Peanut	Meal	0.158	85	15	15	25	15	0	0	0	0	0.00	0.00	0.00	0.00
Wheat	Millec byproducts	0.108	88	40	50	50	50	0	0	0	0	0.00	0.00	0.00	0.00
Canola	Meal	0.097	88	15	15	15	15	0	0	0	0	0.00	0.00	0.00	0.00
Wheat	Grain	0.061	89	50	40	80	80	0	0	0	0	0.00	0.00	0.00	0.00
							Total	100	100	100	100	12.97	20.86	0.45	2.71

Primary Evaluators

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Immediate Office

Date: Mar 13 2006

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Date: January 23/06

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Date: Jan 29/06

In the absence of signatures, this document is considered to be a draft with deliberative material for internal use only.

This DER was originally prepared under contract by Dynamac Corporation (20440 Century Boulevard, Suite 100; Germantown, MD 20874; submitted 05/25/2005). The DER has been reviewed by the Health Effects Division (HED) and Health Canada's Pest Management Regulatory Agency (PMRA). The DER has been revised to reflect current Office of Pesticide Programs (OPP) policies and Directive 98-02.

STUDY REPORT:

46246215 Lemke, V.; Helfrich, K. (2004) JAU6476 480 SC - Magnitude of the Residue In/On Canola. Project Number: J619CN01, RCJAY006, 200464. Unpublished study prepared by Bayer Corp. and ICMS, Inc. 354 p.

46246223 Lenz, C. (2004) JAU6476 480 SC - Magnitude of the Residues in/on Peanuts and Peanut Processed Commodities. Lab Project Number: J619PE02: RCJAY002: 200518. Unpublished study prepared by Bayer CropScience. 447 p.

EXECUTIVE SUMMARY:

Bayer CropScience has submitted a high performance liquid chromatography (with electrospray ionization) and tandem mass spectrometry (LC-MS/MS) data gathering method for the determination of residues of 1*H*-1,2,4-triazole and the triazole conjugates triazolylalanine and triazolylacetic acid in plant commodities.

In the method, crop matrices are extracted with aqueous methanol, and three separate aliquots of the extract are removed for determination of each of the three analytes. Isotopically labelled internal standard is added to each aliquot. For 1*H*-1,2,4-triazole, the aliquot is mixed with dansyl chloride to form the dansyl derivative of 1*H*-1,2,4-triazole, which is partitioned into ethyl acetate and then redissolved in acetonitrile (ACN)/water (1:1, v:v) for LC-MS/MS analysis. For triazolylalanine, the aliquot is cleaned up by solid-phase extraction (SPE), derivatized to the butyl ester using butanolic HCl, and then further derivatized using heptafluorobutyric anhydride (HFBA). The mixture is redissolved in ACN/water (1:1, v:v) for LC-MS/MS analysis. For determination of triazolylacetic acid, the aliquot is cleaned up by SPE, derivatized to the butyl ester using butanolic HCl, and then redissolved in ACN/water (1:1, v:v) for LC-MS/MS analysis. In the crop field trial, processing, and field rotational crop studies, the LOQ has been determined from the lowest spiking level with adequate recovery. Validated LOQs range from 0.01-0.05 ppm for 1*H*-1,2,4-triazole and 0.01-1.5 ppm for triazolylalanine and triazolylacetic acid. The calculated LODs range from 0.001 ppm to values that are greater than the reported LOQs for certain matrices. When the calculated *LOD* exceeded the reported *LOQ*, the *LODs* were set at the LOQ value.

Method validation and concurrent method recovery data for the method demonstrated generally acceptable accuracy/precision for barley (grain, hay, and straw), canola (seed, meal, and refined oil), mustard greens, dried shelled bean, dried shelled pea, peanut (nutmeat, hay, meal, refined oil, dry roasted peanuts, and peanut butter), rice (grain, straw, polished grain, bran, and hulls), turnip (top and root), and wheat (forage, hay, grain, straw, aspirated grain fractions, bran, flour, germ, middlings, and shorts). The spiking levels for these commodities range from 0.01-0.5 ppm for 1*H*-1,2,4-triazole, 0.01-4.5 ppm for triazolylalanine, and 0.01-0.8 ppm for triazolylacetic acid. Recovery ranges (and SD) from these matrices were 59-119% (11%) for 1*H*-1,2,4-triazole, 64-126% (10%) for triazolylalanine, and 67-119% (11%) for triazolylacetic acid.

The spiking levels and samples used in method validation are sufficiently representative of the expected residue levels for the plant commodities. Extraction efficiency has not been demonstrated for plant matrices at this time. The method is not being proposed for enforcement purposes, and as such, independent laboratory validation data is not required.

STUDY/WAIVER ACCEPTABILITY/DEFICIENCIES/CLARIFICATIONS:

Under the conditions and parameters used in the study, the analytical method residue data are classified as scientifically acceptable. No independent laboratory validation data have been submitted. However, since this method is not proposed for enforcement purposes, the data is not required. The applicant has clarified that the lack of extraction efficiency data is due to the fact that this is only a supplemental study that was conducted for the Triazole Task Force to quantify triazole residues found in various studies.

The acceptability of this study for regulatory purposes is addressed in the forthcoming U.S. EPA Residue Chemistry Summary Document, [DP Barcode D303508], and in Canada's Regulatory Decision Document.

COMPLIANCE:

Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided. No deviations from regulatory requirements were reported.

A. BACKGROUND INFORMATION

Prothioconazole is a broad-spectrum systemic fungicide intended for the control of many diseases in different crops such as cereals, oilseed rape or peanuts. The biochemical mode of action is the inhibition of the demethylation of lanosterol or 24-methylene-dihydrolanosterol, which are precursors of sterols in fungi. The chemical has been submitted for joint EPA and PMRA review by Bayer CropScience. The crops proposed for joint review include barley, the dried shell and bean subgroup, the oilseed crop group, and wheat. Uses on peanuts and rice are being proposed for the U.S. only.

TABLE A.1. Test Compound Nomenclature.

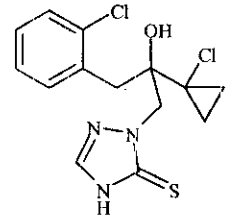
Chemical structure	
Common name	Prothioconazole (ISO accepted)
Company experimental name	JAU6476
IUPAC name	(*RS*)-2-[2-(1-chlorocyclopropyl)-3-(2-chlorophenyl)-2-hydroxypropyl]-2,4-dihydro-1,2,4-triazole-3-thione
CAS name	2-[2-(1-chlorocyclopropyl)-3-(2-chlorophenyl)-2-hydroxypropyl]-1,2-dihydro-3*H*-1,2,4-triazole-3-thione
CAS registry number	178928-70-6
PC Code	113961
End-use product (EP)	Proline® 480 SC (4 lb/gal suspension concentrate), EPA File Symbol 264-IEL

TABLE A.2. Physicochemical Properties of the Technical Grade Test Compound.

Parameter	Value	References
Melting range	139.1 to 144.5°C	MRID 46246003/CES
pH	5.8 (1% solution)	MRID 46246003/CES
Density at 20°C	1.36 g/mL	MRID 46246003/CES
Water solubility at 20°C	pH: mg/L 4: 5 8: 300 9: 2000	MRID 46246003/CES
Solvent solubility at 20°C	Solvent: g/L Acetone: >250 Acetonitrile: 69 Dichloromethane: 88 Dimethysulfoxide: 126 Ethyl acetate: >250 n-Heptane: <0.1 1-Octanol: 58 Polyethylene glycol: >250 2-Propanol: 87 Xylene: 8	MRID 46246003/CES
Vapor pressure at 20 or 25°C	$<4 \times 10^{-7}$ Pa *(calculated from determinations at 70°C)*	MRID 46246003/CES
Dissociation constant, pK_a	6.9 (calculated from K_{OW})	MRID 46246003/CES
Octanol/water partition coefficient, at 20°C	pH: $Log(K_{OW})$ *unbuffered water*: 4.05 4: 4.16 7: 3.82 9: 2.00	MRID 46246003/CES
UV/visible absorption spectrum	Peak maxima at 257 nm. No absorption at > 300 nm.	MRID 46246003/CES

CES - Chemistry Evaluation Section

B. MATERIALS AND METHODS

B.1. Data-Gathering Method

B.1.1. Principle of the Method:

The method was not submitted as a separate study but as an appendix to the crop field trial, processing, and limited field rotational crop studies provided with this submission. The method, entitled "Working Residue Analytical Method for the Determination of Triazole, Triazole Alanine, and Triazole Acetic Acid Residues in Dried Pea, Dried Bean, Rice, Barley, Wheat, Canola, Peanut, Mustard Greens, and Turnip Matrices" (Bayer report No. G200598) is as follows:

Crop matrices are extracted with MeOH:water (4:1,v:v), vacuum filtered, and the extract further diluted with the extraction solution. The extract is divided into 3 separate aliquots to determine each of the 3 analytes. Isotopically labelled internal standard (triazole, triazolylalanine or triazolylacetic acid) is added to each aliquot. A proposed alternate extraction method is similar to the primary method, except that the filtercake is not simply rinsed with the extraction solvent, but is combined with the extraction solvent and blended before the extract is filtered. Oil is extracted with hexane and methanol. The methanol layer is collected, internal standard is added (all three analytes), and the eluent is evaporated to dryness under nitrogen (triazolylacetic acid and triazolylalanine). For 1*H*-1,2,4-triazole, the aliquot is combined with dansyl chloride and sodium bicarbonate to form the dansyl derivative of 1*H*-1,2,4-triazole. Ammonium hydroxide is added, then it is partitioned into ethyl acetate. The ethyl acetate phase is dried over anhydrous sodium sulfate. The dry ethyl acetate phase is evaporated to dryness under nitrogen, and then redissolved in acetonitrile (ACN)/water for LC-MS/MS analysis. For triazolylalanine, the aliquot is cleaned up by solid-phase extraction (SPE) with ACN:water, and derivatized to the butyl ester using 3N HCl in n-butanol. The aliquot is further derivatized using heptafluorobutyric anhydride (HFBA) to form the butyl ester of HFBA. The mixture evaporated to dryness under nitrogen, and then redissolved in ACN/water for LC-MS/MS analysis. For determination of triazolylacetic acid, the aliquot is cleaned up by SPE with ACN:water, then derivatized to the butyl ester using 3N HCl in n-butanol. The mixture evaporated to dryness under nitrogen, and then redissolved in ACN/water for LC-MS/MS analysis.

No LOQs or LODs were reported in the method itself. In the crop field trial, processing, and field rotational crop studies, the LOQ has been determined from the lowest spiking level with adequate recovery. Validated LOQs range from 0.01-0.05 ppm for 1*H*-1,2,4-triazole, and 0.01-1.5 ppm for triazolylalanine and triazolylacetic acid. The calculated LODs range from 0.001 ppm to values that are greater than the reported LOQs for certain matrices. When the calculated LOD exceeded the reported LOQ, the LODs were set at the LOQ value. The LOQs and LODs are reported in TABLE B.1.2.

TABLE B.1.1. Summary Parameters for the Analytical Method Used for the Quantitation of Triazole and Triazole Conjugate Residues in Plant Matrices.	
Method ID	Not assigned; method reported to be contained in Bayer Report No. G200598
Analytes	1*H*-1,2,4-triazole, triazolylalanine, and triazolylacetic acid
Extraction solvent/technique	Samples are soaked in aqueous methanol (MeOH:H_2O, 4:1, v:v) and then blended. The extract is isolated by vacuum filtration (Whatman GF/A filter) and diluted with MeOH:water. The filtercake is rinsed with the extraction solvent, and the extract is further diluted with aqueous methanol. A separate aliquot of the extract is used for determination of each of the three analytes. The appropriate internal standard, [$^{15}N_3$]-1*H*-1,2,4-triazole, [$^{13}C_2$-$^{15}N_3$]-triazolylalanine, or [$^{13}C_2$-$^{15}N_3$]-triazolylacetic acid, is added to the aliquot.
Cleanup/derivatization strategies	For the determination of 1*H*-1,2,4-triazole, the extract aliquot is mixed with dansyl chloride and sodium bicarbonate for 30 minutes to form the dansyl derivative of 1*H*-1,2,4-triazole. After the addition of ammonium hydroxide, the mixture is partitioned with ethyl acetate, dried over anhydrous sodium sulfate, and the dry ethyl acetate phase is evaporated to dryness and redissolved in acetonitrile (ACN) and water for analysis by LC-MS/MS. For determination of triazolylalanine, the extract aliquot is cleaned up by solid-phase extraction (SPE; Bond-Elut Certify II cartridge, conditioned with ACN and aqueous methanol), using ACN:water (4:1, v:v) to elute residues. The eluate is evaporated to dryness and heated with butanolic HCl at 110 °C for 30 minutes to form the butyl ester of triazolylalanine. The aliquot is then evaporated to dryness, redissolved in heptafluorobutyric anhydride (HFBA) to form the butyl ester HFBA (BEHFBA) of triazolylalanine, evaporated to dryness again, and redissolved in ACN:water (1:1, v:v) for analysis by LC-MS/MS. For determination of triazolylacetic acid, the extract aliquot is cleaned up by C18 SPE (cartridge conditioned with ACN and aqueous methanol), using ACN:water (4:1, v:v) to elute residues. The eluate is evaporated to dryness and heated with butanolic HCl at 110 °C for 30 minutes to form the butyl ester of triazolylacetic acid. The aliquot is evaporated to dryness again, and redissolved in ACN:water (1:1, v:v) for analysis by LC-MS/MS.
Instrument/Detector	HPLC utilizing a reverse-phase C18 column and a gradient mobile phase of ACN and 0.1% aqueous formic acid, with tandem mass spectrometry (MS/MS) detection using electrospray ionization and selective reaction monitoring. The ion transitions monitored are: 1*H*-1,2,4-triazole (as dansyl-1,2,4-triazole):303 to 180.6-181.6 (primary); 303 to 169.6-170.6 (secondary) 1*H*-1,2,4-triazole internal standard: 306 to 181.6-182.6 (primary); 306 to 169.6-170.6 (secondary) triazolylalanine (as triazolylalanine BEHFBA): 409 to 69.6-70.6 (primary); 409 to 283.6-284.6 (secondary) triazolylalanine internal standard: 414 to 74.6-75.6 (primary); 414 to 283.6-284.6 (secondary) triazolylacetic acid (as triazolylacetic acid butyl ester): 184 to 69.6-70.6 (primary); 184 to 127.6-128.6 (secondary) triazolylacetic acid internal standard: 189 to 74.6-75.6 (primary); 189 to 132.6-133.6 (secondary)
Standardization method	External and internal standardization. A calibration curve is generated before sample analysis using calibration standards by plotting the ratio of standard peak area to internal standard peak area against standard concentration. Residue values for samples are then determined from the calibration curve. The calibration curves for 1*H*-1,2,4-triazole and triazolylalanine are generated using standards of dansyl-1,2,4-triazole and triazolylalanine BEHFBA, respectively, each calculated in parent equivalents. The calibration curve for triazolylacetic acid is generated from calibration standards of triazolylacetic acid which are derivatized using the procedures described previously for samples.

TABLE B.1.1. Summary Parameters for the Analytical Method Used for the Quantitation of Triazole and Triazole Conjugate Residues in Plant Matrices.	
Stability of std solutions	Not reported.
Retention times	1*H*-1,2,4-triazole (as dansyl-1,2,4-triazole): ~4.5 minutes triazolylalanine (as triazolylalanine BEHFBA): ~3.8 minutes triazolylacetic acid (as triazolylacetic acid butyl ester): ~2.0 minutes

TABLE B.1.2. Limits of Quantitation (LOQ) and Limits of Detection (LOD) for the Analytical Method Used for the Quantitation of Triazole and Triazole Conjugate Residues in Plant Matrices.[1]

Crop	Matrix	Analytes					
		1*H*-1,2,4 Triazole		Triazolylalanine		Triazolylacetic acid	
		LOD	LOQ	LOD	LOQ	LOD	LOQ
Crop Field Trials							
Barley	Straw	0.002	0.01	0.005	0.10	0.048	0.10
	Grain	0.009	0.01	0.050	0.10	0.044	0.10
	Hay	0.002	0.01	0.050	0.05	0.050	0.05
Dried Beans	Dried Beans	0.005	0.01	0.011	0.02	0.02	0.02
Dried Peas	Dried Peas	0.003	0.01	0.019	0.02	0.007	0.02
Canola	Seed	0.014	0.02	0.008	0.025	0.004	0.025
Peanut	Hay	0.009	0.02	0.022	0.100	0.005	0.100
	Nutmeat	0.012	0.02	0.077	0.125	0.067	0.025
Rice	Straw	0.004	0.01	0.028	0.05	0.016	0.05
	Grain	0.004	0.01	0.033	0.05	0.022	0.05
Wheat	Forage	0.006	0.01	0.008	0.01	0.003	0.010
	Hay	0.007	0.01	0.009	0.01	0.023	0.010
	Straw	0.005	0.01	0.008	0.01	0.004	0.025
	Grain	0.001	0.01	0.022	0.01	0.028	0.010
Processing Studies							
Canola	Meal	0.004	0.02	0.033	0.20	0.010	0.20
	Oil	0.004	0.01	0.015	0.02	0.006	0.02
	Seed	0.005	0.02	0.005	0.02	0.016	0.02
Peanut	Nutmeat	0.001	0.01	0.186	0.50	0.003	0.01
	Meal	0.002	0.01	1.135	1.50	0.010	0.01
	Oil	0.003	0.01	0.005	0.01	0.004	0.01

	Dry Roasted	0.020	0.05	0.109	0.50	0.003	0.01
	Peanut Butter	0.011	0.05	0.267	0.50	0.006	0.01
Rice	Bran	0.003	0.01	0.413	0.75	0.081	0.75
	Grain	0.004	0.01	0.033	0.05	0.022	0.05
	Hull	*0.003*	*0.01*	*0.022*	0.05	0.045	0.05
	Polished Grain	0.004	0.01	0.016	0.05	0.037	0.05
Wheat	Aspirated Grain Fraction	0.010	0.01	0.023	0.20	0.044	0.20
	Bran	0.009	0.01	0.172	0.50	0.104	0.50
	Flour	0.012	0.01	0.009	0.30	0.097	0.30
	Germ	0.001	0.01	0.170	0.50	0.071	0.50
	Grain	0.001	0.01	0.022	0.01	0.029	0.01
	Middlings	0.004	0.01	0.282	0.25	0.079	0.25
	Shorts	0.008	0.01	0.131	0.30	0.127	0.30
Rotational Crops							
Mustard Greens	Mustard Greens	0.007	0.01	0.010	0.10	0.013	0.010
Turnips	Tops	0.005	0.01	0.027	0.05	0.009	0.050
	Roots	0.031	0.05	0.036	0.05	0.010	0.050
Wheat	Forage	0.006	0.01	0.008	0.01	0.003	0.010
	Hay	0.015	0.01	0.009	0.01	0.023	0.010
	Straw	0.005	0.01	0.008	0.01	0.004	0.025
	Grain	0.001	0.01	0.022	0.01	0.028	0.010

[1] LODs are generally set at the calculated values. If the calculated LODs are greater than their respective LOQs, the LODs are set at the LOQ value.

B.2. Enforcement Method

The applicant has not proposed this method for determination of triazole and triazole conjugate residues in plant commodities for enforcement purposes.

C. RESULTS AND DISCUSSION

C.1. Data-Gathering Method

In general, separate method validation data were not submitted for the method. The validation data presented in TABLE C.1.1 represent the concurrent method recovery data submitted in conjunction with the crop field trial, processing, and field rotational crop studies associated with this submission. However, wheat grain and wheat processed commodities from the processing study had both concurrent recovery and method validation data submitted.

TABLE C.1.1 Recovery Results from Method Validation of Various Plant Matrices Using the Data-Gathering Analytical Method.[1]

Matrix	Analyte	Spiking Level (ppm)	Sample size (n)	Recoveries Obtained (%)	Mean ± SD [CV] (%)
Barley crop field trial					
Barley grain	1*H*-1,2,4-triazole	0.03	6	71, 71, 71, 73, 74, 83	74 ± 5 [6]
	Triazolylalanine	0.25	3	77, 82, 93	84 ± 8 [10]
		0.75	6	72, 80, 80, 86, 88, 93	83 ± 7 [9]
	Triazolylacetic acid	0.25	3	72, 76, 92	80 ± 11 [13]
		0.75	6	77, 84, 87, 88, 90, 91	86 ± 5 [6]
Barley hay	1*H*-1,2,4-triazole	0.03	6	74, 75, 75, 76, 77, 81	76 ± 3 [3]
		0.20	1	82	NA
	Triazolylalanine	0.05	3	92, 95, 115	101 ± 13 [12]
		0.2	1	86	NA
		0.25	3	93, 96, 97	95 ± 2 [2]
		0.75	5	87, 91, 95, 97, 98	94 ± 5 [5]
	Triazolylacetic acid	0.05	3	73, 97, 113	94 ± 20 [21]
		0.2	1	74	NA
		0.25	3	87, 90, 104	94 ± 9 [10]
		0.75	5	80, 89, 91, 94, 97	90 ± 6 [7]
Barley straw	1*H*-1,2,4-triazole	0.03	5	73, 78, 79, 79, 93	80 ± 7 [9]
		0.20	1	94	NA
	Triazolylalanine	0.20	1	96	NA
		0.25	3	90, 95, 99	95 ± 5 [5]
		0.75	5	84, 86, 95, 103, 105	95 ± 10 [10]
	Triazolylacetic acid	0.20	1	73	NA
		0.25	3	94, 97, 108	100 ± 7 [7]
		0.75	5	68, 86, 90, 94, 96	87 ± 11 [13]
Canola crop field trial					
Canola seed	1*H*-1,2,4-triazole	0.1	7	71, 73, 77, 77, 83, 83, 86	79 ± 6 [7]
	Triazolylalanine	0.05	2	71, 92	82
		0.1	8	72, 74, 81, 84, 89, 95, 96, 102	87 ± 11 [12]
		1.5	3	79, 80, 81	80 ± 1 [1]
	Triazolylacetic acid	0.02	2	78, 81	80
		0.1	7	80, 84, 85, 86, 89, 90, 91	86 ± 4 [4]

TABLE C.1.1. Recovery Results from Method Validation of Various Plant Matrices Using the Data-Gathering Analytical Method.[1]

Matrix	Analyte	Spiking Level (ppm)	Sample size (n)	Recoveries Obtained (%)	Mean ± SD [CV] (%)
Wheat crop field trial					
Wheat forage	1*H*-1,2,4-triazole	0.01	3	59, 71, 73	68 ± 8 [11]
		0.05	3	78, 93, 96	89 ± 10 [11]
		0.10	3	71, 78, 89	79 ± 9 [11]
	Triazolylalanine	0.05	3	84, 88, 98	90 ± 7 [8]
		0.10	3	83, 89, 93	88 ± 5 [6]
	Triazolylacetic acid	0.05	3	72, 74, 87	78± 8 [10]
		0.10	3	79, 82, 84	82 ± 3 [3]
Wheat hay	1*H*-1,2,4-triazole	0.05	9	72, 73, 73, 74, 75, 76, 77, 89, 92	78 ± 7 [9]
		0.10	1	91	NA
	Triazolylalanine	0.025	3	85, 88, 105	93 ± 11 [12]
		0.05	9	77, 80, 82, 83, 85, 86, 87, 87, 97	85 ± 6 [7]
		0.10	1	104	NA
	Triazolylacetic acid	0.01	3	72, 74, 88	78 ± 9 [11]
		0.05	9	71, 76, 76, 77, 85, 86, 92, 100, 110	86 ± 13 [15]
		0.10	1	91	NA
Wheat grain	1*H*-1,2,4-triazole	0.05	6	73, 78, 82, 84, 85, 89	82 ± 6 [7]
	Triazolylalanine	0.05	7	73, 74, 75, 77, 79, 99, 101	83 ± 12 [15]
	Triazolylacetic acid	0.05	7	72, 72, 77, 85, 97, 98, 99	86 ± 12 [14]
Wheat straw	1*H*-1,2,4-triazole	0.05	8	77, 77, 81, 81, 82, 87, 90, 104	85 ± 9 [11]
	Triazolylalanine	0.05	8	76, 77, 80, 82, 90, 91, 93, 95	86 ± 8 [9]
	Triazolylacetic acid	0.05	8	77, 79, 80, 82, 85, 87, 88, 100	85 ± 7 [9]
Canola processing study					
Canola seed	1*H*-1,2,4-triazole	0.02	3	76, 78, 83	79 ± 4 [5]
		0.20	1	100	NA
	Triazolylalanine	0.01	3	79, 82, 91	84 ± 6 [7]
		0.20	4	72, 77, 72, 82	76 ± 5 [6]
	Triazolylacetic acid	0.02	3	109, 116, 95	107 ± 11 [10]
		0.20	1	96	NA
Canola meal	1*H*-1,2,4-triazole	0.02	6	91, 86, 97, 98, 97, 87	93 ± 5 [6]
	Triazolylalanine	0.20	3	79, 79, 83	80 ± 2 [3]
		4.0	3	82, 78, 76	79 ± 3 [4]
	Triazolylacetic acid	0.01	3	105, 73, 85	88 ± 16 [18]
		0.02	6	88, 98, 88, 94, 98, 101	95 ± 6 [6]

TABLE C.1.1. Recovery Results from Method Validation of Various Plant Matrices Using the Data-Gathering Analytical Method.[1]

Matrix	Analyte	Spiking Level (ppm)	Sample size (n)	Recoveries Obtained (%)	Mean ± SD [CV] (%)
Canola refined oil	1*H*-1,2,4-triazole	0.01	3	67, 73, 76	72 ± 5 [6]
		0.02	4	105, 71, 117, 87	95 ± 20 [21]
		0.05	3	99, 86, 88	91 ± 7 [8]
	Triazolylalanine	0.02	3	69, 84, 89	81 ± 10 [13]
		0.05	3	79, 86, 97	87 ± 9 [10]
	Triazolylacetic acid	0.02	3	79, 77, 85	80 ± 4 [5]
		0.05	3	96, 101, 79	92 ± 12 [13]
Peanut processing study					
Peanut nutmeat	1*H*-1,2,4-triazole	0.05	2	103, 92	98
		0.50	1	94	NA
	Triazolylalanine	0.05	1	96	NA
		1.0	1	92	NA
	Triazolylacetic acid	0.05	2	102, 104	103
		0.5	1	92	NA
Peanut meal	1*H*-1,2,4-triazole	0.05	1	94	NA
		0.5	1	96	NA
	Triazolylalanine	0.50	1	96	NA
	Triazolylacetic acid	0.05	1	113	NA
		0.50	1	96	NA
Peanut refined oil	1*H*-1,2,4-triazole	0.05	1	97	NA
	Triazolylalanine	0.05	1	101	NA
	Triazolylacetic acid	0.05	1	102	NA
Dry roasted peanuts	1*H*-1,2,4-triazole	0.05	4	103, 111, 106, 97	104 ± 6 [6]
		0.50	1	92	NA
	Triazolylalanine	1.5	3	99, 98, 92	96 ± 4 [4]
	Triazolylacetic acid	0.05	1	80	NA
		0.50	1	94	NA
Peanut butter	1*H*-1,2,4-triazole	0.05	4	94, 105, 105, 109	103 ± 6 [6]
		0.50	1	99	NA
	Triazolylalanine	1.5	3	105, 99, 104	103 ± 3 [3]
	Triazolylacetic acid	0.05	1	102	NA
		0.50	1	88	NA

TABLE C.1.1. Recovery Results from Method Validation of Various Plant Matrices Using the Data-Gathering Analytical Method.[1]

Matrix	Analyte	Spiking Level (ppm)	Sample size (n)	Recoveries Obtained (%)	Mean ± SD [CV] (%)
Rice processing study					
Rice grain	1*H*-1,2,4-triazole	0.01	3	70, 72, 80	74 ± 5 [7]
		0.05	1	82	NA
		0.1	1	95	NA
	Triazolylalanine	0.05	4	86, 73, 87, 91	84 ± 8 [9]
		0.1	1	70	NA
		0.5	3	100, 97, 100	99 ± 2 [2]
	Triazolylacetic acid	0.05	4	81, 70, 71, 74	74 ± 5 [7]
		0.1	1	86	NA
		0.5	3	81, 83, 82	82 ± 1 [1]
Polished rice grain	1*H*-1,2,4-triazole	0.01	3	85, 99, 92	92 ± 7 [8]
		0.05	4	71, 71, 95, 98	84 ± 15 [18]
	Triazolylalanine	0.05	5	86, 70, 95, 71, 80	80 ± 11 [13]
		0.2	3	77, 79, 87	81 ± 5 [7]
	Triazolylacetic acid	0.05	4	80, 67, 86, 71	76 ± 9 [11]
		0.2	3	70, 73, 70	71 ± 2 [2]
Rice bran	1*H*-1,2,4-triazole	0.01	4	91, 87, 89, 100	92 ± 6 [6]
		0.05	3	93, 96, 85	91 ± 6 [6]
		0.5	1	98	NA
	Triazolylalanine	0.5	1	81	NA
		0.75	3	86, 89, 74	83 ± 8 [10]
		2	3	86, 81, 79	82 ± 4 [4]
	Triazolylacetic acid	0.5	1	97, 93	95
		0.75	3	103, 101, 100	101 ± 2 [2]
		0.75	3	97, 87, 91	92 ± 5 [5]
Rice hulls	1*H*-1,2,4-triazole	0.01	3	77, 77, 72	75 ± 3 [4]
		0.05	4	71, 82, 70, 86	77 ± 8 [10]
	Triazolylalanine	0.02	3	103, 116, 85	101 ± 16 [15]
		0.05	4	81, 64, 96, 79	80 ± 13 [16]
	Triazolylacetic acid	0.02	4	87, 108, 88, 119	101 ± 16 [16]
		0.05	3	96, 113, 108	106 ± 9 [8]

TABLE C.1.1 Recovery Results from Method Validation of Various Plant Matrices Using the Data-Gathering Analytical Method.[1]

Matrix	Analyte	Spiking Level (ppm)	Sample size (n)	Recoveries Obtained (%)	Mean ± SD [CV] (%)
Wheat processing study					
Wheat grain	1*H*-1,2,4-triazole	0.01	3	71, 71, 72	71 ± 1 [1]
		0.5	2	87, 92	90
	Triazolylalanine	0.01	3	88, 90, 85	88 ± 3 [3]
		0.5	3	75, 77, 78	77 ± 2 [2]
		1.4	3	88, 90, 92	90 ± 2 [2]
	Triazolylacetic acid	0.01	3	84, 98, 105	96 ± 11 [11]
		0.5	2	74, 71	73
		0.7	3	79, 80, 80	80 ± 1 [1]
Wheat aspirated grain fractions	1*H*-1,2,4-triazole	0.01	3	92, 88, 113	98 ± 13 [14]
		0.5	3	92, 99, 101	97 ± 5 [5]
	Triazolylalanine	0.1	3	81, 76, 82	80 ± 3 [4]
		0.5	3	87, 80, 84	84 ± 4 [4]
	Triazolylacetic acid	0.2	3	102, 98, 105	102 ± 4 [3]
		0.5	3	97, 91, 92	93 ± 3 [3]
Wheat bran	1*H*-1,2,4-triazole	0.01	3	71, 84, 62	72 ± 11 [15]
		0.5	4	88, 90, 93, 96	92 ± 4 [4]
	Triazolylalanine	0.5	4	91, 89, 72, 84	84 ± 9 [10]
		3.2	3	70, 80, 73	74 ± 5 [7]
		4	3	87, 89, 88	88 ± 1 [1]
	Triazolylacetic acid	0.5	4	89, 84, 78, 89	85 ± 5 [6]
		0.8	3	79, 81, 86	82 ± 4 [4]
Wheat flour	1*H*-1,2,4-triazole	0.01	3	63, 78, 96	79 ± 17 [21]
		0.5	4	103, 97, 100, 87	97 ± 7 [7]
	Triazolylalanine	0.1	3	91, 93, 93	92 ± 1 [1]
		0.5	4	77, 93, 90, 103	91 ± 11 [12]
	Triazolylacetic acid	0.3	3	83, 75, 75	78 ± 5 [6]
		0.5	4	72, 84, 78, 82	79 ± 5 [7]
Wheat germ	1*H*-1,2,4-triazole	0.01	3	82, 81, 81	81 ± 1 [1]
		0.5	4	87, 89, 83, 91	88 ± 3 [4]
	Triazolylalanine	0.5	4	89, 81, 83, 110	91 ± 13 [15]
		4.5	3	93, 90, 84	89 ± 5 [5]
	Triazolylacetic acid	0.3	3	93, 98, 91	94 ± 4 [4]
		0.5	4	81, 77, 80, 97	84 ± 9 [11]

TABLE C.1.1 Recovery Results from Method Validation of Various Plant Matrices Using the Data-Gathering Analytical Method.[1]

Matrix	Analyte	Spiking Level (ppm)	Sample size (n)	Recoveries Obtained (%)	Mean ± SD [CV] (%)
Wheat middlings	1*H*-1,2,4-triazole	0.01	3	89, 85, 95	90 ± 5 [6]
		0.25	5	74, 82, 82, 89, 94	84 ± 8 [9]
		0.5	3	97, 98, 100	98 ± 2 [2]
	Triazolylalanine	0.15	3	73, 119, 71	88 ± 27 [31]
		0.2	3	88, 67, 84	80 ± 11 [14]
		0.5	3	72, 77, 76	75 ± 3 [4]
		1	3	72, 79, 75	75 ± 4 [5]
	Triazolylacetic acid	0.25	3	80, 81, 81	81 ± 1 [1]
		0.3	5	98, 87, 92, 95, 83	91 ± 6 [7]
		0.5	3	73, 81, 71	75 ± 5 [7]
Wheat shorts	1*H*-1,2,4-triazole	0.01	3	98, 93, 77	89 ± 11 [12]
		0.3	3	90, 98, 91	93 ± 4 [5]
		0.5	3	97, 95, 98	97 ± 2 [2]
	Triazolylalanine	0.3	3	73, 83, 72	76 ± 6 [8]
		0.5	3	86, 84, 90	87 ± 3 [4]
		1.5	3	81, 74, 81	79 ± 4 [5]
	Triazolylacetic acid	0.3	6	78, 82, 75, 96, 78, 86	83± 8 [9]
		0.5	3	76, 76, 82	78 ± 3 [4]
Field rotational crop study					
Mustard greens	1*H*-1,2,4-triazole	0.25	6	73, 79, 77, 95, 87, 92	84 ± 9 [11]
	Triazolylalanine	0.25	5	68, 86, 81, 88, 86	82 ± 8 [10]
	Triazolylacetic acid	0.25	5	84, 84, 75, 83, 95	84 ± 7 [8]
Turnip top	1*H*-1,2,4-triazole	0.01	3	59, 81, 106	82 ± 24 [29]
		0.05	3	90, 87, 95	91 ± 4 [4]
	Triazolylalanine	0.05	3	98, 90, 96	95 ± 4 [4]
		0.50	3	88, 81, 92	87 ± 6 [6]
	Triazolylacetic acid	0.05	3	98, 118, 113	110 ± 10 [9]
		0.50	3	98, 88, 100	95 ± 6 [7]
		0.75	3	86, 92, 92	90 ± 3 [4]
Turnip root	1*H*-1,2,4-triazole	0.05	6	88, 102, 102, 97, 97, 93	97 ± 5 [6]
	Triazolylalanine	0.05	3	126, 92, 97	105 ± 18 [17]
		0.50	3	102, 102, 99	101 ± 2 [2]
		0.75	3	95, 93, 102	97 ± 5 [5]
	Triazolylacetic acid	0.05	3	96, 108, 93	99 ± 8 [8]
		0.50	3	104, 103, 101	103 ± 2 [1]

TABLE C.1.1. Recovery Results from Method Validation of Various Plant Matrices Using the Data-Gathering Analytical Method.[1]

Matrix	Analyte	Spiking Level (ppm)	Sample size (n)	Recoveries Obtained (%)	Mean ± SD [CV] (%)
Wheat forage	1*H*-1,2,4-triazole	0.05	1	109	N/A
	Triazolylalanine	0.05	1	96	N/A
Wheat hay	1*H*-1,2,4-triazole	0.05	1	97	N/A
	Triazolylalanine	0.20	1	87	N/A
	Triazolylacetic acid	0.20	1	102	N/A
Wheat straw	1*H*-1,2,4-triazole	0.05	1	93	N/A
	Triazolylalanine	0.20	1	80	N/A
	Triazolylacetic acid	0.20	1	104	N/A
Wheat grain	1*H*-1,2,4-triazole	0.05	1	97	N/A
	Triazolylalanine	0.20	1	71	N/A
	Triazolylacetic acid	0.20	2	89, 82	86

[1] Standards were prepared in water or ACN:water (1:1, v:v). Data reflect concurrent recovery data for all crops except wheat grain and wheat processed commodities from the processing study, for which both concurrent recovery and method validation data were submitted.
[2] Standard deviation (and CV) are not reported for less than three recoveries. N/A = Not applicable.

The spiking levels and samples used in method validation are adequate to bracket expected residue levels. Because of the specificity of LC-MS/MS analyses, an interference study has not been conducted. A second transition for the daughter ions is considered a confirmatory analysis.

TABLE C.1.2. Characteristics for the Data-Gathering Analytical Method Used for the Quantitation of Triazole and Triazole Conjugate Residues in Plant Matrices.

Analytes	1*H*-1,2,4-triazole, triazolylalanine, and triazolylacetic acid
Equipment ID	LC-MS/MS equipment ID not specified in method (TSQ Quantum used for most studies); Phenomenex Synergi MAX-RP, 4 µm (75 mm x 2.0 mm), column
Limit of quantitation (LOQ)	An LOQ was not reported in the method. In the crop field trial, processing, and field rotational crop studies associated with this submission, the LOQ was determined from the lowest spiking level with adequate recovery. See TABLE B.1.2.
Limit of detection (LOD)	An LOD was not reported in the method. For each matrix analyzed in the crop field trial, processing, and field rotational crop studies associated with this submission, the LOD was calculated for each analyte analyzed by multiplying the standard deviation of the LOQ recoveries by the $t_{0.99}$ value for the number of measurements. When the calculated LOD exceeded the reported LOQ, the LOD was set at the LOQ value. See TABLE B.1.2.
Accuracy/Precision	Percent recoveries and coefficients of variance (CVs) indicate generally acceptable accuracy/precision for barley grain, hay, and straw; canola seed, meal, and refined oil; mustard greens; dried shelled bean; dried shelled pea; peanut nutmeat, hay, meal, refined oil, dry roasted peanuts, and peanut butter; rice grain, straw, polished grain, bran, and hulls; turnip top and root; and wheat forage, hay, grain, straw, aspirated grain fractions, bran, flour, germ, middlings, and wheat shorts at spiking levels ranging from 0.01-0.5 ppm for 1*H*-1,2,4-triazole, 0.01-4.5 ppm for triazolylalanine, and 0.01-0.8 ppm for triazolylacetic acid. Recovery ranges (and standard deviations) from these matrices were 59-119% (11%) for 1*H*-1,2,4-triazole, 64-126% (10%) for triazolylalanine, and 67-119% (11%) for triazolylacetic acid. See TABLE C.1.1.

TABLE C.1.2. Characteristics for the Data-Gathering Analytical Method Used for the Quantitation of Triazole and Triazole Conjugate Residues in Plant Matrices.	
Reliability of the Method (ILV)	No independent laboratory validation has been conducted.
Linearity	The method/detector response was linear (coefficient of determination, r^2, was >0.99) for each analyte within the range of 0.05-1.0 ppm (dansyl-1,2,4-triazole) or 0.005-1.0 ppm (triazolylalanine BEHFBA and triazolylacetic acid butyl ester) for all matrices.
Specificity	The control chromatograms generally have no peaks above the chromatographic background, and the spiked sample chromatograms contain only the analyte peak of interest. Peaks are well defined and symmetrical.

The extraction solvents used in the LC-MS/MS method differ from those used in the peanut, sugar beet, and wheat metabolism studies provided with this petition. In the metabolism studies, the majority of the radioactivity generally was extracted using ACN/water containing cysteine HCl (to prevent oxidative decomposition of prothioconazole). Extraction efficiency has not been demonstrated for plant matrices.

C.2. Enforcement Method

This method has not been proposed for the determination of triazole and triazole conjugate residues in plant commodities for enforcement purposes.

C.3. Independent Laboratory Validation

Independent laboratory validation of this method has not been submitted. However, since this method will not be used for enforcement purposes, an ILV is not required at this time (Directive 98-02, Section 3).

D. CONCLUSION

Adequate method validation data have been submitted for the LC-MS/MS method (Bayer Report No. G200598) for the determination of residues of 1*H*-1,2,4-triazole and triazole conjugates triazolylalanine and triazolylacetic acid in plant commodities. The validation data are sufficiently representative of the expected residue levels for the plant commodities included in this petition, and the method adequately quantitated the analytes in the plant matrices.

Extraction efficiency data has not been demonstrated for the method in plant matrices. Since this method is not proposed for enforcement purposes, an independent laboratory validation of the method is not required.

E. REFERENCES

*Please note that studies reporting concurrent recovery and/or method validation data using this analytical method are referenced below.

46246141 Haas, M.; Bornatsch, W. (2000) Metabolism of JAU 6476 in Spring Wheat (after foliar application). Project Number: M/1730851/5, 110880, MR/198/99. Unpublished study prepared by Bayer Ag Institut fuer Ruckstands-Analytik. 149 p.

46246142 Haas, M. (2001) Metabolism of JAU 6476 in Spring Wheat after Seed Dressing. Project Number: M/1730885/2, 110881, MR/467/99. Unpublished study prepared by Bayer Ag Institut fuer Ruckstands-Analytik. 84 p.

46246143 Duah, F.; Lopez, R. (2004) The Metabolism of [Triazole-3, 5-(Carbon 14)] JAU6476 in Wheat. Project Number: J6041601, 200733. Unpublished study prepared by Bayer Corp. 197 p.

46246145 Haas, M. (2001) Metabolism of [Phenyl-UL-(Carbon 14)]JAU6476 in Peanuts. Project Number: M/1730984/2, MR/193/01. Unpublished study prepared by Bayer Ag Institut fuer Ruckstands-Analytik. 130 p.

46246146 Haas, M. (2003) Metabolism of [trizole-UL-(Carbon 14)]JAU6476 in Peanuts. Project Number: M1731145/2, MR/194/02. Unpublished study prepared by Bayer Ag, Institute of Product Info. 145 p.

46246147 Beedle, E.; Ying, S. (2004) The Metabolism of [Triazole-UL-(Carbon 14)]JAU6476 in Sugar Beets. Project Number: J6041603, 200467. Unpublished study prepared by Bayer Corp. 91 p.

46246148 Beedle, E.; Ying, S. (2004) The Metabolism of [Phenyl-UL-(Carbon 14)]JAU6476 in Sugar Beets. Project Number: J6041602, 200466. Unpublished study prepared by Bayer Corp. 86 p.

46246218 Kraai, M. (2004) JAU6476 480 SC - Magnitude of the Residues in/on Wheat Grain, Wheat Aspirated Grain Fractions, and Wheat Processed Commodities. Lab Project Number: J619WH02: RCJAY004: 200521. Unpublished study prepared by Bayer CropScience. 744 p.

46246219 Lemke, V.; Murphy, J. (2004) JAU6476 480 SC - Magnitude of the Residue In/On Wheat. Project Number: J619WH01, RCJAY009, 200524. Unpublished study prepared by Bayer Corp., Battelle and Vaughn Agricultural Research Serv., Ltd. 1270 p.

46246220 Lenz, C. (2004) JAU6476 480 SC - Magnitude of the Residue In/On Barley. Project Number: J619BA01, 200806, RCJAY005. Unpublished study prepared by Bayer Corp., Battelle and Agvise Inc. 797 p.

46246221 Fischer, D.R. (2004) JAU6476 480 SC - Magnitude of the Residue in/on Dried Peas and Dried Beans (Crop Subgroup 6C - Dried, Shelled Pea and Bean - Except Soybean):Lab Project Number: J619BY02; J619DB02; RCJAX007; 200956. Unpublished study prepared by Bayer CropScience. 564 pages.

46246222 Harbin, A. (2004) JAU6476 480 SC - Magnitude of the Residues in/on Rice Grain and Rice Processed Commodities. Lab Project Number: J619RI02: RCJAY003: 200493. Unpublished study prepared by Bayer CropScience. 464 p.

46246224 Beedle, E. (2004) JAU6476 480 SC - Magnitude of the Residues in/on Canola Seed and Canola Processed Commodities. Lab Project Number: J619CN02: RCJAY001: 200953. Unpublished study prepared by Bayer CropScience. 309 p.

46246226 Duah, F.; Kraai, M. (2004) The Accumulation of ((Triazole-3,5-(Carbon 14)) JAU6476 in Confined Rotational Crops. Project Number: J6051601, 200623. Unpublished study prepared by Bayer Corp. 324 p.

46246227 Lemke, V.; Lenz, C.; Murphy, J. (2004) JAU6476 480 SC - Magnitude of the Residue in Field Rotational Crops - Wheat, Mustard Greens, and Turnips. Project Number: 200535, J619RC01, J619RC02. Unpublished study prepared by Bayer Corp., Battelle and Bayer Research Farm. 1202 p.

F. DOCUMENT TRACKING

RDI: Suzan Mathew (PMRA) 23/01/2006; Henri Bietlot (PMRA) 24/01/2006; Stephen Funk (IO)13/03/2006; Leung Cheng (RAB3).
Petition Number: PP#4F6830
DP Barcode: D303508
PC Code: 113961

Template Version September 2003

Primary Evaluators

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In the absence of signatures, this document is considered to be a draft with deliberative material for internal use only.

This DER was originally prepared under contract by Dynamac Corporation (20440 Century Boulevard, Suite 100; Germantown, MD 20874; submitted 04/13/2005). The DER has been rewritten and reviewed by the Health Effects Division (HED) and has been reviewed by Health Canada's Pest Management Regulatory Agency (PMRA). The DER has been revised to reflect current Office of Pesticide Programs (OPP) policies and PMRA Directive 98-02.

STUDY REPORT:

46246202 Weber, H.; Spiegel, K. (2001) [Phenyl-UL-(Carbon 14)]JAU6476: Absorption, Distribution, Excretion, and Metabolism in Laying Hens. Project Number: M81819090, MR-309/01. Unpublished study prepared by Bayer CropScience Ag Development. 142 p.

46246203 Weber, H.; Justus, K. (2003) [Triazole-UL-(Carbon 14)]JAU6476: Absorption, Distribution, Excretion, and Metabolism in Laying Hens. Project Number: M91819118, MEF005/03. Unpublished study prepared by Bayer CropScience Ag Development. 157 p.

EXECUTIVE SUMMARY:

Phenyl-label study

Six laying hens were administered [phenyl-UL-^{14}C]-JAU6476 (0.732 MBq/mg) orally at a mean dose of 9.7 mg/kg b.w. once a day for three consecutive days (corresponding to 171 ppm in the feed). Excreta was collected every 24 hours. Cages were checked for egg production twice daily, and all eggs collected. Five hours after the final dose (53 hours after the initial dose) the hens were sacrificed. Liver (without bile bladder), kidney, leg muscle, breast muscle, skin, subcutaneous fat and eggs from the ovary and oviduct were dissected from the hens. All samples were assayed for total radioactivity by liquid scintillation counting, either directly or by sample combustion. The identification and characterization of metabolites was achieved by high performance liquid chromatography following solvent extraction of tissues.

In [phenyl-UL-^{14}C]-JAU6476 treated hens, the recovered radioactivity accounted for 79.28% of the administered dose. Approximately 78.42% of the administered dose (%AD) was eliminated in the excreta. A total of 0.011 % AD was recovered in eggs, while 0.85% was estimated to have been present in the tissues and organs. A significant portion of the administered dose was absorbed from the intestinal tract prior to excretion, as indicated by the high residue concentrations in the kidney and liver.

Kidney and liver were found to contain the highest total radioactive residues (TRRs), 4.537 ppm and 4.081 ppm, respectively. TRRs in other tissues were 0.597, 0.433, 0.383, 0.107 and 0.058 ppm for eggs from the ovary and oviduct, subcutaneous fat, skin, leg muscle and breast muscle respectively. The major metabolites identified were the parent JAU6476 in muscle (11.33% of the TRRs; 0.010 ppm), fat (30.33% of the TRRs; 0.137 ppm) and liver (24.76% of the TRRs; 0.995 ppm). JAU6476-desthio in fat (28.96% of the TRRs; 0.130 ppm) and eggs (20.13% of the TRRs; 0.007 ppm); JAU6476-*S*- methyl in fat (19.56% of the TRRs; 0.088 ppm); a glucuronide conjugate of the parent JAU6476 in muscle (15.50% of the TRRs; 0.014 ppm), liver (11.93% of the TRRs; 0.479 ppm) and eggs (16.98% of the TRRs; 0.006 ppm); and sulfate conjugates of hydroxylated JAU6476-desthio in liver (11.07% of the TRRs; 0.445 ppm). Minor metabolites (< 10% of the TRRs) included JAU6476-desthio-3,4-dihydroxy-dienyl-glucuronide, JAU6476-4-hydroxy, JAU6476-4- hydroxy-desthio, JAU6476-*N*-glucuronide, JAU6476-hydroxy-glucuronides and JAU6476- dihydroxy-diene. Attempts were made to further release the bound liver residues by ultrasonic and microwave extractions in the presence of methanol and acidic methanol. These efforts released an additional 6.4% (0.257 ppm) of the TRRs in the liver, leaving 14.4% (0.579 ppm) of the TRRs as non-extractable residues. Total accountabilities ranged from 97-101%.

Triazole-label study

Six laying hens were treated with [triazole-UL-^{14}C]- JAU6476 (0.702 Mbq/mg) orally at a mean dose of 10.4 mg/kg b.w. once daily for three consecutive days (corresponding to 163 ppm in the feed). Excreta was collected every 24 hours. Cages were checked for egg production twice daily, and all eggs collected. Five hours after the final dose (53 hours after the initial dose) the hens were sacrificed. Liver (without bile bladder), kidney, leg muscle, breast muscle, skin,

subcutaneous fat and eggs from the ovary and oviduct were dissected from the hens. All samples were assayed for total radioactivity by liquid scintillation counting, either directly or by sample combustion. The identification and characterization of metabolites was achieved by high performance liquid chromatography following solvent extraction of tissues. Mass spectroscopy and ^{1}H-NMR were used to identify a single metabolite in the triazole-label study.

The recovered radioactivity accounted for 66.37% of the administered dose. A large fraction of the administered dose in the [triazole-UL-^{14}C] JAU6476-treated hens was eliminated in the excreta (65.61%). A total of 0.014% of the radioactivity was recovered in eggs, while 0.75% was present in the tissues and organs. A significant portion of the administered dose was absorbed from the intestinal tract prior to excretion, as indicated by the high residue concentrations in the kidney and liver.

Liver and kidney were found to contain the highest mean residues at 3.447 and 3.381 ppm respectively. Mean residues detected in other tissues represented 0.623, 0.342, 0.308, 0.139 and 0.096 ppm for eggs from the ovary and oviduct, subcutaneous fat, skin, leg muscle and breast muscle respectively. The major metabolites identified were parent JAU6476 in fat (15.9% of the TRRs; 0.046 ppm) and liver (30.7% of the TRRs; 1.085 ppm); JAU6476-desthio in fat (26.8% of the TRRs; 0.078 ppm); JAU6476-*S*-methyl in fat (28.5% of the TRRs; 0.083 ppm); JAU6476-*S*-glucuronide in liver (14.9% of the TRRs; 0.526 ppm) and eggs (23.7% of the TRRs; 0.012 ppm); sulfate conjugates of hydroxylated JAU6476-desthio in liver (13.5% of the TRRs, 0.474 ppm); 1*H*-1,2,4-triazole in muscle (18.7% of the TRRs; 0.023 ppm) and eggs (11.4% of the TRRs; 0.006 ppm); and JAU6476-triazolyl-ethanol in muscle (28.3% of the TRRs; 0.035 ppm) and eggs (15.6% of the TRRs; 0.008 ppm). Minor metabolites (<10% of the TRRs) included JAU6476-4-hydroxy, JAU6476-4-hydroxy-desthio, JAU6476-*N*-glucuronide and thiocyanate. Attempts were made to further release the bound liver residues by ultrasonic and microwave extractions in the presence of methanol and acidic methanol. These efforts released an additional 5.7% (0.202 ppm) of the TRRs in the liver, leaving 12.7% (0.448 ppm) of the TRRs as non-extractable residues. Accountabilities ranged from 100-102%.

The fact that the highest mean residues were found in the kidney and liver indicates that a significant fraction of the administered dose is absorbed through the intestine prior to excretion.

JAU6476 was extensively metabolized following the oral administration of [phenyl-UL-^{14}C] JAU6476 or [triazole-UL-^{14}C] JAU6476 to laying hens. The major metabolic pathways were:

- conjugation of the unchanged parent compound with glucuronic acid forming an *S*-glucuronide,
- methylation of the sulfur atom to form JAU6476-*S*-methyl,
- desulfuration of JAU6476 yielding JAU6476-desthio followed by hydroxylation and conjugation with sulfate, and
- cleavage of the chlorobenzylic group of JAU6476-desthio to JAU6476-triazolyl-ethanol and release of 1*H*-1,2,4-triazole.

Several minor metabolic processes were also elucidated. These minor pathways were:

- conjugation of JAU6476 to the *N*-glucuronide,
- hydroxylation of JAU6476 followed by glucuronidation,
- cleavage of the triazolinthione moiety of JAU6476 to yield thiocyanate,
- hydroxylations of JAU6476 and JAU6476-desthio and reduction of the phenyl ring to the corresponding dihydroxy-dienes followed by conjugation with glucuronic acid, and
- further hydroxylation of JAU6476-4-hydroxy-desthio followed by methylation and/or sulfate conjugation.

STUDY/WAIVER ACCEPTABILITY/DEFICIENCIES/CLARIFICATIONS:

The hen metabolism studies are classified as scientifically acceptable.

The acceptability of these studies for regulatory purposes is addressed in the forthcoming U.S. EPA Residue Chemistry Summary Document, DP Barcode D303508, PP#4F6830, and in Canada's Regulatory Decision Document.

COMPLIANCE:

Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided. No deviations from regulatory requirements were reported which would have an impact on the validity of the study.

A. BACKGROUND INFORMATION

Prothioconazole is a broad-spectrum systemic fungicide intended for the control of many diseases in different crops such as cereals, oilseed rape or peanuts. The biochemical mode of action is the inhibition of the demethylation of lanosterol or 24-methylene-dihydrolanosterol, which are precursors of sterols in fungi. The chemical has been submitted for joint EPA and PMRA review by Bayer CropScience. The crops proposed for joint review include barley, the dried shell and bean subgroup, the oilseed crop group, and wheat. Uses on peanuts and rice are being proposed for the U.S. only.

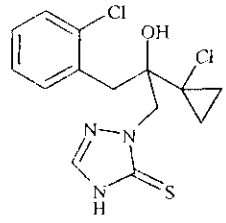
TABLE A.1 Test Compound Nomenclature.	
Chemical structure	
Common name	Prothioconazole (ISO accepted)
Company experimental name	JAU6476
IUPAC name	2-[(2*RS*)-2-(1-chlorocyclopropyl)-3-(2-chlorophenyl)-2-hydroxypropyl]-2*H*-1,2,4-triazole-3(4*H*)-thione
CAS name	2-[2-(1-chlorocyclopropyl)-3-(2-chlorophenyl)-2-hydroxypropyl]-1,2-dihydro-3*H*-1,2,4-triazole-3-thione
CAS registry number	178928-70-6
PC Code	113961
End-use product (EP)	Proline® 480 SC (4 lb/gal suspension concentrate), EPA File Symbol 264-IEL

TABLE A.2. Physicochemical Properties of the Technical Grade Test Compound.			
Parameter	Value		References
Melting range	139.1 to 144.5°C		MRID 46246003/CES
pH	5.8 (1% solution)		MRID 46246003/CES
Density at 20°C	1.36 g/mL		MRID 46246003/CES
Water solubility at 20°C	pH 4 8 9	mg/L 5 300 2000	MRID 46246003/CES
Solvent solubility at 20°C	Solvent Acetone Acetonitrile Dichloromethane Dimethysulfoxide Ethyl acetate n-Heptane 1-Octanol Polyethylene glycol 2-Propanol Xylene	g/L >250 69 88 126 >250 <0.1 58 >250 87 8	MRID 46246003/CES
Vapor pressure at 20 or 25°C	$<4 \times 10^{-7}$ Pa (calculated from determinations at 70°C)		MRID 46246003/CES
Dissociation constant, pK_a	6.9 (calculated from K_{OW})		MRID 46246003/CES
Octanol/water partition coefficient, at 20°C	pH unbuffered water 4 7 9	$Log(K_{OW})$ 4.05 4.16 3.82 2.00	MRID 46246003/CES
UV/visible absorption spectrum	Peak maxima at 257 nm. No absorption at > 300 nm.		MRID 46246003/CES

CES - Chemistry Evaluation Section of PMRA

B. EXPERIMENTAL DESIGN

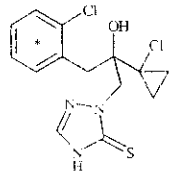
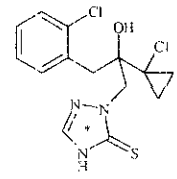
B.1. Livestock

TABLE B.1.1. General Test Animal Information.					
Species	Breed	Age	Weight at study initiation (kg)	Health Status	Description of housing/holding area
Laying hens	White Leghorn (*Gallus domesticus*)	ca. 22 weeks	1.43-1.62 (phenyl) 1.48-1.80 (triazole)	No observable toxicological signs.	Stainless steel metabolism cages in air-conditioned rooms (22 ± 1°C) with 18 hours of illumination. Relative humdity: 70±9% (phenyl); 50±6% (triazole).

TABLE B.1.2. Test Animal Dietary Regime.				
Composition of Diet	Feed consumption (g/day)	Water	Acclimation period	Predosing
Hoelever Laying Hen Complete Feed LA55 - phenyl study LA65 - triazole study	mean food consumption: 90.8 g/day/animal (5.9 - 6.1% of body weight)	Tap water, *ad libitum*	17 days (phenyl-study). Approximately 14 days (triazole-study)	None

TABLE B.1.3. Test Animal Dosing Regime.			
Treatment Type	Feeding Level (ppm test material in food)	Vehicle	Timing/Duration
Oral; dosing level was based on animal weight	171 (phenyl-study) 162 (triazole-study)	Oral administration of test substance in 0.5% aqueous tragacanth suspension, using a knob cannula attached to a glass syringe.	Once per day, after morning egg collection, for three consecutive days.

B.2. Test Materials

TABLE B.2.1. Test Material Characteristics.		
Chemical structure		
Radiolabel position	[phenyl-UL-^{14}C]-prothioconazole	[triazole-UL-^{14}C]-prothioconazole
Lot No.	12268/1	14813/1
Purity	>99% (chemical purity and radio-chemical purity)	>99% (chemical purity and radio-chemical purity)
Specific activity (Bq) [1]	0.732 MBq/mg (4.39 x 10^4 dpm/µg; 19.8 µCi/mg)	0.702 MBq/mg (4.21 x 10^4 dpm/µg; 18.97 µCi/mg)

[1] Bq = disintegrations per second

B.3. Sampling Information

TABLE B.3.1. Sample Collection Information.			
Eggs collected	Excreta collected	Interval from last dose to sacrifice	Tissues harvested and analyzed
Eggs were collected twice daily. The average daily egg production was reported for each of the dosed hens over the total course of the acclimation period plus dosing period; the average daily egg production was 1.02 eggs for the phenyl-label study and 0.99 eggs for the triazole-label study.	Excreta were collected as quantitatively as possible in 24-hour intervals.	5 hours	Liver, muscle (leg and breast), skin without subcutaneous fat, subcutaneous fat, eggs from ovary and oviduct.

B.4. Identification/Characterization of Residues

B.4.1. Sample Handling and Preparation

Eggs were collected twice daily. The white and yolk of each sample were combined, and an aliquot of each sample was radioassayed. All egg samples collected over the duration of the dosing period were then combined, radioassayed, and stored frozen (ca. -18°C) until extraction and analysis. Tissue samples, except skin and fat, were minced after collection. After radioassay, samples of liver, fat, and muscle (both types) from all hens were combined by tissue and then stored frozen (ca. -18°C) until extraction and analysis. Cysteine HCl was added to egg samples and to the extraction mixtures for tissues during sample preparation in order to stabilize the parent compound and the metabolites.

Phenyl-label Study:

Liver and muscle: A subsample was extracted three times with acetonitrile (ACN):water (80:20, v:v) and then twice with ACN:water (50:50, v:v). The first four extracts were combined, concentrated to aqueous, mixed with ACN, and partitioned twice with n-hexane. The n-hexane phases were partitioned twice with methanol. The methanol phases and the ACN/water phase were combined, evaporated to dryness, diluted with buffer solution (pH 3), and cleaned up on an XAD 7 column. The column was rinsed with buffer solution and water and then eluted with methanol. The methanol eluate was evaporated to dryness and redissolved in methanol for HPLC analysis.

A second subsample of liver was subjected to extraction procedures to attempt to release additional residues. The sample was initially extracted as described above (three extractions with 80:20 ACN:water and two extractions with 50:50 ACN:water). The remaining residues were then extracted with methanol and methanol acidified with acetic acid using an ultrasonic bath; these extraction steps were repeated and then the residues were extracted with ACN:water (80:20, v:v), followed by microwave extraction.

Egg: A subsample of egg was extracted three times with ACN. The extracts were combined, concentrated, mixed with methanol, and partitioned twice with hexane. The aqueous phase was evaporated to dryness, and residues were redissolved in buffer solution (pH 3) and cleaned up on an XAD 7 column. The column was rinsed with water and then eluted with methanol. The methanol eluate was evaporated to dryness and redissolved in water for HPLC analysis.

Fat: A subsample of fat was extracted once with ACN and four times with ACN:water (80:20, v:v). The first three extracts were combined, concentrated, mixed with methanol, and partitioned twice with hexane. The methanol/water phase was evaporated to dryness, diluted with buffer solution (pH 3), and cleaned up on an XAD 7 column. The column was rinsed with water and then eluted with methanol. The methanol eluate was evaporated to dryness and redissolved in methanol for HPLC analysis.

The extraction procedures for egg and tissue samples are summarized in FIGURES B.4.1.1-B.4.1.3.

FIGURE B.4.1.1 Extraction process for liver samples containing [phenyl-UL-^{14}C]-JAU6476.

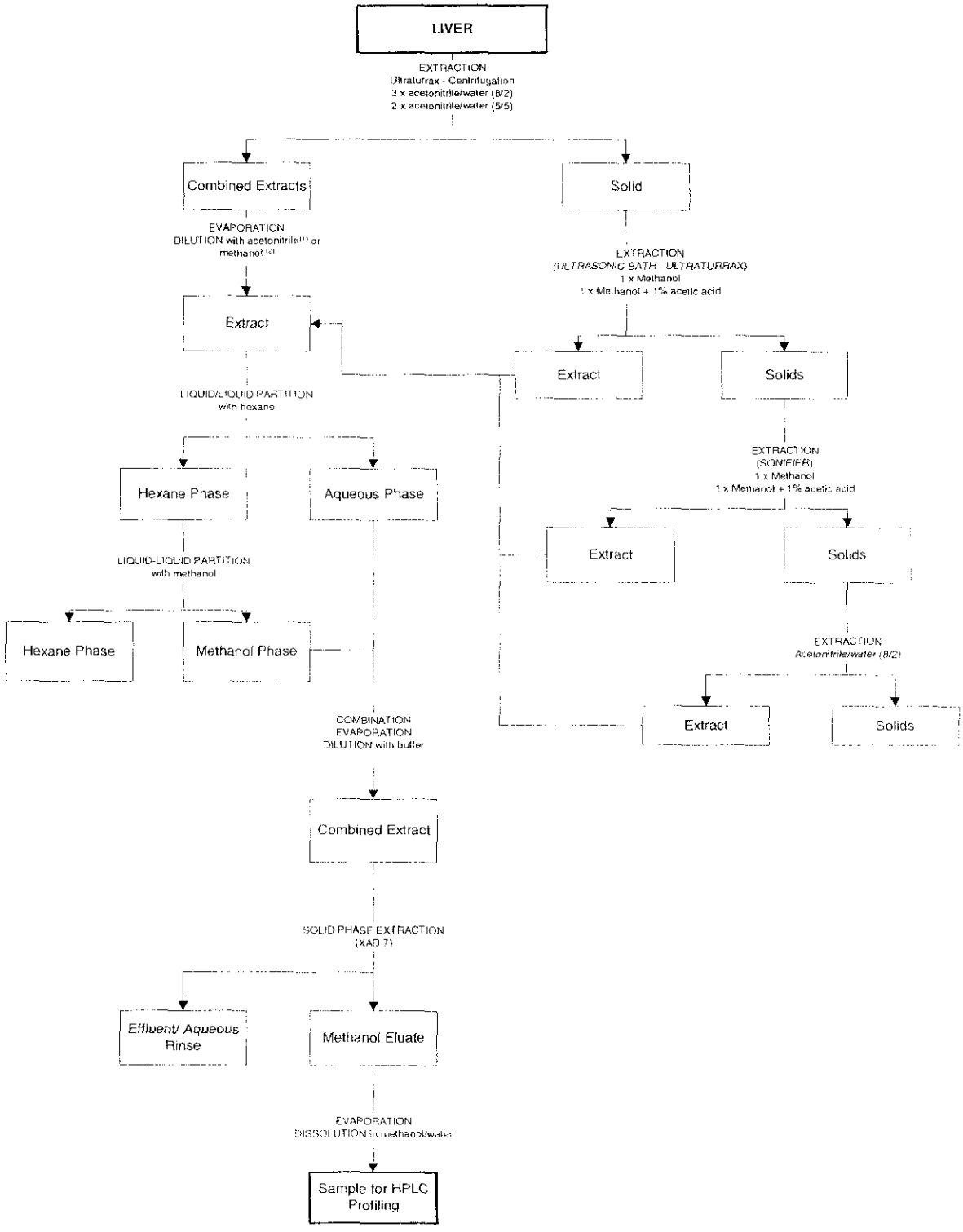


FIGURE B.4.1.2 Extraction process for muscle samples containing [phenyl-UL-^{14}C]-JAU6476.

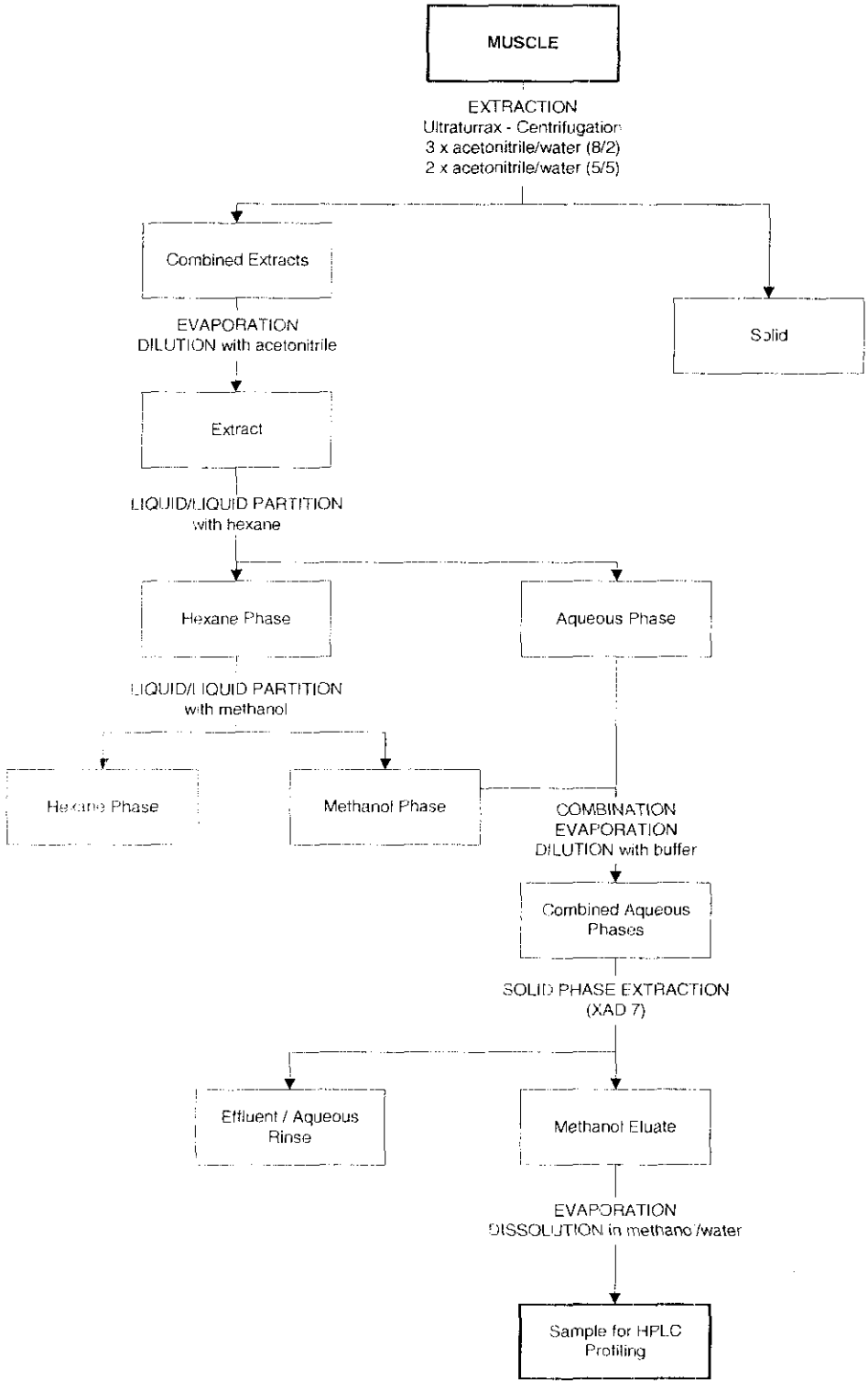
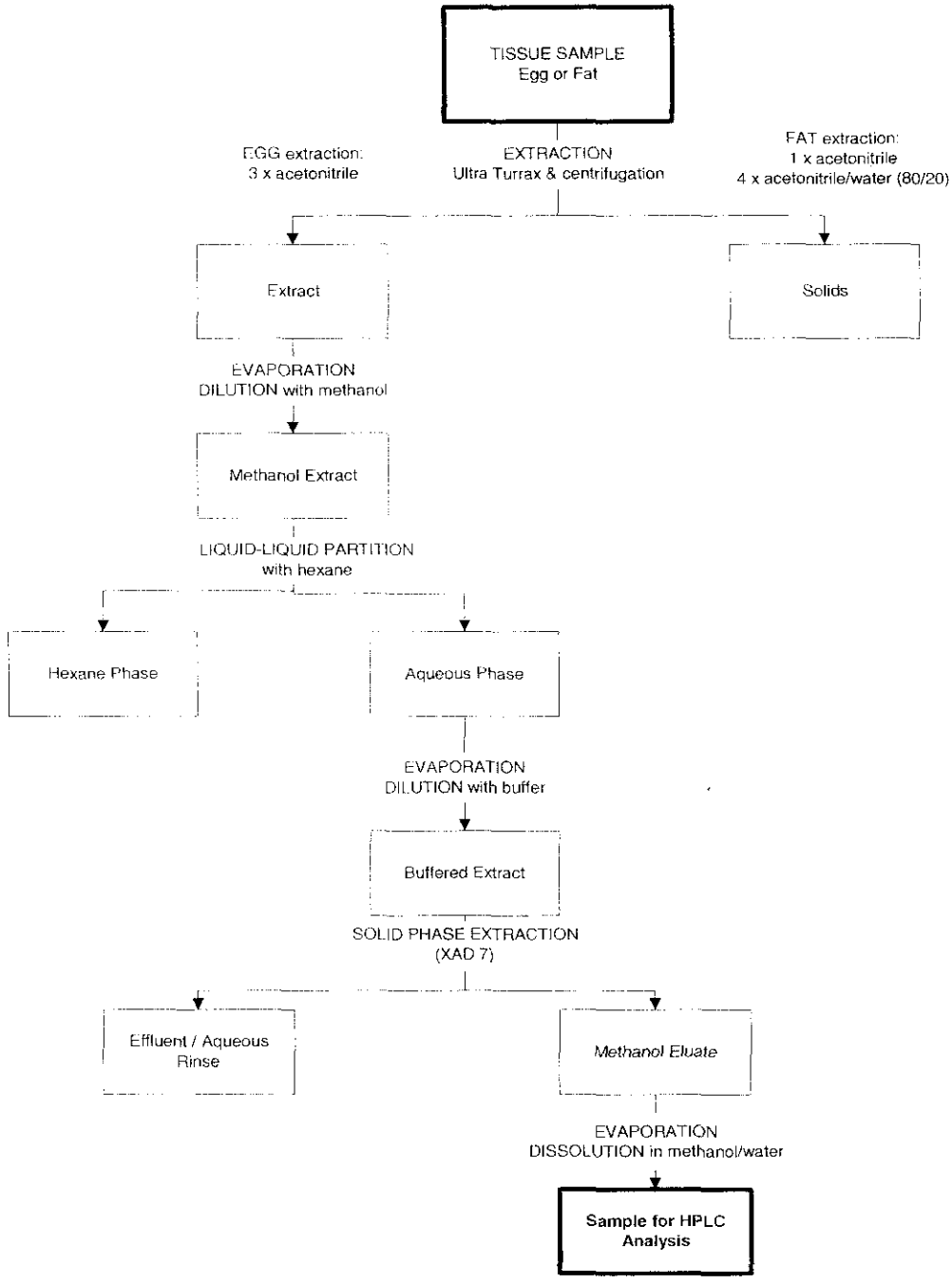


FIGURE B.4.1.3 Extraction process for egg and fat samples containing [phenyl-UL-^{14}C]-JAU6476.



Triazole-label Study:

Liver: A subsample of liver was extracted three times with acetonitrile (ACN):water (80:20, v:v) and then twice with ACN:water (50:50, v:v). The extracts were combined and cleaned up by C18 solid-phase extraction (SPE); the column was rinsed with ACN:water (80:20, v:v) and then eluted with dichloromethane:methanol (1:1, v:v). The effluent and rinse of the column were collected, combined, and concentrated for HPLC analysis.

The non-extractable residues were extracted with methanol and methanol acidified with acetic acid using an ultrasonic bath, and then extracted with methanol and acidified methanol using a microwave (up to 140°C for 10 minutes). All extracts were combined and concentrated for HPLC analysis.

Egg: A subsample of egg was extracted once with ACN and two times with ACN:water (80:20, v:v; water contained 1% cysteine HCl). The first two extracts were combined and cleaned up by C18 SPE; the column was rinsed with ACN:water (80:20, v:v; water) and then eluted with dichloromethane:methanol (1:1, v:v). The effluent and rinse of the column were collected, combined, and concentrated for HPLC analysis.

Muscle: A subsample of muscle was extracted three times with acetonitrile (ACN):water (80:20, v:v) and then twice with ACN:water (50:50, v:v). The first four extracts were combined and cleaned up by C18 SPE; the column was rinsed with ACN:water (80:20, v:v) and then eluted with dichloromethane:methanol (1:1, v:v). The effluent and rinse of the column were collected, combined, and concentrated for HPLC analysis.

Fat: A subsample of fat was extracted five times with ACN:water (80:20, v:v) and then once with n-heptane. The ACN/water extracts were combined and partitioned twice with n-heptane. The n-heptane phases were combined with the n-heptane extract and partitioned three times with ACN:water (80:20, v:v). All ACN/water phases were combined and cleaned up by C18 SPE; the column was rinsed with ACN:water (80:20, v:v) and then eluted with dichloromethane:methanol (1:1, v:v). The effluent and rinse of the column were collected, combined, and concentrated for HPLC analysis.

The extraction procedures for egg and tissue samples are summarized in FIGURES B.4.1.4-B.4.1.6.

FIGURE B.4.1.4 Extraction process for liver samples containing [triazole-UL-^{14}C]-JAU6476

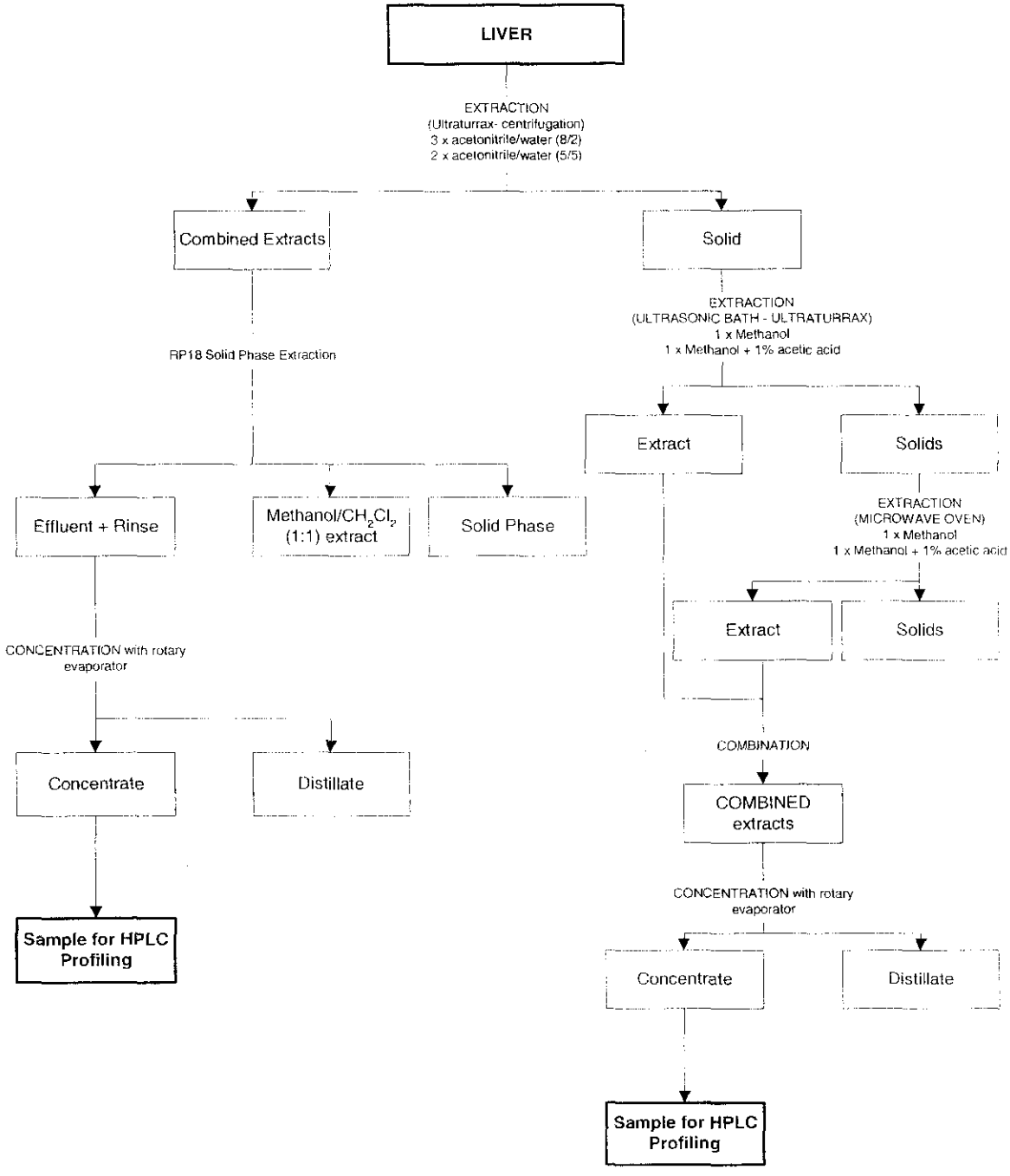


FIGURE B.4.1.5 Extraction process for egg and muscle samples containing [triazole-UL-^{14}C]-JAU6476

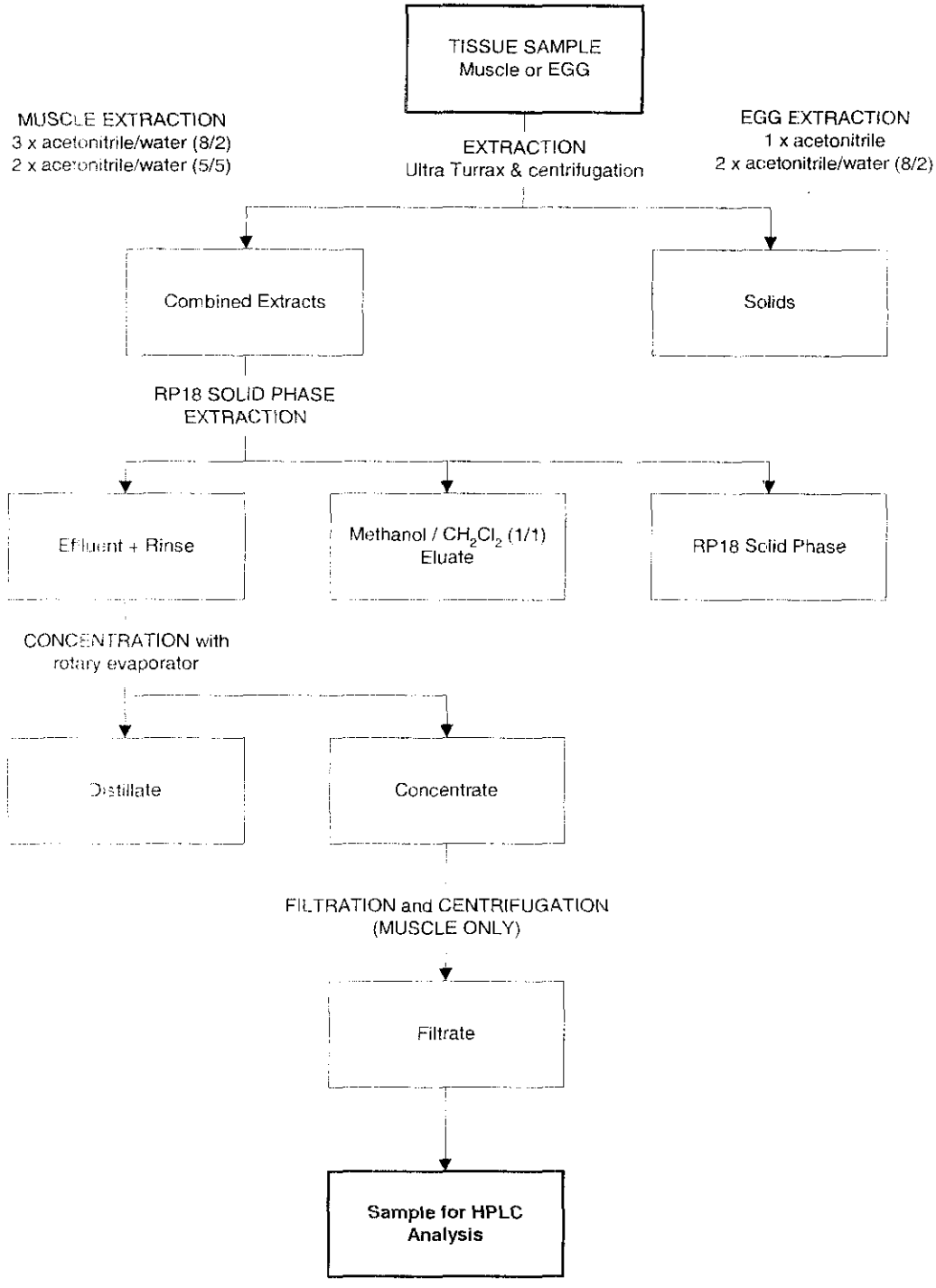
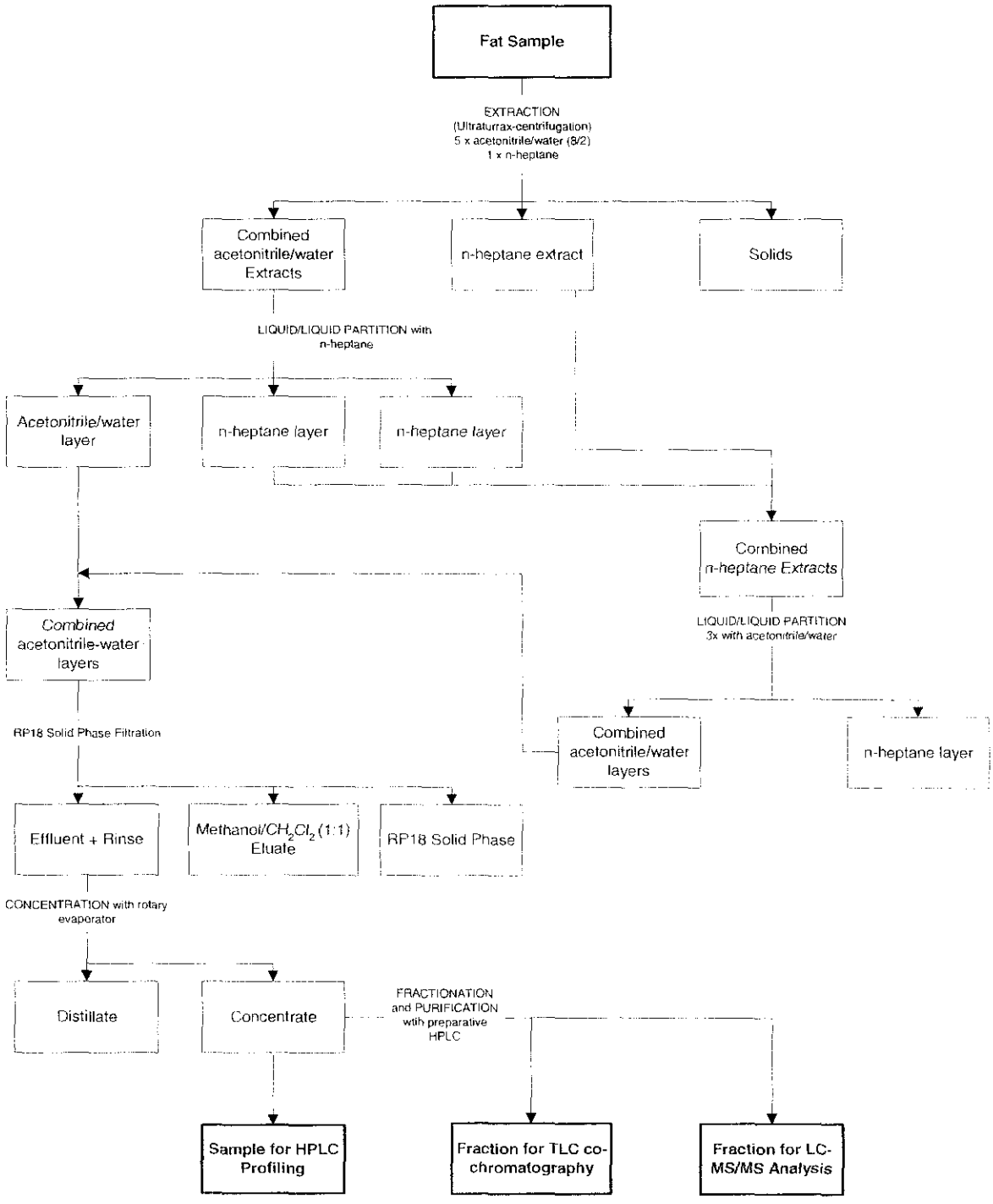


FIGURE B.4.1.6 Extraction process for fat samples containing [triazole-UL- ^{14}C]-JAU6476



B.4.2. Analytical Methodology

The in-life and analytical phases of the studies were conducted by Bayer CropScience (Monheim, Germany). Total radioactive residues (TRRs) were measured (in triplicate) in egg and tissue samples by combustion and liquid scintillation counting (LSC) after freeze drying the sample. For the phenyl-label study, the limits of quantitation (LOQs) for TRR determinations were 0.0003 ppm for eggs, 0.0004 ppm for liver, 0.0002 ppm for muscle, and 0.0008 ppm for fat. For the triazole-label study, the LOQs for TRR determinations were 0.001 ppm for eggs, 0.001 ppm for liver, 0.002 ppm for muscle, and 0.003 ppm for fat. To determine background radioactivity levels for LOQ calculations, the applicant used samples of eggs collected during the acclimation period and samples of tissue collected from hens from a different metabolism study.

Phenyl-label Study:
Extracts and hydrolysates were analyzed by HPLC using a system equipped with a UV detector (230 nm), a radiodetector, a C18 column, and a gradient mobile phase of water and ACN, each containing 1% acetic acid. Metabolites were identified by comparison of retention times or co-chromatography with reference standards.

The extracts used for HPLC profiling were fractionated and subjected to HPTLC analyses. TLC analyses were conducted on silica 60 F_{254} plates using automated multiple development (AMD) with methanol and dichloromethane. The applicant noted that prothioconazole reference standard (non-labeled) and cysteine HCl were spiked to each TLC origin before applying sample solutions to minimize decomposition of prothioconazole and metabolites. Non-labeled standards were visualized under UV light. Radioactive zones were detected using radioluminography. Metabolites were identified by co-chromatography.

Microwave extraction:
6 x 4-5 g of the solids after the methanol extraction steps were mixed with ca. 25 mL ACN/H_2O (80/20) and extracted in a microwave oven.

Conditions of microwave extraction:

pressure:	12.95 (stage 1)	12.95 (stage 2)
run time:	10 min (stage 1)	10 min (stage 2)
time @ p:	15 min (stage 1)	15 min (stage 2)
temperature:	80°C (stage 1)	120°C (stage 2)
power:	40% (stage 1)	50% (stage 2)

Only 1.8% of the TRRs (extract KOE0708.20) could be solubilized by this harsh extraction procedure. Due to the low amounts of radioactivity, the methanol extracts and the microwave extract have not been further analyzed.

Triazole-label Study:
Extracts and hydrolysates were analyzed by HPLC using a system equipped with a UV detector (210 or 254 nm), a radiodetector, and one of the following column/mobile phase combinations: (1) a C18 column and a gradient mobile phase of water and ACN, each containing 1% acetic acid; (2) a C8 column with a gradient mobile phase of water and methanol, each containing ion pairing reagent tetrabutylammonium-hydrogensulfate at 0.005M; (3) a Diol column with a gradient mobile phase of n-hexane and 3% aqueous ammonia in ethanol; (4) a C18 column with a gradient mobile phase of aqueous pH 7 phosphate buffer and ACN; or (5) a C18 column with a gradient mobile phase of water and ACN [systems 3, 4, and 5 were used for metabolite isolation and purification]. Metabolites were identified by comparison of retention times or co-chromatography with reference standards.

The extracts used for HPLC profiling were fractionated and subjected to HPTLC analyses. TLC analyses were conducted on silica 60 F_{254} plates using automated multiple development (AMD) with methanol and dichloromethane, or using one of the following solvent systems: (1) dichloromethane:methanol:aqueous ammonia (80:20:5, v:v:v); ACN:water:aqueous ammonia (80:18:2, v:v:v); or n-butanol:acetic acid:water (60:20:20, v:v:v). The applicant noted that prothioconazole reference standard (non-labeled) and cysteine HCl were spiked to each TLC origin before applying sample solutions to minimize decomposition of prothioconazole and metabolites. Non-labeled standards were visualized under UV light. Radioactive zones were detected using radioluminography. Metabolites were identified by co-chromatography.

LC-MS or LC-MS/MS analyses were used for metabolite confirmation or for identification of metabolites which could not be identified by HPLC or HPTLC. Analyses were conducted using a reverse-phase column, a gradient mobile phase of water and acetonitrile, each containing 0.1% formic acid, and MS or MS/MS detection with electrospray ionization.

The chemical names and structures of the reference standards used in these studies are presented in Appendix 1.

C. RESULTS AND DISCUSSION

Phenyl-label study

The storage intervals and conditions for the hen metabolism studies are presented in TABLE C.1. It was stated that analysis of the extracts was completed within 3 months of sample collection for all matrices. Thus, supporting storage stability data are not required. Total radioactive residues (TRRs) and distribution of TRRs for both radiolabels are presented in FIGURES C.2.1 and C.2.1.1.

TRRs in individual and composited hen eggs and tissues are reported in TABLE C.2.1a. TRRs were 0.0002-0.1648 ppm in eggs, 2.858-5.414 ppm in liver, 3.683-6.501 ppm in kidney, 0.038-0.137 ppm in muscle, and 0.253-0.730 ppm in fat from hens dosed orally with [phenyl-UL-^{14}C]-prothioconazole at 171 ppm in the diet for 3 consecutive days. Radioactivity was highest in liver

and kidney, and lowest in eggs. Residues in eggs did not appear to have reached a plateau by the end of the dosing period; TRRs in eggs collected from the ovary/oviduct at sacrifice were 0.494-0.819 ppm. A large portion of the administered dose, ~67-92%, was excreted. The remaining portion of the administered dose could not be estimated directly because the excreta of birds is comprised of renal and fecal fractions. The high residue concentrations in liver and kidney are indicative of absorption from the intestinal tract prior to excretion.

The distribution of the radioactivity in hen matrices is presented in TABLE C.2.2a. The majority of the radioactivity (~77-95% of the TRRs) was extracted using ACN (egg) or ACN/water (tissues). Non-extractable residues accounted for ≤0.02 ppm (2.6-22.9% of the TRRs) in egg, muscle, and fat, and 14.4% of the TRRs (0.579 ppm) in liver. The applicant normalized the extraction results to 97.2-101.1%; however, accountabilities prior to normalization were 91-118%. Residues were characterized primarily by HPLC analysis, using TLC analyses for confirmation.

The characterization and identification of residues in hen matrices are summarized in TABLE C.2.3a. Approximately 42-84% of the TRRs were identified in hen matrices. Prothioconazole was found to be a major residue in liver at 24.8% of the TRRs (1.00 ppm), in muscle at 11.33% of the TRRs (0.01 ppm), and in fat at 30.33% of the TRRs (0.14 ppm). Prothioconazole was found at lower levels in egg (<4% of the TRRs; 0.001 ppm). JAU6476-desthio was a major residue in egg at 20.1% of the TRRs (0.007 ppm), and fat at 29.0% of the TRRs (0.130 ppm); JAU6476-desthio was found at lower levels in liver and muscle (<8% of the TRRs). JAU6476-*S*-methyl was a major metabolite in fat (19.6% of the TRRs; 0.09 ppm) but was a minor residue in liver, egg, and muscle (<7% of the TRRs; <0.09 ppm). JAU6476-glucuronide (-*O*- or *S*-glucuronide) was a major residue in liver (11.9% of the TRRs; 0.479 ppm), egg (17.0% of the TRRs; 0.006 ppm), and muscle (15.5% of the TRRs, 0.014 ppm); this metabolite was found at ~5% of the TRRs (0.024 ppm) in fat. Additional metabolites identified in hen matrices, each at <10% of the TRRs, were JAU6476-desthio-3,4-dihydroxy-dienyl-glucuronide (liver and muscle), sulfate conjugates of JAU6476-hydroxy-desthio, JAU6476-dihydroxy-desthio, and JAU6476-hydroxy-methoxy-desthio (liver and muscle), JAU6476-dihydroxy-diene (liver and muscle), JAU6476-hydroxy-glucuronides (liver), JAU6476-4-hydroxy-desthio (liver and egg), JAU6476-*N*-glucuronide (liver and muscle), and JAU6476-4-hydroxy (liver and muscle). Unknowns accounted for <6% of the TRRs (≤0.071 ppm) in liver, muscle, and eggs.

The applicant stated that metabolites in egg, muscle, and fat were identified by comparison of the HPLC profile with that of liver, and that metabolites in liver were identified by comparison of the HPLC profile with the HPLC profile of liver from a goat metabolism study with [phenyl-UL-^{14}C]-prothioconazole (MRID 46246150). Identities were then confirmed by HPTLC co-chromatography of the isolated metabolites with reference standards. HPTLC co-chromatography was used to confirm the identification of the following compounds: prothioconazole, JAU6476-glucuronide, JAU6476-desthio, and JAU6476-S-methyl in all matrices; and JAU6476-desthio-3,4-dihydroxy-dienyl-glucuronide and JAU6476-hydroxy-glucuronides in liver.

The applicant noted that sulfate conjugates were identified in liver and muscle by comparing the HPLC profile with that of milk from a goat metabolism study conducted with [phenyl-UL-^{14}C]-JAU6476-desthio; identifications were confirmed by HPTLC. In the goat metabolism study, LC-MS analyses and acid hydrolysis of the milk extract indicated that two isomers of the sulfate conjugate of JAU6476-hydroxy-desthio were possible.

The non-extractable residues of liver accounted for approximately 21% of the TRRs (0.836 ppm). The extraction of liver was repeated, and the solids remaining after extraction were subjected to extraction with methanol and acidic methanol using ultrasonication and microwave extraction, which released 6.4% of the TRRs (0.257 ppm). A last extraction step was performed with acetonitrile/water in a microwave oven, which released an additional 1.8% of the TRRs, leaving 14.4% of the TRRs (0.579 ppm) as non-extractable. The methanol extracts and the microwave extracts were not further analyzed due to the low amounts of radioactivity. It was noted that after the microwave extraction, the solid residue had a gel type consistency and could not be processed further. However, it was proposed by the applicant that additional information about the nature of the residue in methanol extracts can be derived by comparison of the phenyl-label and triazole-label-studies. Identical extraction procedures using acetonitrile/water mixtures were conducted for liver in the laying hen studies with both the phenyl label and the triazole label. The solvent extracts from both studies were analyzed with the same HPLC method and showed very similar metabolic profiles except the very small proportions amounts of label specific metabolites (1*H*-1,2,4-triazole, thiocyanate and JAU6476–triazolyl-ethanol) detected in the triazole-label study. All other metabolites identified were common to both labels. The solids in both studies were further extracted with methanol/water mixtures using similar extraction procedures. These methanol extracts had not been profiled in the phenyl-label study but in the triazole-label study. Label specific polar metabolites together with unknown polar fractions, JAU6476-desthio and parent compound were detected in these extracts. The applicant assumed that similar polar fractions and parent compound represent a major proportion of the methanol extract in the phenyl-label study, as well. It was estimated that sufficient efforts have been made in the study to characterize non-extractable residues in liver.

Triazole-label study

The applicant provided the dates of initial sample extraction and initial and final analysis. The HPLC profiles of the extracts of hen matrices were completed within 116 days of sample collection, and final analyses were conducted within 5 months of collection for egg, muscle, and fat, and within 9 months of collection for liver. The extracts of hen matrices were reanalyzed after 7-8 months of frozen storage to demonstrate storage stability. Comparison of the HPLC profiles indicated that the metabolite profiles were stable in hen liver, egg, muscle, and fat extracts during ~7-8 months of frozen storage. The submitted storage stability information and data are adequate to support the hen metabolism study.

Total radioactive residues (TRRs) in individual and composite hen eggs and tissues are reported in TABLE C.2.1b. TRRs were 0.0002-0.1996 ppm in eggs, 2.548-4.129 ppm in liver, 2.885-4.417 ppm in kidney, 0.071-0.236 ppm in muscle, and 0.214-0.404 ppm in fat from hens dosed

orally with [triazole-^{14}C]-prothioconazole at 163 ppm in the diet for 3 consecutive days. Radioactivity was highest in liver and kidney, and lowest in eggs. Residues in eggs did not appear to have reached a plateau by the end of the dosing period. TRRs in eggs collected from the ovary/oviduct at sacrifice were 0.455-0.898 ppm. A large portion of the administered dose, ~62-72%, was excreted. The remaining portion of the administered dose could not be estimated directly because the excreta of birds is comprised of renal and fecal fractions. The high residue concentrations in liver and kidney are indicative of absorption from the intestinal tract prior to excretion.

The distribution of the radioactivity in hen matrices is presented in TABLE C.2.2b. The majority of the radioactivity (~81-93% of the TRRs) was extracted using ACN/water. Cysteine HCl was added to eggs prior to extraction and to all extraction mixtures in order to stabilize the parent compound and metabolites. Non-extractable residues accounted for <19% of the TRRs (≤0.03 ppm) in egg, muscle, and fat, and 12.7% of the TRRs (0.448 ppm) in liver. The applicant normalized the extraction results for accountabilities of 100.0-102.0%. However, accountabilities prior to normalization were 97-106%. Residues were characterized primarily by HPLC analysis, using TLC analyses for confirmation, and LC-MS/MS analyses for identification of metabolites unique to the triazole study. These methods successfully identified the predominant residues in hen matrices.

Hen liver was extracted with water/acetonitrile to yield an extract containing 79.7% of the TRRs. The solids were further extracted under harsher conditions in 4 steps with methanol using an ultrasonic bath and a microwave oven, yielding an extract representing another 5.7% of the TRRs (0.202 ppm), and leaving 12.7% of the TRRs (0.448 ppm) unextracted in the solids. Compared to the profile of water/acetonitrile extract, the profile of the methanol extract showed qualitatively the same metabolic pattern, only the quantitative proportion was shifted in favor of the more polar metabolites as 1*H*-1,2,4-triazole, thiocyanate, an unknown component and JAU6476-triazolyl-ethanol. From these two extraction experiments, it appears that further extraction would neither reveal new metabolites, nor change the overall quantitative profile of the liver significantly.

The characterization and identification of residues in hen matrices is summarized in TABLE C.2.3b. Approximately 68-82% of the TRRs were identified in hen matrices. Prothioconazole was found to be a major residue in liver, at 30.7% of the TRRs (1.085 ppm), and fat, at 15.9% of the TRRs (0.046 ppm). Prothioconazole was found at lower levels in egg and muscle (<3.4% of the TRRs, ≤0.003 ppm). Free triazole or 1*H*-1,2,4-triazole accounted for a significant portion of radioactivity in egg (11.4% of the TRRs; 0.006 ppm) and muscle (18.7% of the TRRs; 0.023 ppm). 1*H*-1,2,4-triazole was found at lower levels in liver and fat (<2% of the TRRs; ≤0.04 ppm). JAU6476-triazolyl-ethanol was a major metabolite in egg (15.6% of the TRRs, 0.008 ppm) and muscle (28.3% of the TRRs; 0.035 ppm) and was found at lower levels in liver and fat (<4% of the TRRs; ≤0.129 ppm). Sulfate conjugates of JAU6476-hydroxy-desthio, JAU6476-dihydroxy-desthio, and JAU6476-hydroxy-methoxy-desthio together accounted for 12% of the TRRs (0.41 ppm) in liver; these conjugates were detected in fat at <1% of the TRRs (0.002 ppm) but were not found in egg or muscle. JAU6476-*S*-glucuronide was found to be a major

metabolite at 14.9% of the TRRs (0.526 ppm) in liver, 23.7% of the TRRs (0.012 ppm) in egg, 9.8% of the TRRs (0.012 ppm) in muscle, and 7.2% of the TRRs (0.021 ppm) in fat. The metabolites JAU6476-desthio and JAU6476-*S*-methyl were the major metabolites in fat, at 26.8% of the TRRs (0.078 ppm) and 28.5% of the TRRs (0.083 ppm), respectively. These metabolites were found in liver, egg, and muscle at <6.3% of the TRRs (≤0.172 ppm). Additional metabolites identified in hen matrices, each at <10% of the TRRs, were thiocyanate (all matrices), JAU6476-4-hydroxy-desthio (liver); JAU6476-*N*-glucuronide (liver), and JAU6476-4-hydroxy (liver). Unknowns and polar metabolic groups accounted for <11.3% of the TRRs (≤0.02 ppm) in egg, muscle, and fat, and 13.1% of the TRRs (0.463 ppm) in liver (each peak present at <8% of the TRRs).

Metabolites were identified by comparison of the HPLC profile with that of the corresponding extract from the hen metabolism study conducted using [phenyl-^{14}C]-prothioconazole. For the egg extract, the profile was also compared to the HPLC profile of the liver and muscle extracts of the triazole-label study. Identities were then confirmed by HPLC or TLC co-chromatography of the isolated metabolites with reference standards. TLC chromatography was used for quantification of metabolites which co-eluted in HPLC analyses [JAU6476-*S*-glucuronide and JAU6476-4-hydroxy-desthio; and JAU6476-*N*-glucuronide and JAU6476-4-hydroxy]. HPLC co-chromatography was used to identify the following compounds: prothioconazole in liver; thiocyanate in muscle; JAU6476-*S*-glucuronide in liver; JAU6476-4-hydroxy-desthio in liver; JAU6476-*N*-glucuronide in liver; JAU6476-4-hydroxy in liver; JAU6476-desthio in liver and egg; and JAU6476-*S*-methyl in liver. TLC co-chromatography was used to confirm the identification of the following compounds: 1*H*-1,2,4-triazole in egg, muscle, and fat; thiocyanate in egg and fat; sulfate conjugates of JAU6476-hydroxy-desthio, JAU6476-dihydroxy-desthio, and JAU6476-hydroxy-methoxy-desthio in liver; JAU6476-*S*-glucuronide in liver; JAU6476-4-hydroxy-desthio in liver; JAU6476-*N*-glucuronide in liver; and JAU6476-4-hydroxy in liver. All other identifications (with the exception of JAU6476-triazolyl-ethanol) were made by retention time comparisons.

Thiocyanate and 1*H*-1,2,4-triazole co-eluted using the HPLC system used for metabolite profiling; a different system was used to quantify the individual amounts of these metabolites. To confirm the identification of 1*H*-1,2,4-triazole in muscle, the fraction containing that metabolite was isolated and treated with dansyl chloride; the dansyl derivative of 1*H*-1,2,4-triazole was then identified by HPLC co-chromatography with a dansyl derivative synthesized from 1*H*-1,2,4-triazole reference standard.

The fraction containing metabolite JAU6476-triazolyl-ethanol was isolated from muscle, purified by HPLC, and the structure was elucidated using LC-MS/MS and ^{1}H NMR. This metabolite was identified in liver, egg and fat by comparison of the HPLC profile with the profile of muscle.

Based on TLC analyses using co-chromatography with reference standards, the applicant excluded the presence of triazole acetic acid and triazolylalanine in liver, egg, and muscle.

C.1. Storage Stability

Phenyl-label Study: Dates of sample extraction or analysis were provided. It was stated that initial extraction and analysis (HPLC and HPTLC) of the major extracts of hen matrices was completed within 3 months of sample collection (sacrifice of the hen). The metabolite profiles used for identification and quantification were based on these initial analyses. For liver and muscle, second extractions were conducted (to attempt to release additional radioactivity in the case of liver, and to confirm two metabolite identifications in the case of muscle); these extractions and analyses were also completed within approximately 3 months after sample collection. Because all extractions and analyses were completed within 3 months of sample collection, supporting storage stability data are not required.

Triazole-label Study: Samples of hen matrices were stored frozen (ca. -18°C) prior to analysis. The applicant provided dates of initial extraction and initial and final analysis. All samples were initially extracted and profiled within 116 days (3.8 months) of collection, and final analyses were completed within 148 days (4.9 months) of collection for egg, muscle, and fat, and within 279 days (9.2 months) of collection for liver. To demonstrate storage stability, the extracts of all matrices were reanalyzed by HPLC approximately 10 months after sample collection (ca. 7-8 months after initial extraction). Representative chromatograms from the initial and final analyses were included for all matrices. The results of these analyses indicate that the metabolite profiles were stable in extracts during frozen storage for approximately 7-8 months. For the egg extract, some differences in the HPLC profile were observed; however, the differences were not significant.

TABLE C.1. Summary of Storage Conditions.			
Matrix	Storage Temp. (°C)	Actual Storage Duration[1]	Interval of Demonstrated Storage Stability
Phenyl-label Study:			
Egg	ca. -18	≤3 months	Not required
Liver		≤3 months	
Muscle		≤3 months	
Fat		≤3 months	
Triazole-label Study:			
Egg	ca. -18	70-148 days (2.3-4.9 months)	Extract: 241 days (7.9 months)
Liver		70-279 days (2.3-9.2 months)	Extract: 236 days (7.8 months)
Muscle		88-147 days (2.9-4.8 months)	Extract: 210 days (6.9 months)
Fat		116 days (3.8 months)	Extract: 200 days (6.6 months)

[1] The first analyses were conducted within 4-21 days of extraction for the triazole-label study only.

C.2. Identification, Characterization, and Distribution of Residues

TABLE C.2.1a. Total Radioactive Residues (TRRs) from the Phenyl-Label Study in Eggs, Tissue and Excreta.

Matrix	Collection Timing (hours after first dose)	Residues (ppm)						
		Hen No.						
		1	2	3	4	5	6	Mean
Eggs	24	0.0003	0.0002	0.0004	0.0002	0.0235	0.0007	0.004
	48	0.0147	0.0159	0.0218	0.0199	0.0782	0.0248	0.029
	53	0.0722	0.0591	0.0542	0.0657	0.1648	0.1001	0.086
Egg, composite sample	Over duration of study	0.036						
Eggs from ovary/oviduct	At sacrifice	0.642	0.496	0.496	0.494	0.819	0.634	0.597
Liver	At sacrifice	5.211	2.858	4.187	2.934	3.883	5.414	4.081
Liver, composite sample	At sacrifice	4.017						
Kidney	At sacrifice	4.214	3.683	5.242	3.721	3.863	6.501	4.537
Muscle, leg	At sacrifice	0.137	0.068	0.103	0.081	0.114	0.137	0.107
Muscle, breast	At sacrifice	0.071	0.038	0.058	0.043	0.059	0.078	0.058
Muscle, composite sample	At sacrifice	0.089						
Skin, without subcutaneous fat	At sacrifice	0.501	0.251	0.403	0.318	0.384	0.441	0.383
Fat, subcutaneous	At sacrifice	0.387	0.253	0.449	0.311	0.470	0.730	0.433
Fat, composite sample	At sacrifice	0.450						
% of Administered Dose								
Excreta	24	24.28	31.12	34.64	27.66	29.23	29.14	29.34
	48	29.58	34.33	29.75	31.27	30.17	27.66	30.46
	53	13.24	25.65	22.44	23.36	17.60	9.43	18.62
	Total	67.10	91.10	86.83	82.29	77.00	66.23	78.42
Eggs	24	0.00003	0.00002	0.00006	0.00002	0.002	0.00006	0.0004
	48	0.001	0.002	0.002	0.002	0.008	0.002	0.003
	53	0.007	0.006	0.006	0.006	0.011	0.009	0.008
	Total	0.008	0.008	0.008	0.008	0.021	0.011	0.011
Tissue, estimated	At sacrifice	1.04	0.62	0.88	0.70	0.84	0.99	0.85
Total % of Administered Dose	--	68.15	91.73	87.72	83.00	77.86	67.23	79.28

[1] Calculated using body weight and assuming body muscle, fat, and skin (without fat) account for 40%, 12%, and 4% of body weight, respectively.

TABLE C.2.2a. Distribution of the Parent and the Metabolites in Hen Matrices Following Dosing with [Phenyl-UL-^{14}C]-Prothioconazole at 171 ppm in the Diet [1]

Metabolite Fraction	Liver		Egg		Muscle		Fat	
	TRR = 4.017 ppm		TRR = 0.036 ppm		TRR = 0.089 ppm		TRR = 0.450 ppm	
	% TRR	ppm	% TRR	ppm	% TRR	ppm	%TRR	ppm
ACN/water extract	78.5	3.155	77.1	0.027	79.6	0.070	94.9	0.427
-ACN (evaporated)	0.1	0.005	1.9	0.001	1.7	0.001	0.5	0.002
-Hexane phase	1.0	0.040	1.8	0.001	4.4	0.004	5.4	0.024
-Aqueous phase	77.4	3.110	73.3	0.026	73.5	0.065	89.0	0.401
--Methanol XAD eluate	72.4	2.907	53.0	0.019	53.6	0.047	84.1	0.379
Prothioconazole	24.76	0.995	3.57	0.001	11.33	0.010	30.33	0.137
JAU6476-desthio-3,4-dihydroxy-dienyl-glucuronide	2.30	0.092	--	--	0.59*	0.001	--	--
sulfate conjugate of JAU6476-hydroxy-desthio	3.26	0.131	--	--	--	--	--	--
sulfate conjugates of JAU6476-hydroxy-desthio, JAU6476-dihydroxy-desthio, JAU6476-hydroxy-methoxy-desthio	7.81	0.314	--	--	1.52	0.001	--	--
JAU6476-dihydroxy-diene	0.88*	0.035	--	--	1.33*	0.001	--	--
JAU6476-hydroxy-glucuronides	2.55 [2]	0.103	--	--	--	--	--	--
JAU6476-glucuronide	11.93 [3]	0.479	16.98	0.006	15.50	0.014	5.26	0.024
JAU6476-4-hydroxy-desthio	2.71	0.109	3.34*	0.001	--	--	--	
JAU6476-N-glucuronide	1.08*	0.043	--	--	0.64*	0.001	--	--
JAU6476-4-hydroxy	0.72*	0.029	--	--	1.62*	0.001	--	--
JAU6476-desthio	4.17	0.167	20.13	0.007	7.16	0.006	28.96	0.130
JAU6476-S-methyl	2.24	0.090	1.88	0.001	6.42	0.006	19.56	0.088
Unknowns	1.76	0.071	6.03	0.002	1.71	0.002	--	--
Polar metabolite	6.19	0.249	--	--	5.82	0.005	--	--
Electronic spike			1.03	0.000				
--XAD effluent/rinse	5.0	0.203	20.3	0.007	19.9	0.018	4.9	0.022
ACN/water extract	0.6	0.026	--	--	1.0	0.001	2.5	0.012
Non-extractable	20.8 [4]	0.836	22.9	0.008	19.4	0.017	2.6	0.012
Methanol /Acetic Acid (Sonication)	3.3	0.133						
Methanol /Acetic Acid (Microwave)	3.1	0.124						
Non-extractable	14.4	0.579	22.9	0.008	19.4	0.017	2.6	0.012

[1] *Shading indicates that the extraction step and/or characterization analysis was not conducted for the matrix in question.*

[2] HPLC analyses indicated the presence of JAU6476-4-hydroxy-glucuronide and another JAU6476-hydroxy-glucuronide.

[3] May have co-eluted with JAU6476-3-hydroxy-desthio.

[4] In a separate experiment, the non-extractable liver residues were re-extracted with methanol and methanol/acetic acid in an ultrasonic bath and in a microwave. These extractions solubilized additional residues leaving 14.4% of the TRRs as non-extractable.

* These are considered characterized peaks, as they were identified by HPLC only (no HPTLC confirmation).

TABLE C.2.3a. Summary of Characterization and Identification of Radioactive Residues in Hen Matrices Following Dosing with [Phenyl-UL-^{14}C]-prothioconazole at 171 ppm in the Diet.

Compound	Liver		Egg		Muscle		Fat	
	TRR = 4.017 ppm		TRR = 0.036 ppm		TRR = 0.089 ppm		TRR = 0.450 ppm	
	% TRR	ppm	% TRR	ppm	% TRR	ppm	%TRR	ppm
Identified								
Prothioconazole	24.76	1.00	3.57	0.001	11.33	0.01	30.33	0.14
JAU6476-desthio-3,4-dihydroxy-dienyl-glucuronide	2.30	0.09	--	--	0.59*	0.00	--	--
sulfate conjugate of JAU6476-hydroxy-desthio	3.26	0.13	--	--	--	--	--	--
sulfate conjugates of JAU6476-hydroxy-desthio, JAU6476-dihydroxy-desthio, JAU6476-hydroxy-methoxy-desthio	7.81	0.31	--	--	1.52	0.00	--	--
JAU6476-dihydroxy-diene	0.88*	0.035	--	--	1.33*	0.001	--	--
JAU6476-hydroxy-glucuronides	2.55	0.10	--	--	--	--	--	--
JAU6476-*O*- or S-glucuronide	11.93 [1]	0.48	16.98	0.006	15.50	0.01	5.26	0.02
JAU6476-4-hydroxy-desthio	2.71	0.11	3.34*	0.001	--	--	--	--
JAU6476-N-glucuronide	1.08*	0.043	--	--	0.64*	0.001	--	--
JAU6476-4-hydroxy	0.72*	0.029	--	--	1.62*	0.001	--	--
JAU6476-desthio	4.17	0.17	20.13	0.007	7.16	0.01	28.96	0.13
JAU6476-*S*-methyl	2.24	0.09	1.88	0.001	6.42	0.01	19.56	0.09
Characterized								
Unknowns	1.76	0.07	6.03	0.002	1.71	0.00	--	--
Polar metabolite	6.19	0.249	--	--	5.82	0.01	--	--
Characterized	2.68	0.107	3.34	0.001	4.18	0.01	--	--
Fractions not analyzed	13.10	0.531	24.00	0.009	27.00	0.02	13.30	0.06
Total identified	61.7	2.480	42.6	0.015	41.9	0.037	84.1	0.379
Total characterized	23.7	0.958	33.4	0.012	38.7	0.036	13.3	0.060
Total extractable	85.4	3.438	76.0	0.027	80.6	0.073	97.4	0.439
Electronic spike	--	--	1.0	0.000	--	--	--	--
Non-extractable (PES) [2]	14.4	0.579	22.9	0.008	19.4	0.017	2.6	0.012
Accountability	100.0		97.2		101.1		100.0	

[1] May have co-eluted with JAU6476-3-hydroxy-desthio.

[2] In a separate experiment, the non-extractable liver residues were re-extracted with methanol and methanol/acetic acid in an ultrasonic bath and in a microwave. These extractions solubilized additional residues leaving 14.4% of the TRRs as non-extractable.

[3] Accountability = (Total extractable + Total unextractable)/(absolute values of the TRRs (ppm)) * 100.

* These are considered characterized peaks, as they were identified by HPLC only (no HPTLC confirmation). Therefore they are tallied under total characterized instead of total identified.

TABLE C.2.1b. Total Radioactive Residues (TRRs) from the Triazole-Label Study in Eggs, Tissue and Excreta.

Matrix	Collection Timing (hours after first dose)	Residues (ppm)						
		Hen No.						
		1	2	3	4	5	6	Mean
Eggs	24	0.0025	0.0006	0.0002	0.0022	0.0002	0.0004	0.001
	48	0.1996	0.0526	0.0348	0.0413	0.0391	0.0313	0.066
	53	--	0.1303	0.0876	0.1072	0.1079	0.0845	0.104
Egg, composite sample	Over duration of study	0.050						
Eggs from ovary/oviduct	At sacrifice	0.626	0.681	0.480	0.898	0.597	0.455	0.623
Liver	At sacrifice	3.281	4.112	4.129	3.355	3.259	2.548	3.447
Liver, composite sample	At sacrifice	3.531						
Kidney	At sacrifice	2.973	3.664	4.417	2.885	3.127	3.220	3.381
Muscle, leg	At sacrifice	0.121	0.236	0.144	0.133	0.110	0.089	0.139
Muscle, breast	At sacrifice	0.099	0.125	0.103	0.098	0.082	0.071	0.096
Muscle, composite sample	At sacrifice	0.122						
Skin, without subcutaneous fat	At sacrifice	0.263	0.373	0.315	0.342	0.307	0.249	0.308
Fat, subcutaneous	At sacrifice	0.214	0.399	0.371	0.377	0.404	0.289	0.342
Fat, composite sample	At sacrifice	0.290						
% of Administered Dose								
Excreta	24	27.40	25.28	23.51	29.47	28.74	25.37	26.63
	48	31.76	34.26	31.21	25.79	31.21	33.15	31.23
	53	3.85	2.43	7.36	15.35	11.06	6.46	7.75
	Total	63.01	61.97	62.08	70.61	71.01	64.98	65.61
Eggs	24	0.00026	0.00005	0.00003	0.00022	0.00002	0.00004	0.0001
	48	0.0102	0.0045	0.0037	0.0041	0.0038	0.0034	0.0050
	53	--	0.0111	0.0096	0.0105	0.0109	0.0089	0.0102
	Total	0.0105	0.0157	0.0133	0.0148	0.0147	0.0123	0.0136
Tissue, estimated	At sacrifice	0.64	0.93	0.85	0.77	0.66	0.63	0.75
Total % of Administered Dose	--	63.66	62.92	62.94	71.39	71.68	65.62	66.37

[1] Calculated using body weight and assuming body muscle, fat, and skin (without fat) account for 40%, 12%, and 4% of body weight, respectively.

TABLE C.2.2b. Distribution of the Parent and Metabolites in Hen Matrices Following Dosing with [Triazole-^{14}C]-*Prothioconazole at 163 ppm in the Diet.* [1]

Metabolite Fraction	Liver		Egg		Muscle		Fat	
	TRR = 3.531 ppm		TRR = 0.050 ppm		TRR = 0.122 ppm		TRR = 0.290 ppm	
	% TRR	ppm	% TRR	ppm	% TRR	ppm	%TRR	ppm
ACN/water	81.6	2.881	81.8	0.041	81.1	0.099	92.7	0.269
n-Heptane							5.3	0.015
-ACN/water							89.5	0.259
--C18 SPE effluent/rinse	79.7	2.815	81.8	0.041	78.9	0.097	88.2	0.256
Prothioconazole	29.7	1.047	3.4	0.002	2.5	0.003	15.9	0.046
1*H*-1,2,4-triazole	0.4	0.016	11.4	0.006	18.7	0.023	1.5	0.004
Thiocyanate	0.3	0.010	9.8	0.005	4.0	0.055		
JAU6476-triazolyl-ethanol	2.7	0.097	15.6	0.008	28.3	0.035	1.6	0.005
sulfate conjugate of JAU6476-hydroxy-desthio	1.6	0.055	--	--	--	--	--	--
sulfate conjugates of JAU6476-hydroxy-desthio, JAU6476-dihydroxy-desthio, JAU6476-hydroxy-methoxy-desthio	11.9	0.419	--	--	--	--	0.7	0.002
JAU6476-*S*-glucuronide	14.9 [2]	0.526	23.7 [3]	0.012	9.8	0.012	7.2	0.021
JAU6476-4-hydroxy-desthio	0.9	0.031	--	--	--	--	--	--
JAU6476-*N*-glucuronide	0.2	0.006	--	--	--	--	--	--
JAU6476-4-hydroxy	0.3	0.011	--	--	--	--	--	--
JAU6476-desthio	4.7	0.164	6.2	0.003	2.1	0.003	26.8	0.078
JAU6476-*S*-methyl	1.7	0.059	1.2	0.001	2.2	0.003	28.5	0.083
Polar metabolic group	7.8	0.275	3.0	0.001	5.9	0.007	1.2	0.004
Unknowns	2.8	0.098	7.4	0.004	5.4	0.007	4.7	0.013
--C18 SPE eluate	1.6	0.056	0.0	0.0	2.2	0.003	1.2	0.004
--C18 SPE solids	0.3	0.010	0.0	0.0	0.0	0.0	0.0	0.0
-*N*-Heptane							8.5	0.025
ACN/water			2.5	0.001	0.0	0.0		
Non-extractable	--	--	15.7	0.008	18.9	0.023	2.1	0.006
-Methanol/acetic acid	5.7	0.202						
Prothioconazole	1.1	0.038						
1*H*-1,2,4-triazole	0.6	0.021						
Thiocyanate	0.4	0.013						
JAU6476-triazolyl ethanol	0.9	0.032						
JAU6476-desthio	0.2	0.008						
Unknowns/unassigned	2.6	0.090						
-Solids	12.7	0.448						

[1] Shading indicates that the extraction step and/or characterization analysis was not conducted for the matrix in question.
[2] May contain trace amounts of JAU6476-3-hydroxy-desthio.
[3] May contain a minor amount of JAU6476-4-hydroxy-desthio and a trace amount of JAU6476-3-hydroxy-desthio.

TABLE C.2.3b. Summary of Characterization and Identification of Radioactive Residues in Hen Matrices Following Dosing with [Triazole-^{14}C]-Prothioconazole at 163 ppm in the Diet.

Compound	Liver		Egg		Muscle		Fat	
	TRR = 3.531 ppm		TRR = 0.050 ppm		TRR = 0.122 ppm		TRR = 0.290 ppm	
	% TRR	ppm	% TRR	ppm	% TRR	ppm	%TRR	ppm
Identified								
Prothioconazole	30.7	1.085	3.4	0.002	2.5	0.003	15.9	0.046
1*H*-1,2,4-triazole	1.0	0.037	11.4	0.006	18.7	0.023	1.5 [1]	0.004 [1]
Thiocyanate	0.7	0.023	9.8	0.005	4.0	0.005		
JAU6476-triazolyl-ethanol	3.6	0.129	15.6	0.008	28.3	0.035	1.6	0.005
sulfate conjugate of JAU6476-hydroxy-desthio	1.6	0.055	--	--	--	--	--	--
sulfate conjugates of JAU6476-hydroxy-desthio, JAU6476-dihydroxy-desthio, JAU6476-hydroxy-methoxy-desthio	11.9	0.419	--	--	--	--	0.7	0.002
JAU6476-*S*-glucuronide	14.9 [2]	0.526 [2]	23.7 [3]	0.012 [3]	9.8	0.012	7.2	0.021
JAU6476-4-hydroxy-desthio	0.9	0.031	--	--	--	--	--	--
JAU6476-*N*-glucuronide	0.2	0.006	--	--	--	--	--	--
JAU6476-4-hydroxy	0.3	0.011	--	--	--	--	--	--
JAU6476-desthio	4.9	0.172	6.2	0.003	2.1	0.003	26.8	0.078
JAU6476-*S*-methyl	1.7	0.059	1.2	0.001	2.2	0.003	28.5	0.083
Characterized								
Unknowns	5.3	0.188	7.4	0.004	5.4	0.007	4.7	0.013
Polar metabolic group	7.8	0.275	3.0	0.001	5.9	0.007	1.2	0.004
Fractions not analyzed	1.9	0.066	2.5	0.001	2.2	0.003	9.7	0.029
Total identified	72.4	2.553	71.3	0.037	67.6	0.083	82.3	0.239
Total Characterized	15.0	0.529	12.9	0.006	13.5	0.017	15.6	0.046
Total extractable	87.4	3.082	84.2	0.043	81.1	0.101	97.9	0.285
Unextractable (PES) [4]	12.7	0.448	15.7	0.008	18.9	0.023	2.1	0.006
Accountability [5]	100.0		102.0		101.6		100.3	

[1] For fat, the values of 1*H*-1,2,4-triazole and thiocyanate could only be determined as the sum of both metabolites.
[2] May contain a trace amount of JAU6476-3-hydroxy-desthio.
[3] May contain a minor amount of JAU6476-4-hydroxy-desthio and a trace amount of JAU6476-3-hydroxy-desthio.
[4] Residues remaining after exhaustive extractions.
[5] Accountability = (Total extractable + Total unextractable)/(absolute values of the TRRs (ppm)) * 100.

FIGURE C.2.1 Total Radioactive Residues from the Phenyl- and Triazole-Label Studies

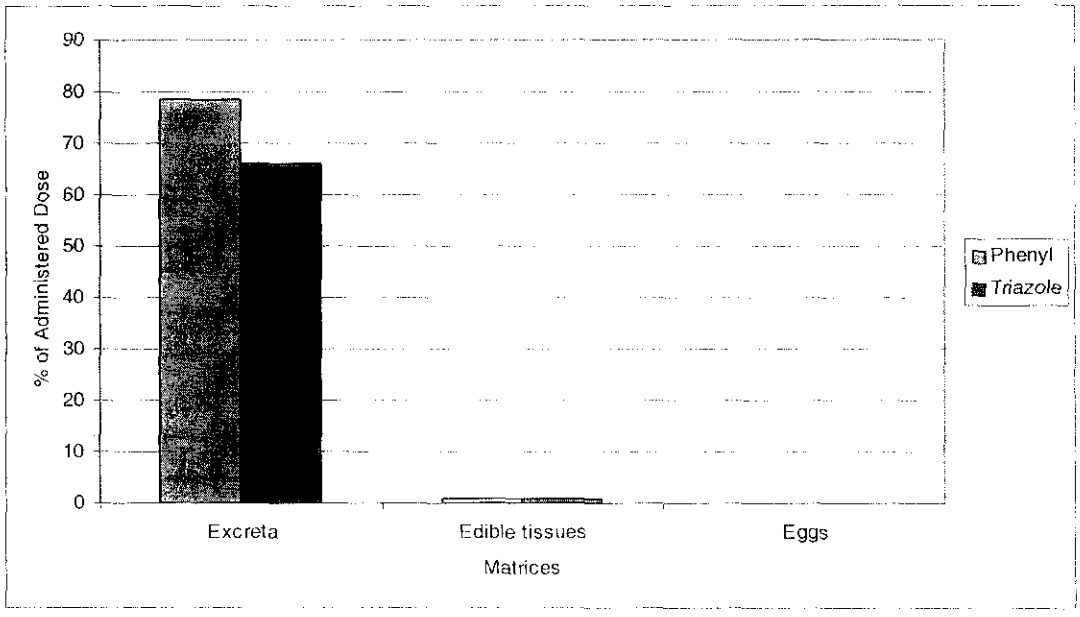
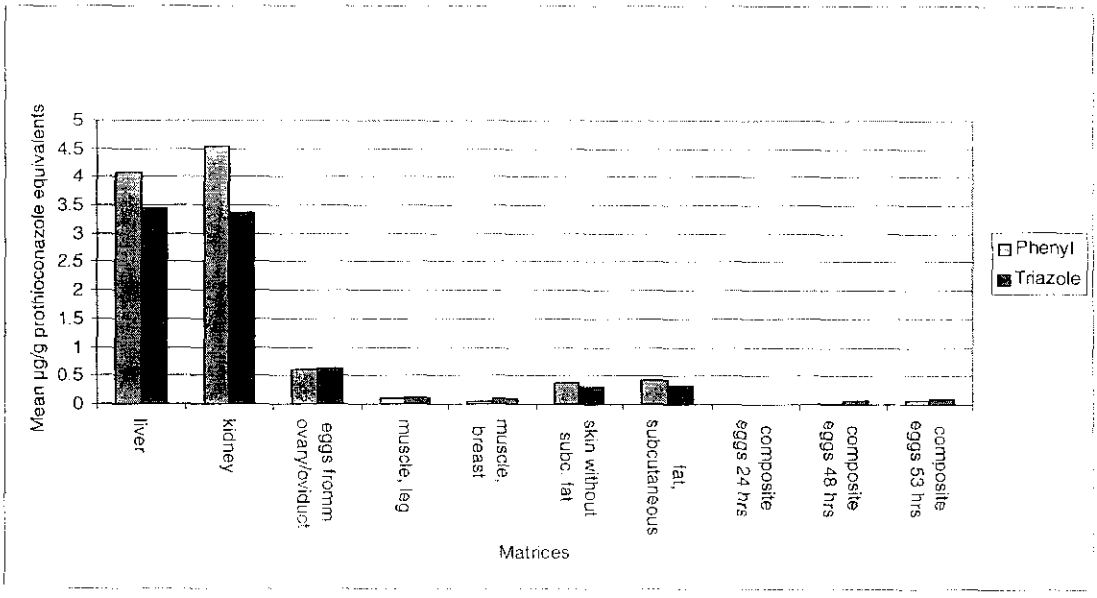


FIGURE C.2.1.1 Distribution of Total Radioactive Residues from the Phenyl- and Triazole-label Studies



C.3. Proposed Metabolic Profile

The proposed metabolic pathway for prothioconazole (JAU6476) is shown in FIGURE C.3.1. The major routes of metabolism appeared to be glucuronide conjugation of the parent JAU6476 to yield JAU6476-*S*-glucuronide (and to a lesser extent JAU6476-*N*-glucuronide and JAU6476-*O*-glucuronide); cleavage of the aliphatic carbon chain to yield 1*H*-1,2,4-triazole and JAU6476-triazolyl-ethanol; cleavage of the triazole ring to yield thiocyanate; methylation of the triazolinethione moiety to yield JAU6476-*S*-methyl; desulfuration of the parent compound to yield JAU6476-desthio and the subsequent hydroxylation or oxidation of the chlorophenyl moiety on JAU6476-desthio and conjugation with sulfate to yield a variety of sulfate conjugates of hydroxylated JAU6476-desthio. Minor metabolic routes include the hydroxylation of the parent JAU6476 to yield JAU6476-4-hydroxy (and possibly JAU6476-3-hydroxy) and its subsequent conjugation with glucuronide and oxidation of the phenyl ring of the parent JAU6476 to yield JAU6476-dihydroxy-diene.

FIGURE C.3.1. Proposed Metabolic Profile of [phenyl-UL-^{14}C] and [triazole-UL-^{14}C]-Prothioconazole in Laying Hen.

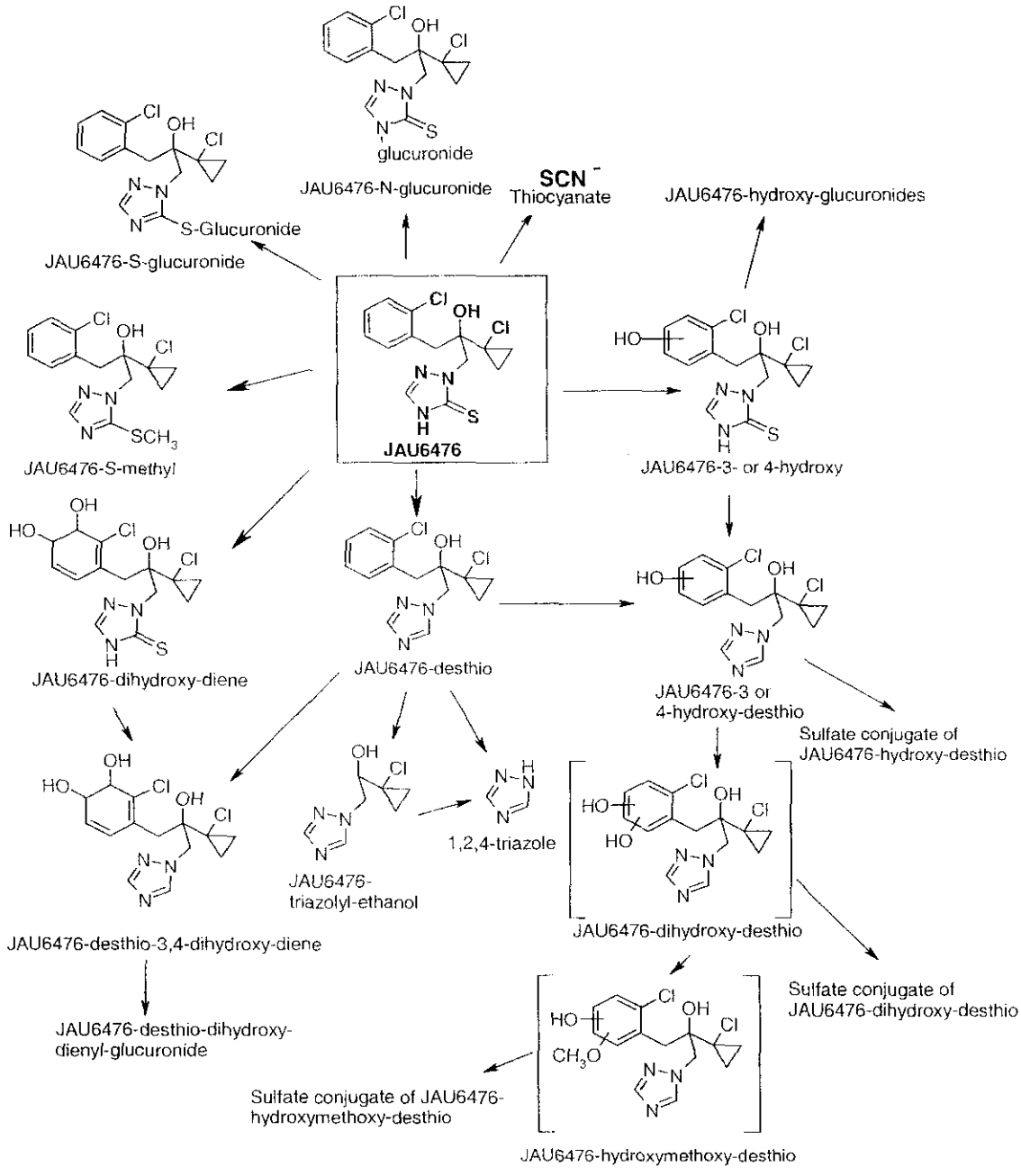


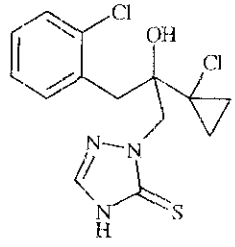
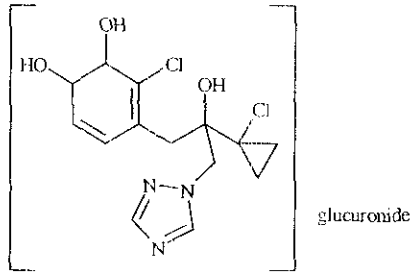
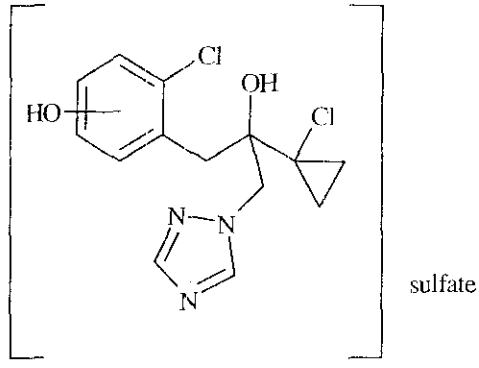
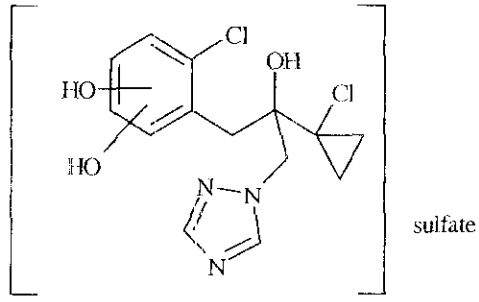
TABLE C.3.1. Identification of Compounds from Metabolism Studies.		
Common name/code FIGURE C.3. ID No.	Chemical name	Chemical structure
Prothioconazole: JAU6476	2-[2-(1-chlorocyclopropyl)-3-(2-chlorophenyl)-2-hydroxypropyl]-1,2-dihydro-3*H*-1,2,4-triazole-3-thione	
JAU6476-desthio-3,4-dihydroxy-diene-glucuronide		 glucuronide
Sulfate conjugate of JAU6476-hydroxy-desthio		 sulfate
Sulfate conjugate of JAU6476-dihydroxy-desthio		 sulfate

TABLE C.3.1. Identification of Compounds from Metabolism Studies.

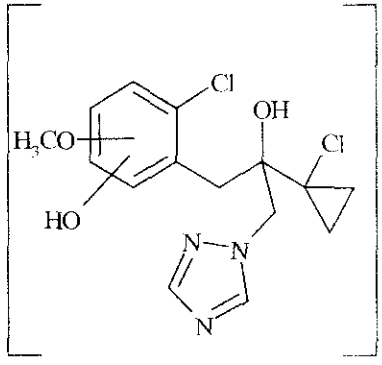
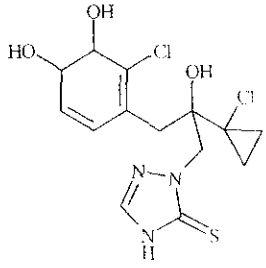
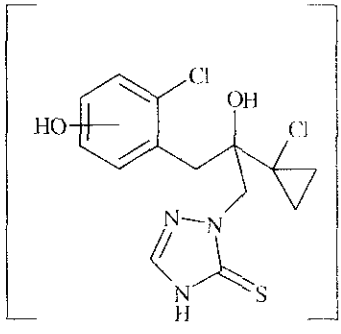
Common name/code FIGURE C.3.1 ID No.	Chemical name	Chemical structure
Sulfate conjugate of JAU6476-hydroxy-methoxy-desthio		 sulfate
JAU6476-dihydroxy-diene	2-[2-(1-chlorocyclopropyl)-3-(2-chloro-3,4-dihydroxycyclohexa-1,5-dien-1-yl)-2-hydroxypropyl]-2,4-dihydro-3*H*-1,2,4-triazole-3-thione [1]	
JAU6476-hydroxy-glucuronide		 glucuronide

TABLE C.3.1. Identification of Compounds from Metabolism Studies.

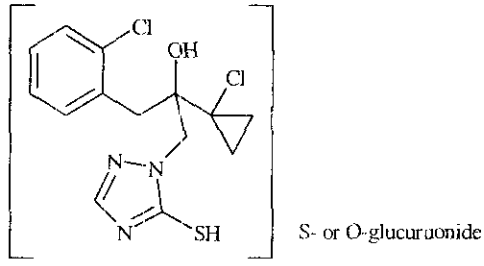
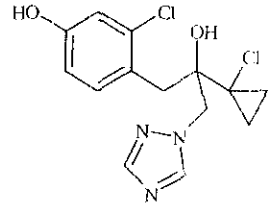
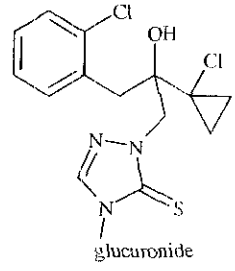
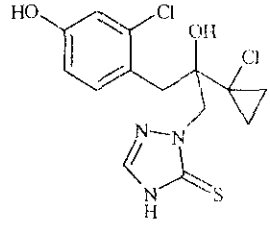
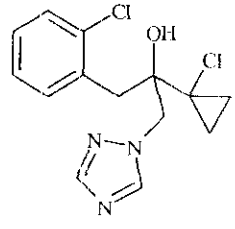
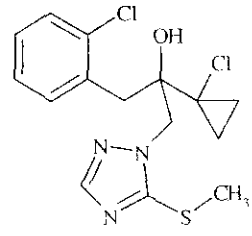
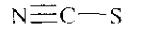
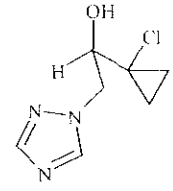
Common name/code FIGURE C.3.1 ID No.	Chemical name	Chemical structure
JAU6476-*S* or *O*-glucuronide		 S- or O-glucuronide
JAU6476-4-hydroxy-desthio	α-(1-chlorocyclopropyl)-α-[(2-chloro-4-hydroxyphenyl)methyl]-1*H*-1,2,4-triazole-1-ethanol	
JAU6476-*N*-glucuronide		
JAU6476-4-hydroxy	2-[2-(1-chlorocyclopropyl)-3-(2-chloro-4-hydroxyphenyl)-2-hydroxypropyl]-1,2-dihydro-3*H*-1,2,4-triazole-3-thione	
JAU6476-desthio	α-(1-chlorocyclopropyl)-α-[(2-chlorophenyl)methyl]-1*H*-1,2,4-triazole-1-ethanol	

TABLE C.3.1. Identification of Compounds from Metabolism Studies.		
Common name/code FIGURE C.3.1 ID No.	Chemical name	Chemical structure
JAU6476-*S*-methyl	α-(1-chlorocyclopropyl)-α-[(2-chlorophenyl)methyl]-3-(methylthio)-1*H*-1,2,4-triazole-1-ethanol	
Free triazole or 1*H*-1,2,4-triazole	1*H*-1,2,4-triazole	
Thiocyanate	thiocyanate ion	
JAU6476-triazolyl ethanol	1-(1-chlorocyclopropyl)-2-(1*H*-1,2,4-triazol-1-yl)ethanol	

[1] Chemical name generated using ACD chemical naming software.

D. CONCLUSION

Two groups of six laying hens received three consecutive daily administrations of either [phenyl -UL-^{14}C]-JAU6476 or [triazole-UL-^{14}C]-JAU6476. The majority of the administered dose (AD) was eliminated in the excreta within five hours of the final administration (78.42% and 65.61% for the phenyl-label and triazole-label treated groups, respectively). Only minor fractions of the total administered dose were detected in eggs (0.011% and 0.014% for the phenyl-label and triazole-label treated groups, respectively) and edible body tissues (0.85% and 0.75% in the phenyl-label and triazole-label treated groups, respectively). The largest concentrations of ^{14}C residues were detected in the liver and kidney, which is not unexpected due to the extensive metabolism and apparent rapid excretion observed with JAU6476. A major metabolite identified in muscle, liver and fat was the unchanged parent, JAU6476. Other major metabolites detected were JAU6476-glucuronide in muscle, liver and eggs; JAU6476-desthio in fat and eggs and JAU6476-*S*-methyl in fat. The polar metabolites 1*H*-1,2,4-triazole, thiocyanate and JAU6476-triazolyl-ethanol were only detected in the [triazole- UL-^{14}C]-JAU6476 study and represented a major component of the TRRs in muscle and eggs.

E. REFERENCES

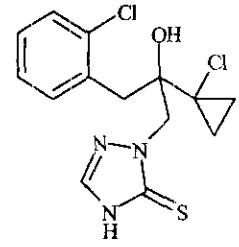
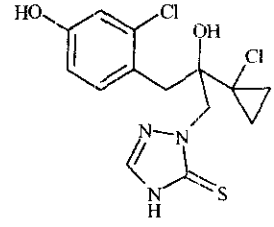
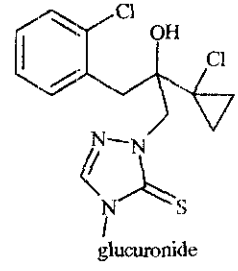
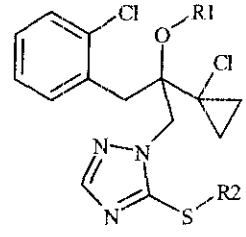
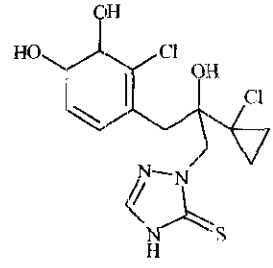
46246150 Weber, H.; Spiegel, K. (2001) [Phenyl-UL-(Carbon 14)]JAU6476: Absorption, Distribution, Excretion, and Metabolism in the Lactating Goat. Project Number: M/91819082.

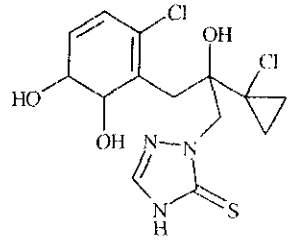
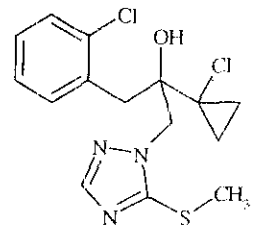
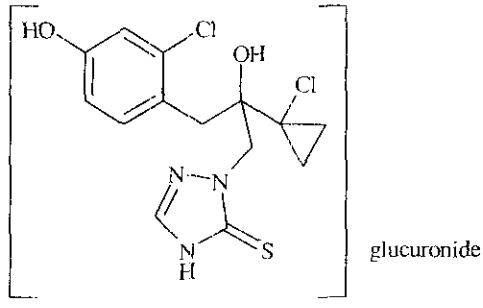
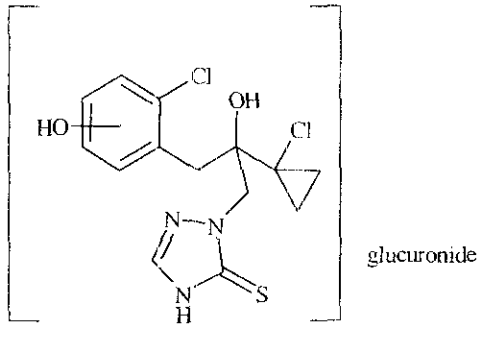
MR/092/01. Unpublished study prepared by Bayer Ag Institut fuer Ruckstands-Analytik. 205 p.

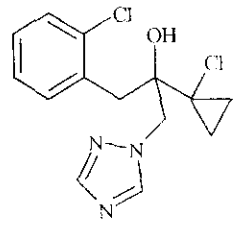
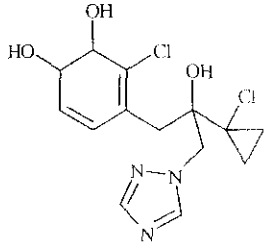
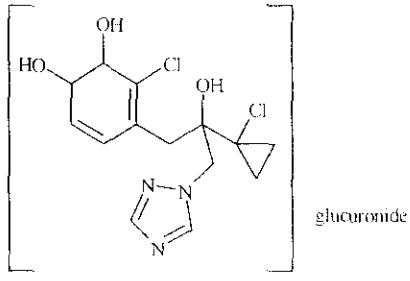
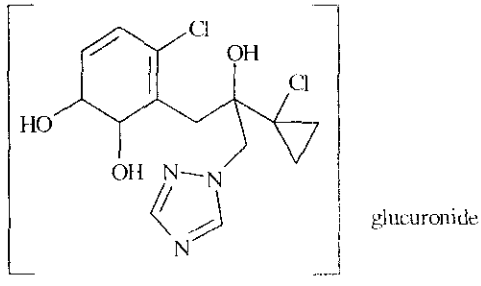
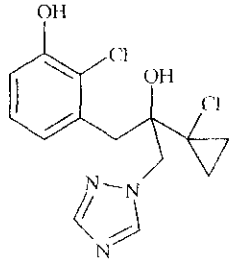
F. DOCUMENT TRACKING

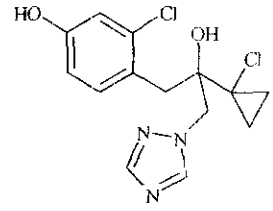
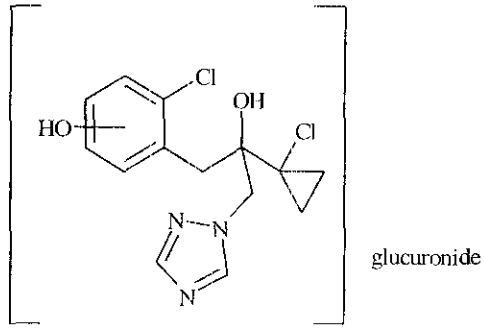
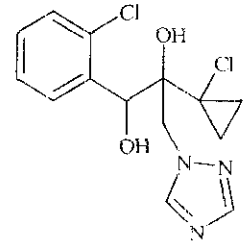
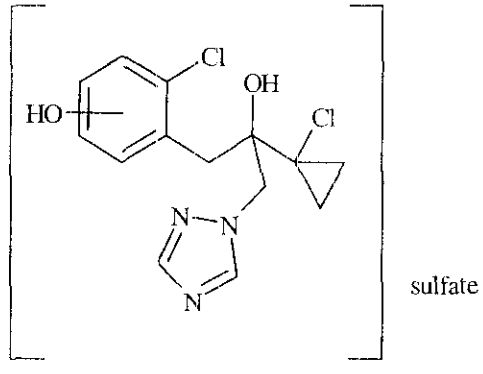
RDI: Louise Croteau, Suzan Mathew (PMRA) 23/01/2006; Henri Bietlot (PMRA) 24/01/2006; Stephen Funk (IO)13/03/2006; Leung Cheng (RAB3).
Petition Number: PP#4F6830
DP Barcode: D303508
PC Code: 113961

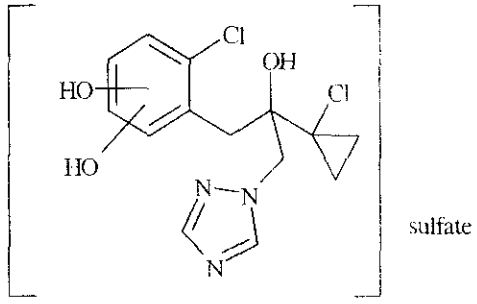
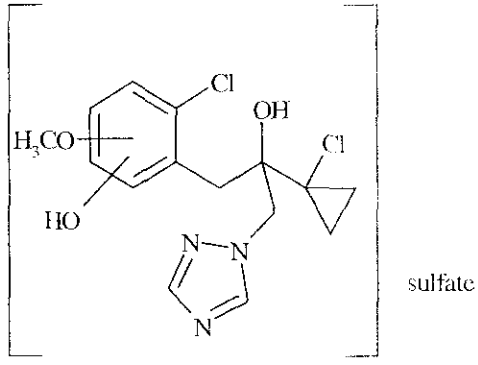
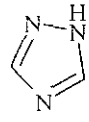
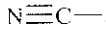
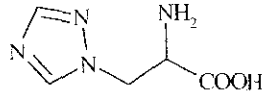
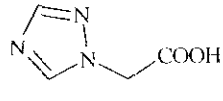
Template Version September 2003

APPENDIX I. Chemical Names and Structures of Reference Standards Used in Hen Metabolism Study.		
Common name; Company code	Chemical name	Chemical structure
Prothioconazole; JAU6476 [1]	2-[2-(1-chlorocyclopropyl)-3-(2-chlorophenyl)-2-hydroxypropyl]-1,2-dihydro-3*H*-1,2,4-triazole-3-thione	
JAU6476-4-hydroxy [2]	2-[2-(1-chlorocyclopropyl)-3-(2-chloro-4-hydroxyphenyl)-2-hydroxypropyl]-1,2-dihydro-3*H*-1,2,4-triazole-3-thione	
JAU6476-*N*-glucuronide [3]		
JAU6476-glucuronide [3] (position of conjugation not determined definitely)	[JAU6476-*S*-glucuronide: R1 = H, R2 = glucuronic acid JAU6476-*O*-glucuronide: R1 = glucuronic acid, R2 = H]	
JAU6476-3,4-dihydroxy-diene [3]	2-[2-(1-chlorocyclopropyl)-3-(2-chloro-3,4-dihydroxycyclohexa-1,5-dien-1-yl)-2-hydroxypropyl]-2,4-dihydro-3*H*-1,2,4-triazole-3-thione [4]	

APPENDIX 1. Chemical Names and Structures of Reference Standards Used in Hen Metabolism Study.		
Common name; Company code	Chemical name	Chemical structure
JAU6476-5,6-dihydroxy-diene [3]	2-[2-(1-chlorocyclopropyl)-3-(2-chloro-5,6-dihydroxycyclohexa-1,3-dien-1-yl)-2-hydroxypropyl]-2,4-dihydro-3*H*-1,2,4-triazole-3-thione [4]	
JAU6476-*S*-methyl [3]	α-(1-chlorocyclopropyl)-α-[(2-chlorophenyl)methyl]-3-(methylthio)-1*H*-1,2,4-triazole-1-ethanol	
JAU6476-4-hydroxy-glucuronide [3]		
JAU6476-hydroxy-glucuronide [3]		

APPENDIX I. Chemical Names and Structures of Reference Standards Used in Hen Metabolism Study.		
Common name; Company code	Chemical name	Chemical structure
JAU6476-desthio [3] (unlabeled standard also used)	α-(1-chlorocyclopropyl)-α-[(2-chlorophenyl)methyl]-1*H*-1,2,4-triazole-1-ethanol	
JAU6476-desthio-3,4-dihydroxy-diene [2,5]	3-chloro-4-[2-(1-chlorocyclopropyl)-2-hydroxy-3-(1*H*-1,2,4-triazol-1-yl)propyl]cyclohexa-3,5-diene-1,2-diol [4]	
JAU6476-desthio-3,4-dihydroxy-dienyl-glucuronide [5]		
JAU6476-desthio-5,6-dihydroxy-dienyl-glucuronide [2]		
JAU6476-3-hydroxy-desthio [3] (unlabeled standard also used)	α-(1-chlorocyclopropyl)-α-[(2-chloro-3-hydroxyphenyl)methyl]-1*H*-1,2,4-triazole-1-ethanol	

APPENDIX I. Chemical Names and Structures of Reference Standards Used in Hen Metabolism Study.		
Common name: Company code	Chemical name	Chemical structure
JAU6476-4-hydroxy-desthio [3] (unlabeled standard also used)	α-(1-chlorocyclopropyl)-α-[(2-chloro-4-hydroxyphenyl)methyl]-1*H*-1,2,4-triazole-1-ethanol	
JAU6476-hydroxy-desthio-glucuronide [5]		
JAU6476-α-hydroxy-desthio[6] (unlabeled standard also used)	2-(1-chlorocyclopropyl)-1-(2-chlorophenyl)-3-(1*H*-1,2,4-triazol-1-yl)-1,2-propanediol	
Sulfate conjugate of JAU6476-hydroxy-desthio [5]		

APPENDIX I. Chemical Names and Structures of Reference Standards Used in Hen Metabolism Study.		
Common name; Company code	Chemical name	Chemical structure
Sulfate conjugate of JAU6476-dihydroxy-desthio [5]		 sulfate
Sulfate conjugate of JAU6476-hydroxy-methoxy-desthio [5]		 sulfate
Free triazole or *1H*-1,2,4-triazole [1]	1*H*-1,2,4-triazole	
Thiocyanate (potassium salt) [1]	thiocyanate ion	
Triazolyl alanine [5]	3-(1*H*-1,2,4-triazol-1-yl)-alanine	
Triazolyl acetic acid [1]	1*H*-1,2,4-triazol-1-yl-acetic acid [6]	

[1] *Standard was radiolabeled.*
[2] Isolated and identified in goat metabolism study (refer to the DER for MRID 46246150).
[3] Isolated and identified in rat metabolism study (MRID 46246421).
[4] Chemical name generated using ACD chemical naming software.
[5] Isolated and identified in goat metabolism study with desthio metabolite.
[6] Isolated and identified in wheat metabolism study (refer to the DER for MRID 46246141).

Primary Evaluators

Stephen Funk, Ph.D., Senior Chemist
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Date:

Henri P Bietlot, Acting Section Head
FREAS, HED
Date: Jan 27/06

In the absence of signatures, this document is considered to be a draft with deliberative material for internal use only.

This DER was originally prepared under contract by Dynamac Corporation (20440 Century Boulevard, Suite 100; Germantown, MD 20874; submitted 05/05/2005). The DER has been reviewed by the Health Effects Division (HED) and the Pest Management Regulatory Agency (PMRA) of Health Canada. The DER has been revised to reflect current Office of Pesticide Programs (OPP) policies, and PMRA Directive 98-02.

STUDY REPORTS:

46246141 Haas, M.; Bornatsch, W. (2000) Metabolism of JAU6476 in Spring Wheat (after foliar application). Project Number: M/1730851/5, 110880, MR/198/99. Unpublished study prepared by Bayer Ag Institut fuer Ruckstands-Analytik. 149 p.

46246143 Duah, F.; Lopez, R. (2004) The Metabolism of [Triazole-3, 5-(Carbon 14)] JAU6476 in Wheat. Project Number: J6041601, 200733. Unpublished study prepared by Bayer Corp. 197 p

EXECUTIVE SUMMARY:

Phenyl-label Study

Bayer CropScience has submitted studies investigating the metabolism of [phenyl-UL-^{14}C]-prothioconazole (specific activity 27.6 mCi/mmol) in wheat as a foliar treatment. The radiolabeled test substances were formulated as emulsifiable concentrate (EC) formulations and applied as two foliar broadcast sprays to wheat plants grown outdoors in planting containers at the beginning of tillering (BBCH 32) and at full flowering (BBCH 65). Applications were made at 0.193 lb a.i./A (216 g a.i./ha) and 0.178 lb a.i./A (199 g a.i./ha), with a 17-day re-treatment interval, for a total seasonal application of 0.371 lb a.i./A (415 g a.i./ha). Forage and hay were harvested 6 and 26 days, respectively, and grain and straw were harvested 48 days following the second application.

Total radioactive residues (TRRs) were 10.45 ppm (forage), 8.90 ppm (hay), 26.74 ppm (straw), and 0.08 ppm (grain) following foliar application of [phenyl-UL-^{14}C]-prothioconazole.

Extraction with organic solvents released the majority of the radioactivity (~61-85% of the TRRs) in all wheat matrices. Cysteine HCl was added to all extracting solvents and to extracts during concentration procedures to prevent oxidative decomposition of prothioconazole. Accelerated solvent extraction (ASE) released an additional ~5-13% of the TRRs (0.02-2.57 ppm) from all matrices. Acid hydrolysis with HCl:dioxane released ~7-8% of the TRRs (0.64-2.18 ppm) in hay and straw. Enzyme hydrolysis of the grain with diastase released an additional ~15% of the TRRs (0.01 ppm). Non-extractable residues remaining following extraction/hydrolysis accounted for <4% of the TRRs (≤0.83 ppm) in forage, hay, and straw; and accounted for 18% of the TRRs (0.013 ppm) in grain. Accountabilities were approximately 99-100% for all matrices. Residues were identified primarily by LC-MS, LC-MS/MS, and/or NMR spectroscopy with confirmatory analysis by HPLC and/or TLC co-chromatography. These methods successfully identified the predominant residues in wheat forage, hay, straw, and grain. Adequate storage stability data were submitted demonstrating the stability of prothioconazole and the major metabolite JAU6476-desthio in wheat matrices.

Approximately 67-73% of the TRRs were identified in all wheat matrices except in grain where only 34% of the TRRs were identified. Prothioconazole was identified at low levels (1-4% of the TRRs, <0.01-0.98 ppm) in all wheat matrices. Metabolite JAU6476-desthio was the major identified residue, accounting for 35% of the TRRs (3.70 ppm) in forage, 19% of the TRRs (1.64 ppm) in hay, 22% of the TRRs (5.95 ppm) in straw, and 16% of the TRRs (0.014 ppm) in grain.

All remaining metabolites were identified at <10% of the TRRs. In the phenyl-label study, metabolites JAU6476-α-OH-desthio and JAU6476-triazolinone were identified in all wheat matrices at ~1-9% of the TRRs (<0.01-1.64 ppm). The metabolites JAU6476-3-OH-desthio, JAU6476-4-OH-desthio, JAU6476-6-OH-desthio and JAU6476 sulfonic acid were identified as 1-9% of the TRRs (0.11-2.24 ppm) in forage, hay and straw. In hay, straw and grain, the metabolites JAU6476-α-acetoxy-desthio (along with benzylpropyldiol) and benzylpropyldiol glucoside were identified at <5% of the TRRs (≤0.55 ppm). JAU6476 disulfide, JAU6476-OH-

desthio (comprised of the 3-OH, 4-OH and/or 6-OH-desthio isomers) and two JAU6476-OH-desthio glucoside isomers were tentatively identified at <6% of the TRRs (≤1.08 ppm) each. The third JAU6476-OH-desthio glucoside isomer was tentatively identified in all matrices except grain at <3% of the TRRs (≤0.35 ppm). JAU6476-desthio glucoside was only tentatively identified in hay and straw at <7% of the TRRs (≤1.79 ppm). Characterized radioactivity remaining at the TLC origins accounted for ~12-20% of the TRRs (0.01-3.28 ppm). Unassigned radioactivity was reported as characterized at ~3-10% of the TRRs (<0.01-2.29 ppm). Unknowns accounted for 2-6% of the TRRs (<0.01-0.83 ppm) in each matrix. In grain, ~15% of the TRRs (0.01 ppm) were characterized based on diastase hydrolysis. Another 8% of the TRRs (<0.01 ppm) were extracted by ASE but not analyzed, and 6% of the TRRs (<0.01 ppm) were characterized as polar and aqueous soluble.

Triazole-labeled Study

Bayer CropScience has submitted studies investigating the metabolism of [triazole-3,5-^{14}C]-prothioconazole (specific activity 18.3 mCi/mmol) in wheat as a foliar treatment. The radiolabeled test substances were formulated as emulsifiable concentrate (EC) formulations and applied as two foliar broadcast sprays to wheat plants grown outdoors in planting containers at the beginning of tillering (BBCH 32) and at full flowering (BBCH 65). Applications were made at 0.159 lb a.i./A (178 g a.i./ha) and 0.260 lb a.i./A (292 g a.i./ha), with a 23-day re-treatment interval, for a total seasonal application of 0.420 lb a.i./A (470 g a.i./ha). Wheat forage was harvested 6 days after the second application, 29 days for hay and 64 days for grain and straw.

Total radioactive residues (TRRs) were 7.96 ppm (forage), 11.18 ppm (hay), 7.94 ppm (straw) and 4.97 ppm (grain) following foliar application of [triazole-3,5-^{14}C]-prothioconazole.

Extraction with organic solvents released the majority of the radioactivity (~65-81% of the TRRs) in all wheat matrices. Accelerated solvent extraction (ASE) released an additional ~8-24% of the TRRs (0.61-1.58 ppm) from all matrices. Acid hydrolysis with HCl:methanol and or HCl:dioxane released ~6-11% of the TRRs (0.48-1.05 ppm) in forage, hay and straw. Acid hydrolysis with HCl:dioxane released 1% of the TRRs (0.041 ppm) for grain. Non-extractable residues remaining following extraction/hydrolysis accounted for <6% of the TRRs (<0.45 ppm) in forage, hay, and straw, and 0.1% of the TRRs (<0.01 ppm) in grain. Extraction results were normalized; therefore, accountabilities were 100%. Residues were identified primarily by LC-MS, LC-MS/MS, and/or NMR spectroscopy with confirmatory analysis by HPLC and/or TLC co-chromatography. These methods successfully identified the predominant residues in wheat forage, hay, straw, and grain. Adequate storage stability data were submitted demonstrating the stability of prothioconazole and the major metabolite JAU6476-desthio in wheat matrices.

Approximately 57-91% of the TRRs were identified in all wheat matrices. Prothioconazole was identified at low levels (3-7% of the TRRs, 0.38-0.53 ppm) in forage, hay and straw. However, in grain, neither prothioconazole nor any metabolites unique to prothioconazole were identified. Metabolite JAU6476-desthio was identified as 9-19% of the TRRs (0.74-1.50 ppm) in forage, hay and straw. In grain, the predominant metabolites were triazolylalanine (TA) at 71% of the TRRs (3.54 ppm) followed by triazolylacetic acid (TAA) accounting for 19% of the TRRs (0.95

ppm) with triazolylhydroxypropionic acid (THPA) constituting the remaining identified residue at 0.4% of the TRRs (0.02 ppm). The triazole-specific metabolite, TA, was also a major residue in hay (25% of the TRRs; 2.77 ppm), and accounted for 12% of the TRRs (0.95 ppm) in forage and 4% of the TRRs (0.32 ppm) in straw. TAA was identified at <5% of the TRRs (<0.5 ppm) in forage, hay, and straw, and THPA was identified at <8% of the TRRs (<0.85 ppm) in these same matrices. Free triazole or *1H*-1,2,4-triazole was not identified in any wheat matrix.

All remaining metabolites were identified at <10% of the TRRs. In the triazole-label study, JAU6476-α-OH-desthio and JAU6476-triazolinone were identified at 1-9% of the TRRs (0.08-0.78 ppm) in forage, hay and straw. JAU6476-OH-desthio and JAU6476-α-acetoxy-desthio were identified in forage and straw at 2-6% of the TRRs (0.16-0.49 ppm). Triazolyl-ethanol-glucoside, JAU6476-OH-desthio-glucoside isomers, unresolved metabolites (TA, TAA, and/or THPA), and JAU6476-OH-desthio-malonyl-glucoside isomers were identified in forage, hay and straw each at <4% of the TRRs (<0.30 ppm). Unresolved glucoside isomers were found in forage and hay at <5% of the TRRs (<0.52 ppm). Triazolyl-ethanol (found in straw), JAU6476-desthio-malonyl-glucoside (found in forage), JAU6476-desthio-phenyl-cysteine isomers and JAU6476-diOH-desthio-malonyl-glucoside (both found in forage) were identified each at <3% of the TRRs (<0.18 ppm). Unknowns accounted for 6-22% of the TRRs (0.3-1.9 ppm) in each matrix. However, these consisted of multiple components, each generally <3% of the TRRs (≤0.36 ppm). The remaining radioactivity was characterized as HCl hydrolysates at ~6% of the TRRs (<0.49 ppm).

Based on the results of the wheat metabolism study, the applicant concluded that prothioconazole is initially metabolized in wheat by oxidation and loss of sulfur to form JAU6476-desthio, after which two major metabolic processes occur: (1) hydroxylation of the phenyl ring and or benzylic carbon to form isomers of JAU6476-OH-desthio, JAU6476-diOH desthio, and JAU6476-α-OH-desthio, followed by conjugation to form the corresponding glucosides, malonyl-glucosides and acetate; and (2) release of the triazole moiety to form TA and THPA and further metabolism of the triazole conjugates to form TAA. The applicant noted that the absence of 1,2,4-triazole in any wheat matrix suggested that immediate or very rapid conjugation of released triazole occurred. The following minor metabolic pathways were reported: formation of JAU6476-triazolinone and JAU6476-desthio-phenyl-cysteine; conjugation of JAU6476-desthio with glucose and malonic acid; oxidation of the sulfur atom of prothioconazole to form JAU6476 sulfonic acid; cleavage of the benzylic group to form triazolyl ethanol and its glucoside; and conjugation of the benzylpropyldiol portion of the remaining molecule.

STUDY/WAIVER ACCEPTABILITY/DEFICIENCIES/CLARIFICATIONS:

The wheat metabolism data are classified as scientifically acceptable.

The acceptability of these studies for regulatory purposes is addressed in the forthcoming U.S. EPA Residue Chemistry Summary Document, DP Barcode D303508, PP#4F6830, and in Canada's Regulatory Decision Document.

COMPLIANCE:

Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided. No deviations from regulatory requirements were reported.

A. BACKGROUND INFORMATION

Prothioconazole is a broad-spectrum systemic fungicide intended for the control of many diseases in different crops such as cereals, oilseed rape or peanuts. The biochemical mode of action is the inhibition of the demethylation of lanosterol or 24-methylene-dihydrolanosterol, which are precursors of sterols in fungi. The chemical has been submitted for joint EPA and PMRA review by Bayer CropScience. The crops proposed for joint review include barley, the dried shell and bean subgroup, the oilseed crop group, and wheat. Uses on peanuts and rice are being proposed for the U.S. only.

TABLE A.1. Test Compound Nomenclature.

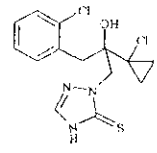
Chemical structure	
Common name	Prothioconazole (ISO accepted)
Company experimental name	JAU6476
IUPAC name	2-[(2*RS*)-2-(1-chlorocyclopropyl)-3-(2-chlorophenyl)-2-hydroxypropyl]-2*H*-1,2,4-triazole-3(4*H*)-thione
CAS name	2-[2-(1-chlorocyclopropyl)-3-(2-chlorophenyl)-2-hydroxypropyl]-1,2-dihydro-3*H*-1,2,4-triazole-3-thione
CAS registry number	178928-70-6
PC Code	113961
End-use product (EP)	Proline® 480 SC (4 lb/gal suspension concentrate), EPA File Symbol 264-IEL

TABLE A.2. Physicochemical Properties of Prothioconazole

Parameter	Value	Reference
Melting range	139.1 to 144.5°C	MRID 46246003 / CES[1]
pH	5.8 (1% solution)	MRID 46246003 / CES
Density at 20°C	1.36 g/mL	MRID 46246003 / CES
Water solubility at 20°C	pH: mg/L 4: 5 8: 300 9: 2000	MRID 46246003 / CES
Solvent solubility at 20°C	Solvent: g/L Acetone: >250 Acetonitrile: 69 Dichloromethane: 88 Dimethysulfoxide: 126 Ethyl acetate: >250 *n*-Heptane: <0.1 1-Octanol: 58 Polyethylene glycol: >250 2-Propanol: 87 Xylene: 8	MRID 46246003 / CES
Vapor pressure at 20 or 25°C	$<4 \times 10^{-7}$ Pa (calculated from determinations at 70 °C)	MRID 46246003 / CES
Dissociation constant, pK_a	6.9 (calculated from K_{OW})	MRID 46246003 / CES
Octanol/water partition coefficient, Log(K_{OW}), at 20°C	pH: Log Kow unbuffered water: 4.05 pH 4: 4.16 pH 7: 3.82 pH 9: 2.00	MRID 46246003 / CES
UV/visible absorption spectrum	Peak maxima at 257 nm	MRID 46246003 / CES

1 CES: Chemistry Evaluation Section of PMRA

B. EXPERIMENTAL DESIGN

B.1. Test Site and Crop Information

TABLE B.1.1. Test Site Information.

Testing Environment	Soil characteristics			
	Type	%OM	pH	CEC (meq/100g)
[Phenyl-UL-^{14}C]-Prothioconazole				
Plants were grown in 1-m^2 planting containers at outdoor test plots (Leverkusen, Germany).	*Sandy loam*	1.98	6.3	10.0
Temperature/rainfall: Average monthly temperatures and sunshine hours were reported for the study period. Plants were watered as needed, and maintenance pesticides and fertilizers were applied. No unusual weather conditions were reported.				

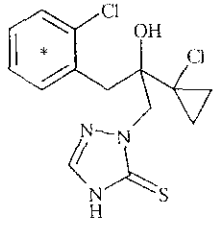
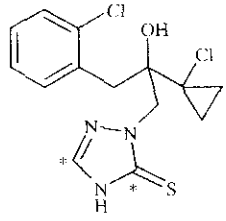
TABLE B.1.1. Test Site Information.

Testing Environment	Soil characteristics			
	Type	%OM	pH	CEC (meq/100g)
[Triazole-3,5-^{14}C]-Prothioconazole				
Plants were grown outdoors in a 21.77 ft^2 metal stock tub (Stilwell, KS); the tub was moved into a greenhouse one day prior to application of test substance and was moved outdoors one day after application.	Sandy loam	5.9	7.4	14.9
Temperature/rainfall: No temperature or rainfall data were provided; however, the applicant stated that plants were watered, fertilized, hand-weeded, and sprayed with maintenance chemicals as needed to maintain healthy plant growth. Rain water was drained from a valve in the bottom of the tub when necessary. No unusual weather conditions were reported.				

TABLE B.1.2. Crop Information.

Crop; crop group	Variety	Growth stage at application	Growth stage at harvest	Harvested RAC	Harvesting procedure
[Phenyl-UL-^{14}C]-Prothioconazole					
Wheat; Grain, cereal (Crop group 15) and Grain cereal, forage, fodder and straw, (Crop Group 16)	Spring wheat, var. Kadett	1: BBCH 32 (beginning of tillering) 2: BBCH 65 (full flowering)	BBCH 69 (early hay)	Forage	Plants were removed by cutting at the soil surface.
			BBCH 83 (early dough)	Hay	
			BBCH 89 (Mature)	Grain and straw	Wheat ears were removed by cutting from the stalks; straw was then cut at the soil surface.
[Triazole-3,5-^{14}C]-Prothioconazole					
Wheat; Grain, cereal (Crop group 15) and Grain, cereal, forage, fodder and straw, (Crop Group 16)	Spring wheat, var. Butte	1: BBCH 32 (beginning of tillering; node 2 ≥ 1) 2: BBCH 65 (full flowering)	BBCH 65 (full flowering)	Forage	Plants were harvested using "good agricultural practices."
			BBCH 83 to BBCh 85	Hay	
			BBCH 89 (Mature)	Grain and straw	

B.2. Test Materials

TABLE B.2.1.	Test Material Characteristics	
Chemical structure		
Radiolabel position	[phenyl-UL-^{14}C]-prothioconazole	[triazole-3,5-^{14}C]-prothioconazole
Lot No.	11403/1	C-885 and C-885A
Purity	>99% radiochemical purity; >99% chemical purity	94.56% (C-885) and 100% (C-885A) radiochemical purity
Specific activity	27.6 mCi/mmol	18.3 mCi/mmol

B.3. Study Use Pattern

TABLE B.3.1.	Use Pattern Information	
Chemical name	[phenyl-UL-^{14}C]-prothioconazole	[triazole-3,5-^{14}C]-prothioconazole
Application method	The radiolabeled test substance was formulated as an EC with formulation blank, then diluted with water and applied using a computer-controlled track sprayer.	The radiolabeled test substance was combined with nonradiolabeled prothioconazole, evaporated under a stream of nitrogen, and formulated as a suspension concentrate with formulation blank. The formulated test substance was diluted with water and added to a spray bottle containing additional formulation blank prior to application.
Application rate	Two applications at 0.193 lb a.i./A (216 g a.i./ha) and 0.178 lb a.i./A (199 g a.i./ha) for a total application rate of 0.371 lb a.i./A (415 g a.i./ha)	Two applications at 0.159 lb a.i./A (178 g a.i./ha) and 0.260 lb a.i./A (292 g a.i./ha) for a total application rate of 0.420 lb a.i./A (470 g a.i./ha)
Number of applications	2	2
Timing of applications	Applications were made at BBCH 32 and BBCH 65; 75 and 92 days after planting, 17-day re-treatment interval.	Applications were made at BBCH 32 and BBCH 65 with a 23-day re-treatment interval.
PHI[1] (Days)	Forage: 6 Hay: 26 Grain and straw: 48	6 29 64

[1] PHI = pre-harvest interval.

B.4. Identification/Characterization of Residues

B.4.1. Sample Handling and Preparation

Phenyl-label study
Wheat grain was separated from the chaff by hand, then frozen in liquid nitrogen and homogenized. Samples of forage, hay, and straw (including chaff) were chopped into 1-cm pieces, then frozen in liquid nitrogen and homogenized. Following processing, wheat samples were stored frozen -20°C. Extracts were stored either refrigerated (4°C) or frozen (-20°C) for longer periods.

General extraction procedures: Cysteine HCl was added to all extracting solvents and to extracts during concentration procedures to prevent oxidative decomposition of prothioconazole. Extracts were centrifuged to remove precipitated material including cysteine HCl. Following partitioning with dichloromethane (DCM), a small volume of acetonitrile (ACN) was added to each DCM phase prior to concentration.

Forage: Subsamples were extracted three tiems with ACN:water (80:20, v:v), then vacuum filtered through Celite. The filtrates were combined, concentrated to aqueous, and partitioned with DCM (three times); the resulting DCM phases were combined and concentrated. Non-extractable residues following ACN:water extraction (from vacuum filtration, including Celite) were subjected to accelerated solvent extraction (ASE) using ACN:water (65:35, v:v for forage and 80:20, v:v for all other matrices) at 50 and 100°C (two extractions each temperature).

Hay and straw: Subsamples were extracted three times with ACN:water (80:20, v:v), then vacuum filtered through Celite. The remaining non-extractable residues (including Celite) were subjected to ASE as described above for forage, except that ACN:water (80:20, v:v) was used as the extraction solvent. The initial extracts and the ASE extracts were combined, concentrated to aqueous, and partitioned with DCM (three times); the resulting DCM phases were combined and concentrated.

A subsample of the non-extractable residues following ASE was subjected to acid hydrolysis using dioxane:2N HCl (9:1, v:v) under reflux for 2 hours. The hydrolysate was isolated by filtration and concentrated, and the remaining non-extractable residues were washed with water (yielding a water dissolvable slurry) and then lyophilized prior to combustion and liquid scintillation counting (LSC).

For isolation and identification of metabolites, a second subsample of straw was soaked overnight in water (at 4°C), then extracted four times with ACN:water (80:20, v:v). Following partitioning with DCM (three times) as described above for forage, the resulting aqueous phase was partitioned with n-butanol (three times).

Grain: Subsamples were extracted three times with ACN:water (80:20, v:v), filtered, and partitioned with DCM as described above for forage. The resulting non-extractable residues were subjected to ASE as described above for hay.

For grain, separate subsamples of the non-extractable residues following extraction with ACN:water were subjected to: (1) acid hydrolysis using dioxane:2N HCl as described above for hay; and (2) enzyme hydrolysis with diastase (α-amylase) in citrate:NaOH buffer, pH 6, containing NaN_3, at room temperature for 9 days; the mixture was filtered on days 2, 3, 4, 7, and 9, and fresh enzyme solution was added to the remaining solids. The applicant noted that the solids remaining after acid hydrolysis were solubilized in the water wash, and that minimal solids remained following enzyme hydrolysis.

The extraction procedures for wheat matrices are summarized in FIGURES B.4.1.1 to B.4.1.4

FIGURE B.4.1.1 Extraction Procedure for Forage

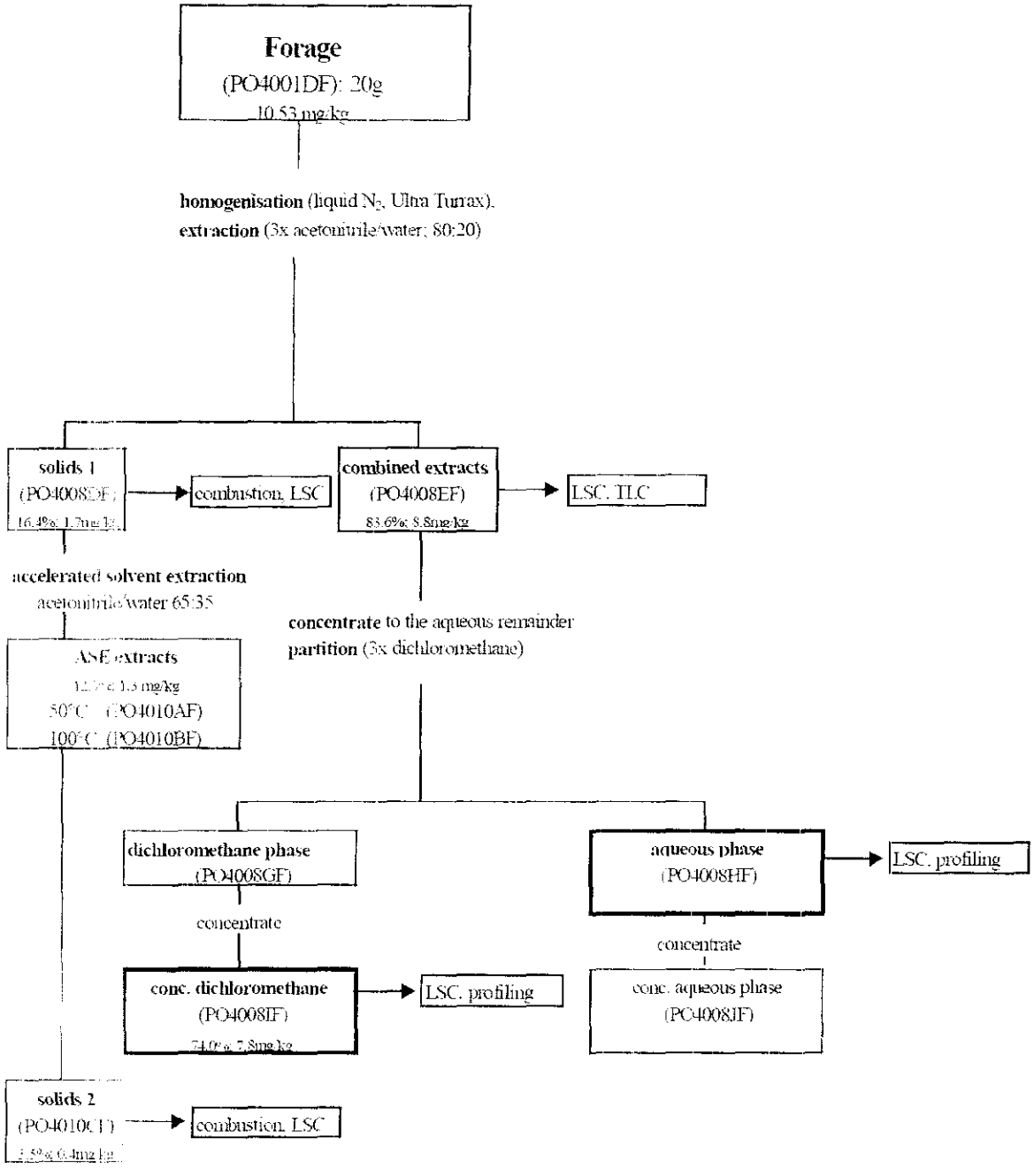


FIGURE B.4.1.2 Extraction Procedure for Hay

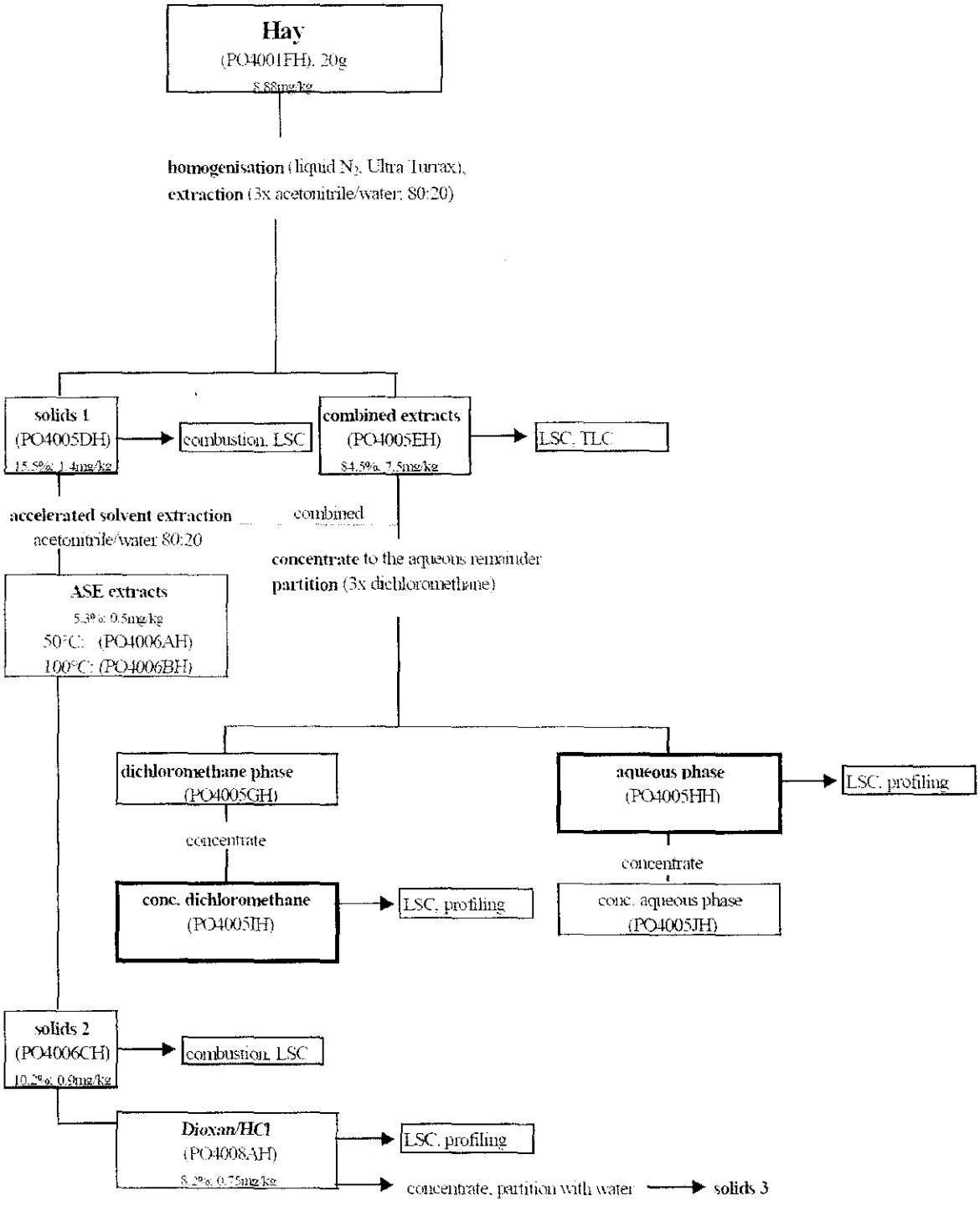


FIGURE B.4.1.3 Extraction Procedure for Straw

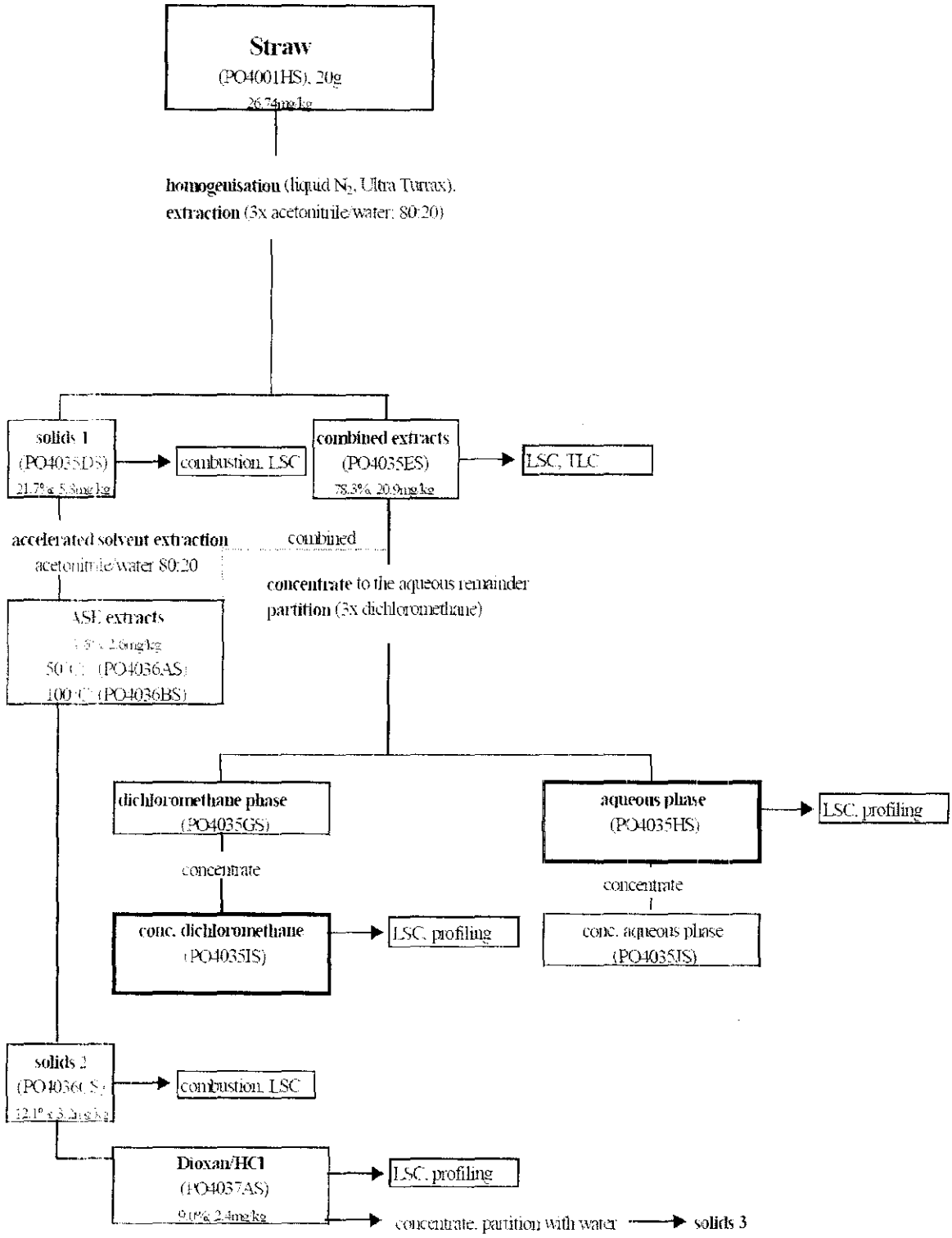
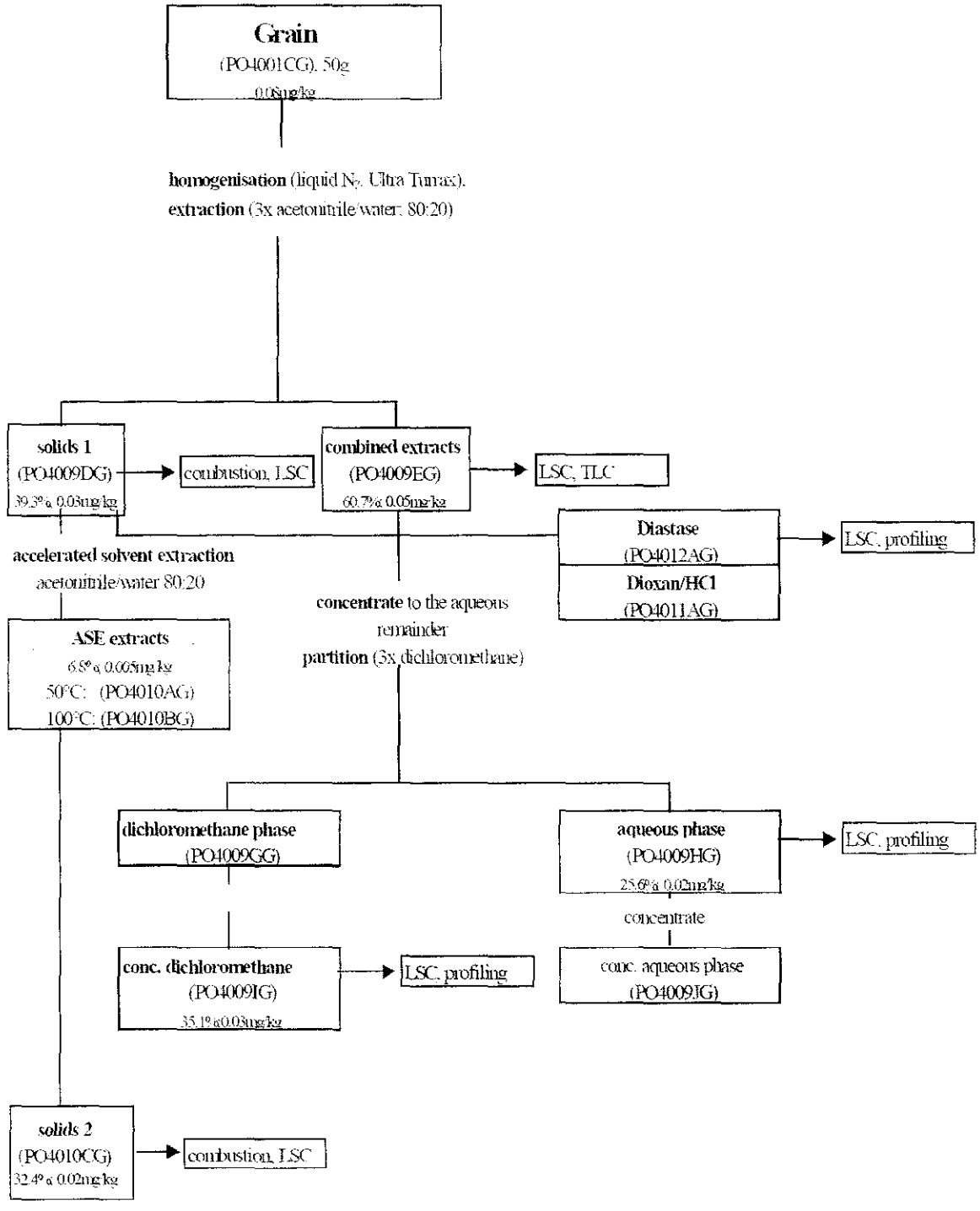


FIGURE B.4.1.4 Extraction Procedure for Grain



Triazole-label study

Wheat forage and straw were cut into small pieces using a knife or scissors immediately following harvest. Hay was dried in the greenhouse for 3 days prior to processing. Wheat grain was separated from the wheat head and chaff by hand. Plant samples were stored frozen (-20 ± 5 °C) prior to further processing. Chopped samples of forage, hay, and straw were homogenized in the presence of dry ice; grain was homogenized using liquid nitrogen. Following processing wheat samples were stored frozen (-20 ± 5°C).

Subsamples of forage and hay were extracted 3x with acetonitrile (ACN):water (4:1, v:v), then centrifuged; subsamples of straw and grain were sequentially extracted with methanol (1x) followed by ACN:water (4:1, v:v; three times), then centrifuged. Cysteine HCl was added to extracting solvents at 1 mg/mL. The supernatants for each matrix were combined, evaporated to dryness under vacuum, redissolved in methanol:water (1:1, v:v), and reserved for HPLC analysis. Non-extractable residues following extraction were subjected to accelerated solvent extraction (ASE) with ACN:water (65:35, v:v) at 50 and 100°C (two extractions each temperature). For hay, straw, and grain, a second ASE extraction procedure was performed with water at 150°C.

The non-extractable residues of wheat forage following ASE were subjected to acid hydrolysis with 2N HCl in methanol:water (1:1, v:v) at reflux (90°C) for 4 hours. After cooling, the hydrolysate was adjusted to pH 7, vacuum filtered, and the filtrate was evaporated to dryness under vacuum. Remaining non-extractable residues of forage and the non-extractable residues of hay, straw, and grain following ASE were subjected to hydrolysis with dioxane:2N HCl (4:1, v:v) under reflux for ~7 hours (grain was refluxed for ~16 hours), then centrifuged. Non-extractable residues were washed with methanol, and the methanol was added to the hydrolysate. Hydrolysates were evaporated to dryness, and those containing sufficient radioactivity for HPLC analysis were dissolved in water and subjected to solid phase extraction (SPE) clean-up on a C-18 cartridge; residues were eluted with ACN:water (1:1 and 4:1, v:v), ACN, and methanol:water. The eluates were combined and evaporated to dryness and dissolved in ACN:0.1% trifluoroacetic acid (1:1, v:v) for HPLC analysis; SPE was repeated if necessary.

The extraction procedures for wheat matrices are summarized in FIGURES B.4.1.5 to B.4.1.8.

FIGURE B.4.1.5 Extraction Procedure for Forage

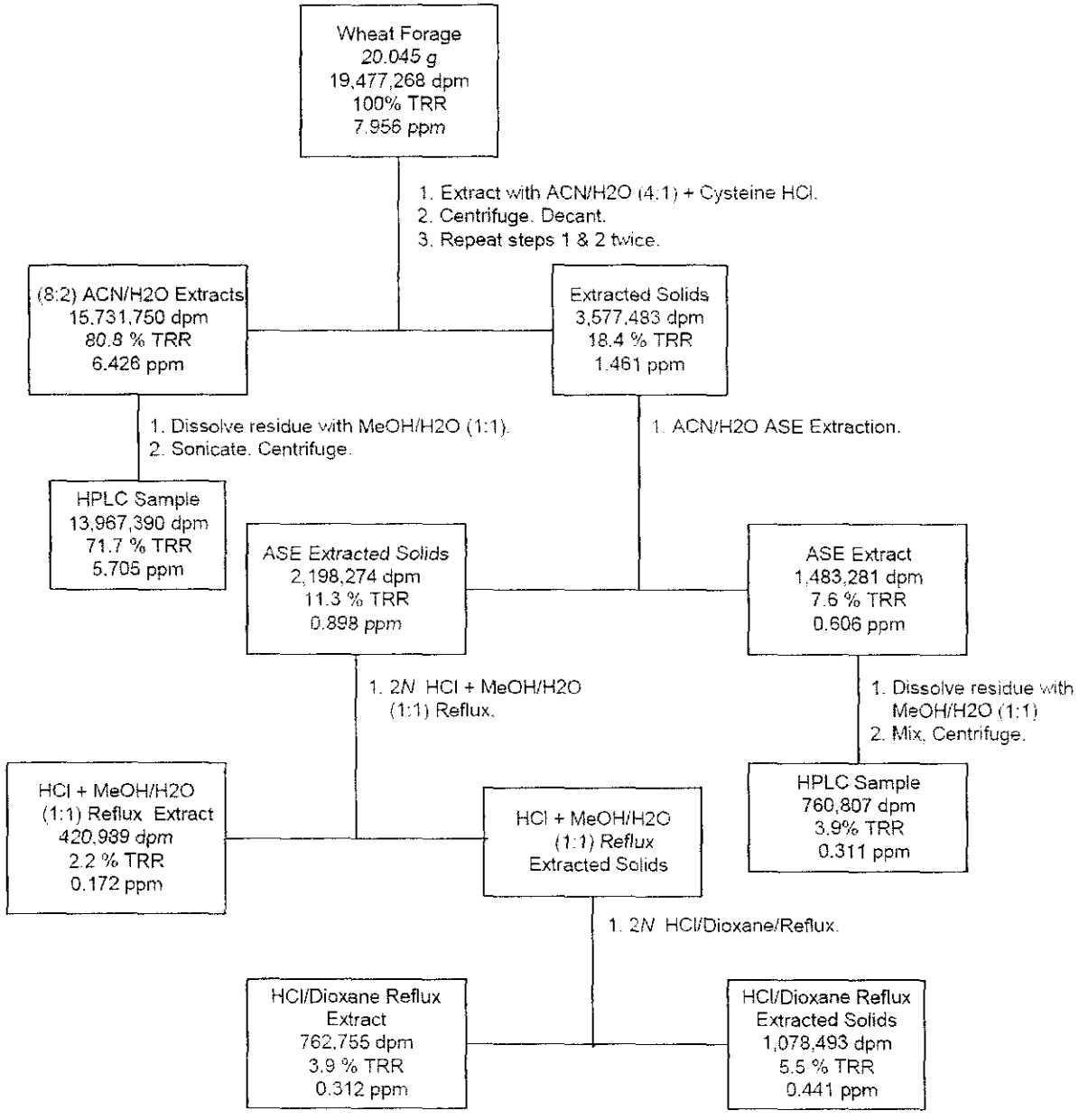


FIGURE B.4.1.6 Extraction Procedure for Hay

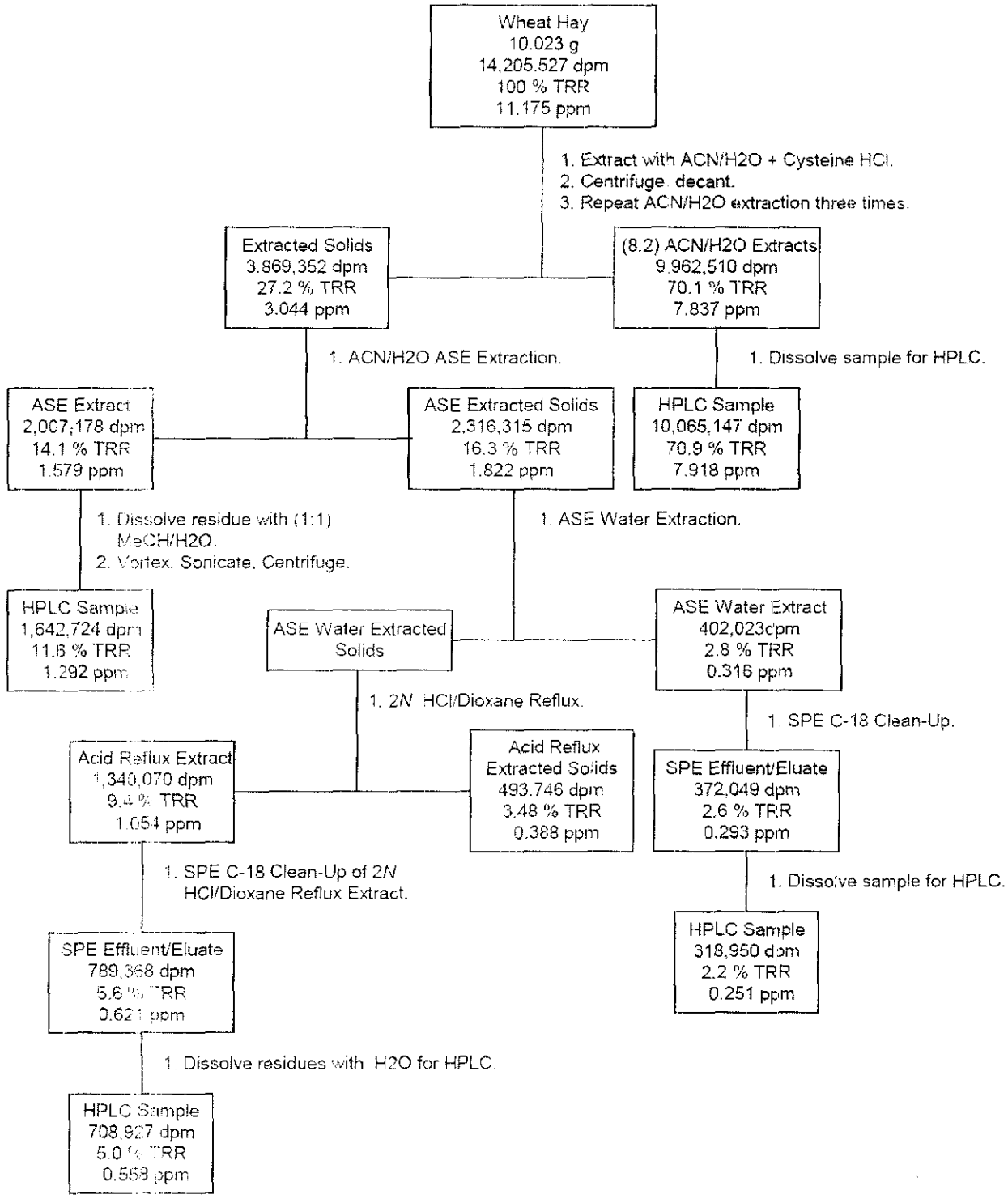


FIGURE B.4.1.7 Extraction Procedure for Straw

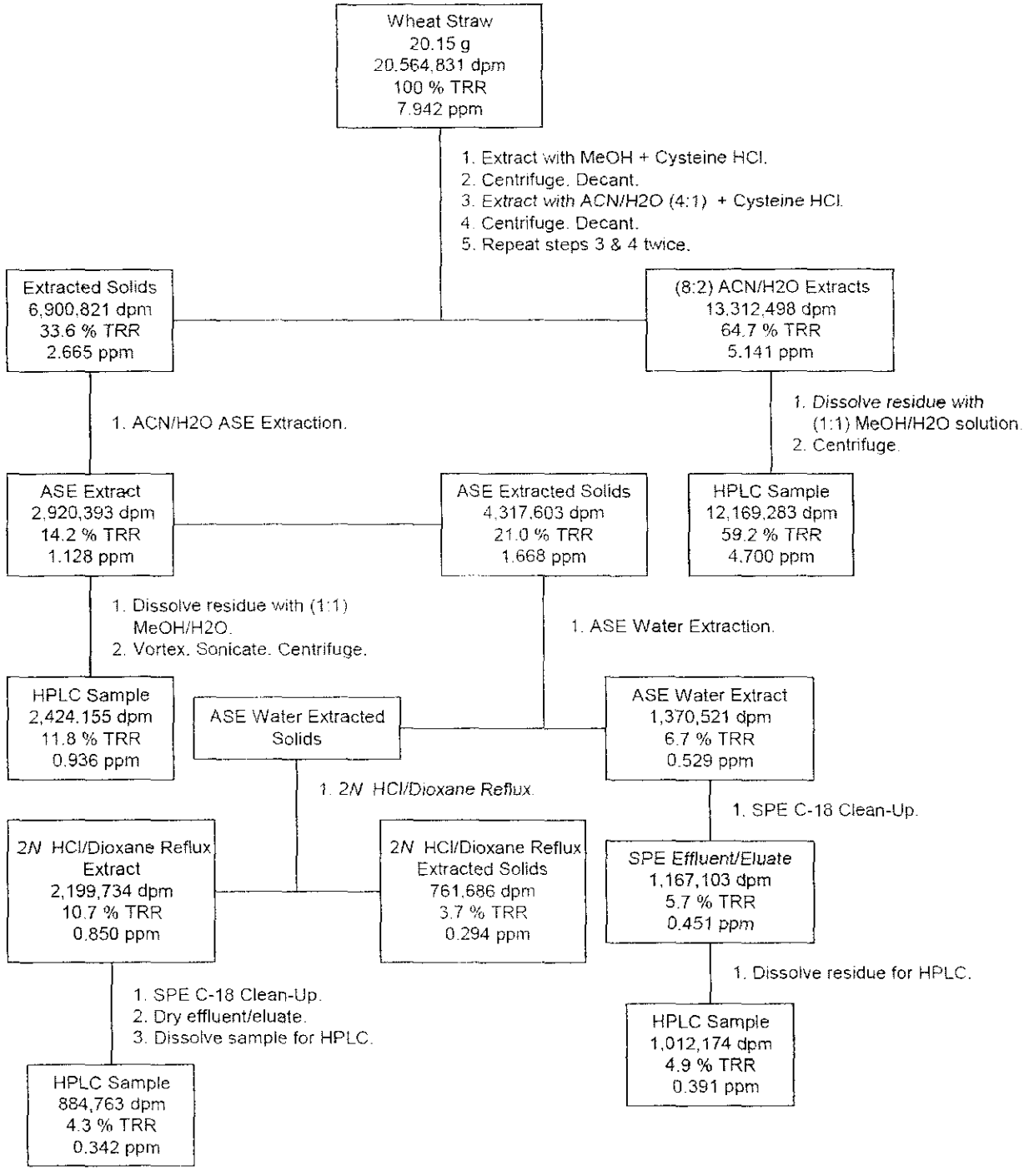
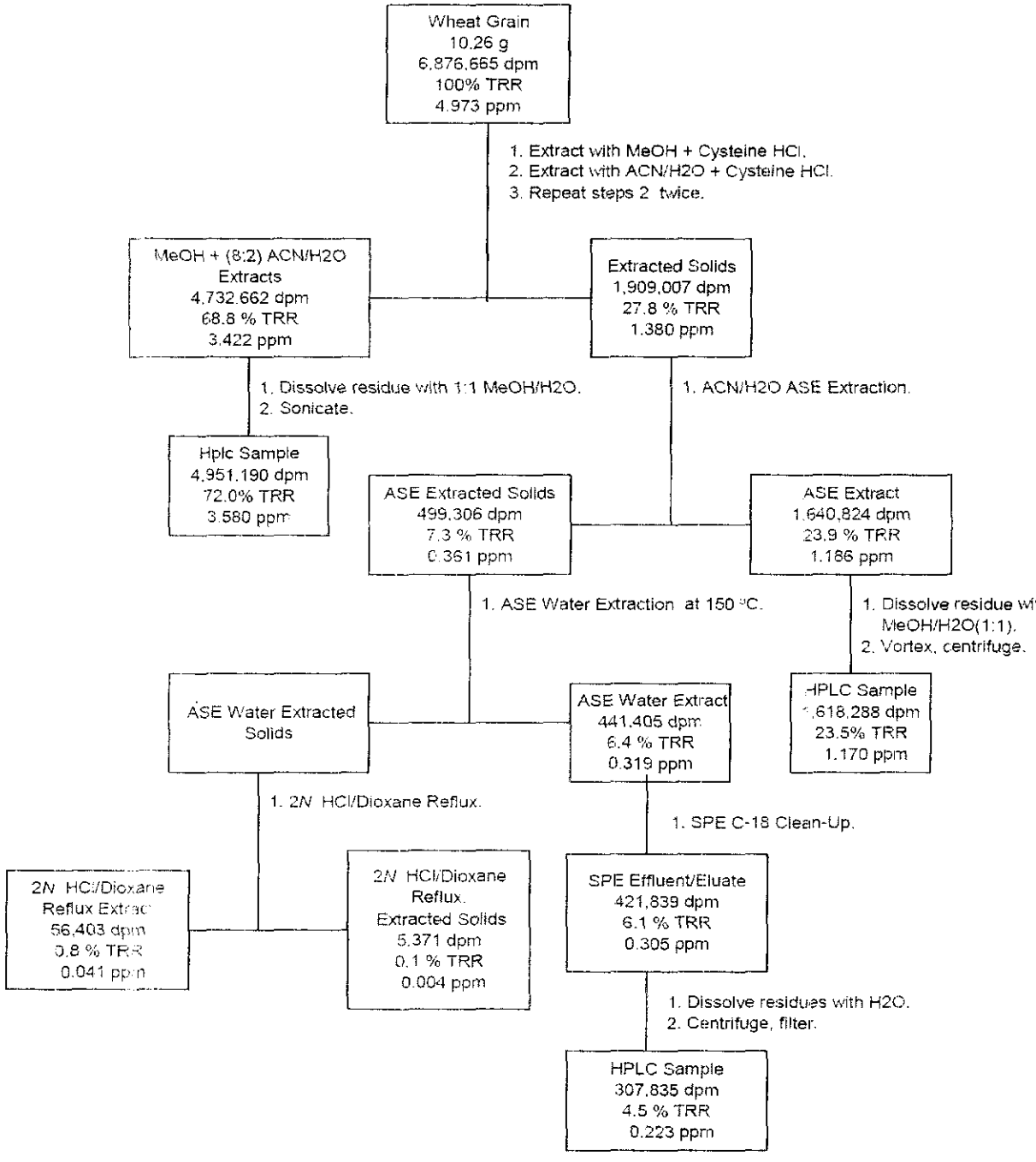


FIGURE B.4.1.8 Extraction Procedure for Grain



B.4.2. Analytical Methodology

Phenyl-label study

Total radioactive residues (TRRs) in wheat matrices were determined by summing radioactivity in extractable and non-extractable residues following extraction with ACN:water. Extracts and hydrolysates were radioassayed by liquid scintillation counting (LSC), and non-extractable residues were radioassayed by combustion/LSC. The limit of detection was reported as twice background.

High Performance Liquid Chromatography (HPLC)
Extracts and hydrolysates of wheat matrices were subjected to reverse or normal phase HPLC and/or TLC analysis for isolation, purification, and/or identification of metabolites. HPLC analyses were conducted on systems equipped with a UV detector and a flow-through radiodetector. The following column/mobile phase combinations were used: (1) C18 column (Lichrospher 100 RP 18e or Phenomenex ODS 30) with a gradient mobile phase of water and ACN, each containing 0.1% acetic acid; and (2) silica column (Lichrospher 100 Diol) with a gradient mobile phase of n-hexane and aqueous ethanol containing ammonia.

Thin Layer Chromatography (TLC)
TLC analyses were conducted using silica gel Si60 F_{254} plates and three solvent systems: DCM:methanol:25% ammonia (90:10:1, v:v:v; SS1); n-butanol:water:acetic acid (4:1:1, v:v:v; SS2); and ethyl acetate:isopropanol:water (65:23:12, v:v:v; SS3). The applicant noted that prothioconazole reference standard and cysteine HCl were spiked to each TLC origin before applying sample solutions to minimize decomposition of prothioconazole. Radioactive areas were detected and quantitatively evaluated using bioimaging, and nonradioactive reference standards were visualized under UV light (254 nm). Metabolites were identified by co-chromatography and/or retention time or R_f value comparisons with reference standards, or by comparison of metabolite patterns between like extracts of different matrices.

Individual metabolites were isolated from the organic and aqueous phases of straw (second subsample) using silica gel SPE columns and micropreparative HPLC using one or both of the systems described above. The DCM and n-butanol phases of straw were evaporated and dissolved in ethyl acetate:isopropanol:water (SS3 above), then purified by silica gel SPE; residues were eluted with SS3 and separated by fraction collection. Selected fractions were combined and concentrated to dryness. Isolated fractions from the DCM phase that contained radioactivity were dissolved in 90% ACN, then subjected to micropreparative HPLC for isolation of individual metabolites. One representative fraction from the n-butanol phase that contained significant radioactivity was applied to a C18 SPE column, and nine separate fractions were sequentially eluted with a stepwise gradient of ACN and water, each containing 0.1% acetic acid. Fractions containing radioactivity were subjected to micropreparative HPLC which resulted in the isolation of four major peaks. Remaining fractions from the initial SPE purification of the n-butanol phase were either characterized based on their similarity to the representative fraction or subjected to enzyme and acid hydrolysis. A methanol washing solution that was collected from

the column after residues were eluted was subjected to SPE analysis for characterization of polar metabolites. Isolated metabolites from both phases were analyzed by LC-MS, LC-MS/MS, and/or ^{1}H-NMR for structure elucidation/identification. Selected metabolites in forage, hay, and grain were identified by comparison of chromatographic characteristics with those of the isolated metabolites of straw.

Mass Spectrometry (MS)
LC-MS and LC-MS/MS analyses were conducted using a reverse-phase column, a gradient mobile phase of 1% acetic acid in water and ACN, and MS or MS/MS detection with positive or negative electro-spray ionization (ESI). For further characterization of one glucoside metabolite that could not be conclusively identified, LC-MS analysis was conducted using a C18 column and a gradient mobile phase of water and ACN, each containing 0.1% formic acid.

Hydrolysis
The aqueous phase of the second straw subsample was subjected to acid and enzyme hydrolysis. Acid hydrolyses were conducted using 1N and 6N HCl (pH 1, at 100°C for 24 hours) and enzyme hydrolyses were conducted using β-glucosidase (in 0.2M sodium acetate buffer, pH 5, at 37°C for 24 hours) and cellulase (in 0.1M potassium phosphate buffer, pH 4.5, at 37°C for 24 hours). The resulting hydrolysates were analyzed by HPLC or TLC.

Triazole-label Study
Total radioactive residues (TRRs) in wheat matrices were determined by combustion/LSC. Extracts and hydrolysates were radioassayed by LSC, and non-extractable residues were radioassayed by combustion/LSC. The reported limit of detection for solid samples was 0.003 ppm.

High Performance Liquid Chromatography (HPLC)
Initial HPLC analyses of wheat extracts and preparative HPLC analysis for separation of selected metabolites were conducted on systems equipped with a variable wavelength UV detector and a flow-through radiodetector. Initial analysis of wheat extracts was conducted on a system equipped with a C18 column and using a gradient mobile phase of ACN and 0.1% aqueous trifluoroacetic acid. The reported limit of detection was 0.003 ppm. Metabolites were identified by co-chromatography and/or retention time comparisons with reference standards.

A second HPLC system with ion-pairing was used to separate triazolylalanine (TA) from triazolylacetic acid (TAA) and triazolylhydroxypropionic acid (THPA). The system was equipped with a C8 column and used a gradient mobile phase of water and methanol, each containing 0.005M pentyltriethylammonium phosphate as the ion-pairing agent. Isolated TA was derivatized to dansyl-TA by heating with dansyl chloride and sodium bicarbonate at 50°C for 7 hours. A second aliquot of TA was purified by sequential SAX/SCX SPE prior to derivatization with heptafluorobutyric anhydride. The SAX cartridge was sequentially eluted with methanol and acetic acid methanol, and the SCX cartridge was sequentially eluted with methanol and ammonium hydroxide:methanol. The purified residue was dried under a stream of nitrogen, then

redissolved in n-butanolic HCl and heated at 110°C for 1 hour. The resulting sample was dried under nitrogen and heated with heptafluorobutyric anhydride at 110-120°C for 10-15 minutes.

The HPLC fraction containing TAA and THPA was heated with n-butanolic HCl at 120°C for 30 minutes to form the butyl ester derivatives of TAA and THPA. Following esterification, THPA butyl ester was separated from TAA butyl ester on a third HPLC system equipped with a C18 column and using a gradient mobile phase of ACN and 0.1% aqueous acetic acid. This system was also used for isolation of the dansyl- and the heptafluorobutyric-TA derivatives for LC-MS.

A fourth HPLC system was used to separate components eluting between 36-49 minutes in wheat forage, hay, and straw. The HPLC system was equipped with a C8 column and used a gradient mobile phase of ACN and 0.1% aqueous acetic acid. To further purify metabolites for MS analyses, isolated components were subjected to preparative HPLC using a C18 or C8 column and a gradient mobile phase of 0.1% acetic acid in water and methanol.

Mass Spectrometry (MS)
LC-MS and LC-MS/MS analyses were conducted using a C8 column, a gradient mobile phase of 0.1% formic acid and methanol, and MS or MS/MS detection with positive and negative electro-spray ionization.

C. RESULTS AND DISCUSSION

Phenyl-label study
Total radioactive residues (TRRs) in wheat matrices are reported in TABLE C.2.1 and represented in FIGURE C.2.1. TRRs were determined by summing extractable and non-extractable radioactivity and were 10.45 ppm in forage, 8.90 ppm in hay, 26.74 ppm in straw and 0.08 ppm in grain.

The distribution of the radioactivity in wheat matrices is presented in TABLE C.2.2. Solvent extraction with ACN:water (80:20) released the majority of the radioactivity (~60.5-84.5% of the TRRs; 0.05-20.94 ppm) in all wheat matrices. Cysteine HCl was added to all extracting solvents and to extracts during concentration procedures to prevent oxidative decomposition of prothioconazole. Accelerated solvent extraction (ASE) with ACN:water (65:35, v:v for forage and 80:20, v:v for all other matrices) at 50°C and 100°C released an additional ~5.3-12.9% of the TRRs (0.02-2.57 ppm) from all matrices; acid hydrolysis with dioxane:HCl released 7.2-8.2% of the TRRs (0.64-2.18 ppm) in hay and straw, and enzyme hydrolysis with diastase released ~14.7% of the TRRs (0.01 ppm) from grain. When the extractable residues were partitioned with DCM, the majority of the TRRs partitioned into the DCM phase. Non-extractable residues remaining following extraction/hydrolysis accounted for <4% of the TRRs (≤0.83 ppm) in forage, hay, and straw, and <18% (<0.02 ppm) in grain. Because TRRs were determined by summing extractable and non-extractable radioactivity, accountabilities were ~99-100%. Residues were identified primarily by LC-MS, LC-MS/MS, and/or NMR spectroscopy with confirmatory analysis by HPLC and/or TLC co-chromatography. These methods successfully identified the predominant residues in wheat forage, hay, straw, and grain.

The characterization and identification of residues in wheat matrices are summarized in TABLE C.2.4. Approximately 67-73% of the TRRs were identified in wheat forage, hay, and straw, and 33.7% of the TRRs were identified in grain. Prothioconazole was identified at low levels (1.0-3.7% of the TRRs, <0.01-0.98 ppm) in all wheat matrices. Metabolite JAU6476-desthio was the major identified residue, accounting for 35.4% of the TRRs (3.70 ppm) in forage, 18.5% of the TRRs (1.64 ppm) in hay, 22.3% of the TRRs (5.95 ppm) in straw, and 15.9% of the TRRs (0.014 ppm) in grain. All remaining metabolites were identified at <10% of the TRRs; residue levels were generally comparable in forage, hay, and straw, and much lower in grain. Metabolites JAU6476-α-OH-desthio and JAU6476-triazolinone were identified in all wheat matrices at 2.8-9.4% of the TRRs (0.002-1.54 ppm) and 1.3-6.9% of the TRRs (0.001-1.64 ppm), respectively. Three additional metabolites were tentatively identified in all wheat matrices: JAU6476 disulfide at ≤2.5% of the TRRs (≤0.45 ppm), and two JAU6476-OH-desthio glucoside isomers each at ≤5.6% of the TRRs (≤1.08 ppm) each. Five metabolites were identified in forage, hay, and straw, but not in grain: JAU6476-3-OH-desthio, JAU6476-4-OH-desthio, and JAU6476-6-OH-desthio at 1.1-8.5% of the TRRs (0.11-0.76 ppm); JAU6476-3-OH-desthio and/or JAU6476-4-OH-desthio were tentatively identified in grain at 1.1% of the TRRs (0.0010 ppm), JAU6476 sulfonic acid at 3.3-8.4% of the TRRs (0.29-2.24 ppm), and JAU6476-OH-desthio glucoside (Isomer 3; tentative identification) at ≤2.4% of the TRRs (≤0.35 ppm). Metabolites JAU6476-α-acetoxy-desthio (with benzylpropyldiol in straw) and benzylpropyldiol glucoside were identified in hay, straw, and grain at ≤4.6% (<0.55 ppm) and ≤1.8% (≤0.47 ppm) of the TRRs, respectively, and metabolite JAU6476-desthio glucoside was tentatively identified in hay and straw only, at 2.2% (0.19 ppm) and 6.7% (1.79 ppm) of the TRRs, respectively.

Remaining radioactivity in wheat matrices was characterized as: (1) radioactivity remaining at the TLC origins (12.3-20.1% of the TRRs, 0.01-3.28 ppm); (2) unassigned or diffuse radioactivity (2.5-9.6% of the TRRs, <0.01-2.29 ppm); and (3) discrete unknowns, one to two in each matrix, each accounting for ≤4.0% of the TRRs (≤1.08 ppm). In grain, 14.7% of the TRRs (0.01 ppm) was characterized based on diastase hydrolysis, 7.9% of the TRRs (0.006 ppm) was extracted by ASE but not analyzed, and 6.3% of the TRRs (0.005 ppm) was characterized as polar and aqueous soluble.

Identification of prothioconazole in wheat straw was confirmed by TLC and HPLC co-chromatography. Metabolites JAU6476-desthio and JAU6476-triazolinone were identified by LC-MS and LC-MS/MS, and identification was confirmed by HPLC and/or TLC co-chromatography. Metabolites JAU6476-α-OH-desthio, JAU6476-3-OH-desthio, JAU6476-4-OH-desthio, JAU6476-6-OH-desthio, and JAU6476 sulfonic acid were identified by LC-MS, LC-MS/MS and NMR spectroscopy and confirmed by TLC co-chromatography. Metabolite JAU6476-α-acetoxy-desthio was identified by LC-MS, LC-MS/MS, and NMR spectroscopy; the applicant noted that the isolated straw fraction containing this metabolite additionally contained another metabolite that was postulated to be a JAU6476-α-OH-desthio diastereomer. Benzylpropyldiol glucoside was identified by LC-MS and NMR spectroscopy and was confirmed as a glucoside on the basis of its polarity compared to other metabolites on TLC analysis. Benzylpropyldiol was identified by LC-MS and LC-MS/MS spectroscopy but could not be separated from JAU6476-α-acetoxy-desthio on TLC analysis of the straw extract.

Metabolite JAU6476 disulfide was tentatively identified in wheat straw by TLC co-chromatography. Further attempts to isolate/purify this dimer resulted in decomposition to prothioconazole and JAU6476-desthio. The structures of the JAU6476-OH-desthio glucosides (Isomers 1 and 2) were tentatively resolved by LC-MS and LC-MS/MS in the positive ESI mode; LC-MS and LC-MS/MS in the positive and negative ESI modes were used to resolve the structure of Isomer 3. Tentative identification of an additional JAU6476-OH-desthio metabolite was made in the DCM extract of straw by LC-MS and LC-MS/MS; TLC analysis confirmed that the metabolite was not JAU6476-3-, 4-, or 5-OH-desthio, and the applicant concluded that it was probably a diastereomer of JAU6476-α-OH-desthio.

The applicant stated that because radioactivity remaining at the TLC origin accounted for a relatively high percentage of the TRR in the DCM phase of hay following initial analysis using SS1, the radioactive zone was isolated by preparative TLC and rechromatographed using SS2 and SS3. Eight areas of radioactivity were observed, five of which corresponded to metabolites that had already been identified in hay. No unknown metabolites were observed when this fraction was analyzed by HPLC. No supporting chromatograms or quantitative data for these analyses were included in the submission.

Triazole-label

Total radioactive residues (TRRs) in wheat matrices are reported in TABLE C.2.1 and represented in FIGURE C.2.1. TRRs were 7.956 ppm in forage, 11.175 ppm in hay, 7.942 ppm in straw, and 4.973 ppm in grain.

The distribution of the radioactivity in wheat matrices is presented in TABLE C.2.3. Solvent extraction with methanol and/or ACN:water released the majority of the TRRs (64.7-80.8%) in all matrices; cysteine HCl was added to extracting solvents to prevent oxidative decomposition of prothioconazole. Accelerated solvent extraction (ASE) with ACN:water at 50°C and 100°C released an additional ~7.6-23.9% of the TRRs (0.606-1.579 ppm) from all matrices, and subsequent ASE with water released ~2.8-6.7% of the TRRs (0.316-0.529 ppm) in hay, straw, and grain. Acid hydrolysis with HCl:methanol and/or HCl:dioxane released 6.1% of the TRRs (0.484 ppm) in forage, 9.4-10.7% of the TRRs (0.85-1.054 ppm) in hay and straw, and 1% of the TRRs (0.041 ppm) in grain. Non-extractable residues remaining following extraction/hydrolysis accounted for <6% of the TRRs (≤0.441 ppm) in forage, hay, straw, and grain. Extraction results were normalized; therefore, accountabilities were 100%. Residues were identified/confirmed by HPLC and/or by LC-MS and LC-MS/MS. These methods successfully identified the predominant residues in wheat forage, hay, straw, and grain.

The characterization and identification of residues in wheat matrices are summarized in TABLE C.2.5. Approximately 57.3-90.5% of the TRRs were identified in wheat matrices. The metabolite profiles were similar in forage, hay, and straw, and differed significantly from the metabolite profile in grain, in which neither prothioconazole nor any metabolites unique to prothioconazole were identified. In wheat forage, hay, and straw, prothioconazole was identified at 3.4-6.7% of the TRRs (0.378-0.529 ppm); metabolite JAU6476-desthio was the major identified residue in forage and straw at 18.8% of the TRRs (1.496 ppm) and 9.3% of the TRRs

(0.744 ppm), respectively, and was a significant component in hay at 11.9% of the TRRs (1.332 ppm). In grain, the major identified metabolite was triazolylalanine (TA) at 71.1% of the TRRs (3.537 ppm); triazolylacetic acid (TAA) accounted for 19.0% of the TRRs (0.946 ppm) in grain, and triazolylhydroxypropionic acid (THPA) constituted the remaining identified residue at 0.4% of the TRRs (0.022 ppm). TA was also the major identified residue in hay at 24.8% of the TRRs (2.769 ppm), and accounted for 12.0% of the TRRs (0.951 ppm) in forage and 4.1% of the TRRs (0.321 ppm) in straw. TAA was identified at 1.4% of the TRRs (0.108 ppm) in forage, 4.5% of the TRRs (0.499 ppm) in hay and 4.6% of the TRRs (0.365 ppm) in straw while THPA was identified at 2.8% of the TRRs (0.220 ppm), 7.6% of the TRRs (0.849 ppm) and 7.7% of the TRRs (0.609 ppm), respectively, in these same wheat matrices. Free triazole or *1H*-1,2,4-triazole was not identified in any wheat matrix.

All remaining metabolites were identified at <10% of the TRRs in forage, hay, and straw. Metabolites JAU6476-α-OH-desthio and JAU6476-triazolinone were identified in forage, hay, and straw at 6.5-8.5% of the TRRs (0.509-0.774 ppm) and 1.0-1.8% of the TRRs (0.083-0.145 ppm), respectively. JAU6476-OH-desthio (3-OH and/or 4-OH isomers) and JAU6476-α-acetoxy-desthio were identified in forage and straw at 2.0-6.1% of the TRRs (0.162-0.485 ppm) and 2.1-2.9% of the TRRs (0.167-0.233 ppm), respectively. Triazolyl-ethanol was identified in straw only at 1.6% of the TRRs (0.124 ppm), and triazolyl-ethanol glucoside was identified in forage, hay, and straw at 1.0-2.1% of the TRRs (0.081-0.227 ppm). Remaining identified components in forage, hay, and straw consisted of JAU6476-phenyl-cysteine isomers (forage; 2.2% of the TRRs; 0.177 ppm) and glucoside and malonyl-glucoside isomers of JAU6476-desthio, JAU6476-OH-desthio, JAU6476-OH-sulfonic acid, and JAU6476-diOH-desthio at ≤3.8% of the TRRs (≤0.299 ppm) each in any matrix. Discrete unknowns accounted for a total of 10.6% (0.860 ppm), 17.1% (1.899 ppm), 22.3% (1.762 ppm), and 6.3% (0.310 ppm) of the TRRs, respectively, in forage, hay, straw, and grain. Except for one unknown in straw that was present at 6.9% of the TRRs (0.549 ppm), all unknowns were present at ≤3.2% of the TRRs (0.358 ppm) each.

Prothioconazole, and metabolites JAU6476-desthio, JAU6476-α-OH-desthio, and JAU6476-triazolinone, were identified by HPLC co-chromatography and/or retention time comparisons with reference standards, and identification was confirmed by LC-MS analysis. Metabolite TA was identified by HPLC co-chromatography, and identification was confirmed by co-chromatography and LC-MS of the dansyl- or heptafluorobutyric derivatives. Metabolites THPA and TAA were identified by HPLC co-chromatography and LC-MS of the butyl ester derivatives, except in grain, where THPA was tentatively identified on the basis of retention time comparison with the butyl ester standard.

The following metabolites were identified by LC-MS of isolated and purified fractions of the initial extracts: JAU6476-OH-desthio isomers and JAU6476-α-acetoxy-desthio (in forage and straw; identification in hay was by retention time comparison with standard), triazolyl-ethanol (tentative identification), triazolyl-ethanol-glucoside, JAU6476-OH-desthio-glucoside isomers, JAU6476-OH-sulfonic acid-glucoside, JAU6476-OH-desthio-malonyl-glucoside isomers,

JAU6476-desthio-malonyl-glucoside, JAU6476-desthio-phenyl-cysteine, and JAU6476-diOH-desthio-malonyl-glucoside (tentative identification).

C.1. Storage Stability

Phenyl-label study

Processed wheat samples were stored frozen at -20°C. Extracts were stored either refrigerated (4°C) or frozen (-20°C). The extraction, profiling and quantitation of metabolites were obtained from analyses which were performed between 12 and 57 days after harvest of all wheat matrices. A TLC chromatogram reflecting analysis of the initial extracts was provided. To demonstrate the stability of residues of JAU6476 during frozen storage, the applicant provided data reflecting analysis for JAU6476 and JAU6476-desthio in samples of forage, hay, straw, and grain that were re-extracted and re-analysed following frozen storage for 461 days (forage), 518 days (hay), 167 and 538 days (straw), and 462 days (grain). A chromatogram was provided reflecting co-chromatography of the initial and final extracts for all matrices. No significant changes were observed in the levels of prothioconazole and JAU6476-desthio in samples analyzed following up to 538 days (17.7 months) of frozen storage. These data are sufficient to support the sample storage conditions and intervals of the submitted study.

All lab work[1] was completed within 762 days (25.0 months) of harvest of forage and 720 days (23.7 months) of harvest of straw and grain, however, no further profiling and metabolite quantitation occurred during this time.

TABLE C.1.1 Summary of Storage Conditions.

Matrix	Storage Temp.	Storage Duration				Interval of Demonstrated Storage Stability
		1st Extraction/Analysis	2nd Extraction	3rd Extraction	Study Completion	
Forage	-20°C	18 days	461 days (15.1 months)	NP	762 days (25.0 months)	461 days (15.1 months)
Hay		12 days	518 days (17.0 months)	NP	742 days (24.4 months)	518 days (17.0 months)
Straw		57 days	167 (5.6 months)	538 days (17.7 months)	720 days (23.7 months)	538 days (17.7 months)
Grain		57 days	462 days (15.2 months)	NP	720 days (23.7 months)	462 days (15.2 months)

Triazole-label study

Processed wheat samples were stored frozen (-20 ± 5°C). The applicant provided the dates of sample collection, extraction, and analysis for the wheat samples (RAC and extracts). Since the storage duration of the RACs was within a month, no additional storage stability data was necessary. For the extracts that were stored up to 473 days, residue profiles indicated no significant changes.

[1]The applicant defined lab work as all experimental activities and all entries into the raw data notebook beginning on the date of first application of test material (May 27, 1997) to the wheat plants and ending at 720-762 days for all matrices and 785 days for the entire study.

TABLE C.1.2	Summary of Storage Conditions.				
Matrix	Storage Temp.	Actual Storage Duration (Days)			Interval of Demonstrated Storage Stability (days)
		RAC	Extracts		
Forage	-20 ± 5°C	2	ACN:H_2O ACN:H_2O ASE 2N HCl MeOH:H_2O reflux 2N HCl:Dioxane reflux	2 34 398[1] 399[1]	not required
Hay		4	ACN:H_2O ACN:H_2O ASE 2N HCl MeOH:H_2O reflux 2N HCl:Dioxane reflux	4 7 454[1] 473[1]	not required
Straw		6	ACN:H_2O ACN:H_2O ASE 2N HCl MeOH:H_2O reflux 2N HCl:Dioxane reflux	7 20 432[1] 441[1]	not required
Grain		15	ACN:H_2O ACN:H_2O ASE 2N HCl MeOH:H_2O reflux 2N HCl:Dioxane reflux	18 19 448[1] not analyzed	not required

[1] Although the initial extractions/analysis/sample collection, which were performed within 34 days of harvest, yielded 79% to 93% of the TRRs in the wheat matrices, later attempts to extract more radioactivity with more stringent extraction methods (2N HCl MeOH:H_2O reflux, 2N HCl:Dioxane reflux) were made only to determine if additional bound and unchanged JAU6476 residues could be released. The results of the analysis of some of the later extracted samples showed residue profiles which were very similar to the profiles for the samples extracted within 34 days of harvest. No previously unidentified residue was found in the later extracts and the residues identified in the later extracts represented only less than 1 to 3% of the TRRs in the corresponding matrices.

C.2 Identification, Characterization, and Distribution of Residues

TABLE C.2.1	Total Radioactive Residues (TRRs) in Wheat Matrices.				
Matrix	Timing and Applic. No.	PHI (days)	[Phenyl-UL-^{14}C]-Prothioconazole, ppm	PHI (days)	[Triazole-3,5-^{14}C]-Prothioconazole, ppm
Forage	2 foliar applications	6	10.45	6	7.956
Hay		26	8.90	29	11.175
Straw		48	26.74	64	7.942
Grain		48	0.08	64	4.973

FIGURE C.2.1 Distribution of TRRs in wheat matrices.

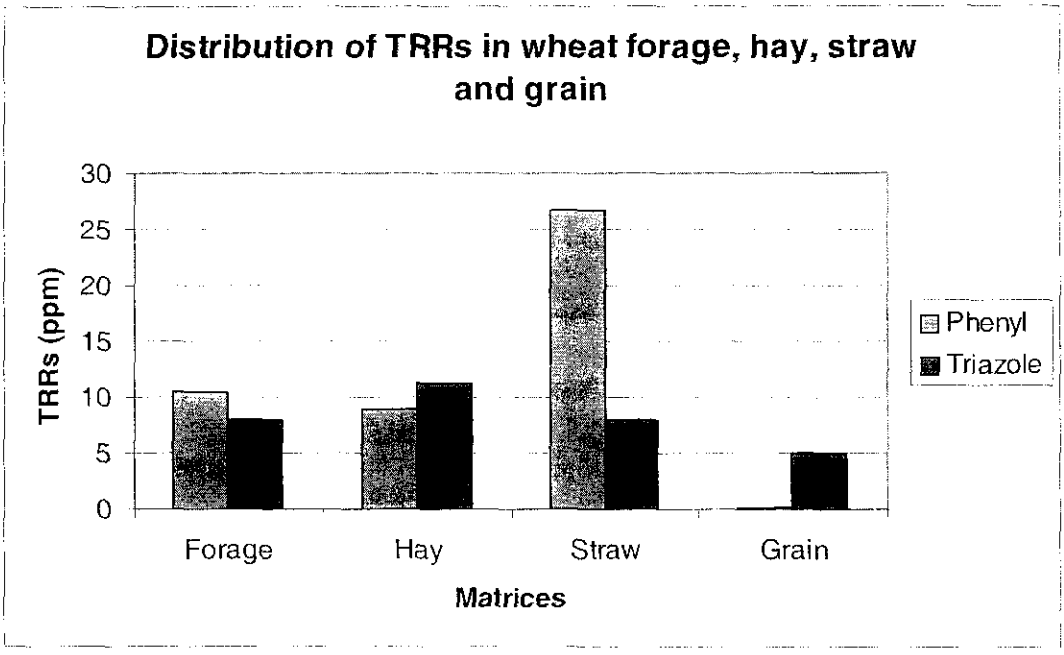


TABLE C.2.2. Distribution of the Parent and the Metabolites in Wheat Matrices Following Foliar Application of [Phenyl-UL-^{14}C]-Prothioconazole at 0.371 lb a.i./A (415 g a.i./ha). [1]

Metabolite Fraction	Forage		Hay		Straw		Grain	
	TRR = 10.45 ppm		TRR = 8.90 ppm		TRR = 26.74 ppm		TRR = 0.08 ppm	
	%TRR	ppm	%TRR	ppm	%TRR	ppm	%TRR	ppm
ACN:water	83.5	8.73	84.5	7.53	78.3	20.94	60.5	0.05
Combined ACN:water + ASE			89.8	8.00	87.9	23.52		
DCM	65.1	6.80	76.7	6.83	61.1	16.33	34.2	0.03
Prothioconazole	2.3	0.24	1.2	0.11	2.1	0.56	1.0	0.0009
JAU6476-desthio	34.3	3.59	17.3	1.54	20.8	5.55	15.9	0.0139
JAU6476-α-OH-desthio	4.5	0.47	9.4	0.83	5.8	1.54	2.8	0.0024
JAU6476-3-OH-desthio	2.4	0.25	8.5	0.75	2.9	0.76	1.1 [2]	0.0010
JAU6476-4-OH-desthio	1.2	0.13	6.7	0.60	2.7	0.72		
JAU6476-6-OH-desthio	1.1	0.12	1.2	0.11	1.2	0.32	--	--
JAU6476-triazolinone	6.6	0.69	4.7	0.42	5.6	1.51	1.3	0.0012
JAU6476 sulfonic acid	5.6	0.59	3.0	0.26	7.2	1.91	--	--
JAU6476-α-acetoxy-desthio	--	--	4.4	0.39	1.5	0.40	0.4	0.0003
Benzylpropyldiol	--	--	--	--			--	--
Benzylpropyldiol glucoside	--	--	0.7	0.06	1.8	0.47	1.5	0.0013
JAU6476 disulfide [2]	2.3	0.24	0.4	0.04	1.3	0.36	1.3	0.0011
TLC origin	3.0	0.31	14.0	1.25	5.5	1.46	8.8	0.0077
Unassigned [3]	1.7	0.18	5.2	0.46	2.9	0.77	--	--
Aqueous	19.2	2.01	13.1	1.17	26.9	7.19	26.3	0.02
JAU6476 sulfonic acid	1.2	0.12	0.3	0.03	1.2	0.33	--	--
JAU6476-OH-desthio glucoside (Isomer 1) [2]	2.4	0.25	1.7	0.16	4.0	1.08	5.6	0.0042
JAU6476-OH-desthio glucoside (Isomer 2) [2]	3.8	0.40			2.0	0.53	2.8	0.0020
JAU6476-OH-desthio glucoside (Isomer 3) [2]	2.4	0.26	0.9	0.08	1.3	0.35	--	--
JAU6476-desthio glucoside [2]	--	--	2.2	0.19	6.7	1.79	--	--
Unknown FO 9	4.9	0.51	--	--	--	--	--	--
Unknown FO 14	0.7	0.07	--	--	--	--	--	--
Unknown HA 11	--	--	1.2	0.11	--	--	--	--
Unknown HA 16	--	--	0.4	0.04	--	--	--	--
Unknown ST 11	--	--	--	--	1.6	0.42	--	--
Unknown ST 17	--	--	--	--	1.5	0.41	--	--
Unknown GR 11	--	--	--	--	--	--	2.6	0.0020
Polar radioactivity	--	--	--	--	--	--	6.3	0.0048
TLC origin	0.6	0.07	2.1	0.18	2.8	0.75	5.4	0.0041
Unassigned [5]	3.2	0.34	4.4	0.39	5.7	1.52	2.5	0.0019

TABLE C.2.2. Distribution of the Parent and the Metabolites in Wheat Matrices Following Foliar Application of [Phenyl-UL-^{14}C]-Prothioconazole at 0.371 lb a.i./A (415 g a.i./ha). [1]

Metabolite Fraction	Forage		Hay		Straw		Grain	
	TRR = 10.45 ppm		TRR = 8.90 ppm		TRR = 26.74 ppm		TRR = 0.08 ppm	
	%TRR	ppm	%TRR	ppm	%TRR	ppm	%TRR	ppm
Non-extractable	16.5	1.72	15.5	1.38	21.7	5.80	39.5	0.03
ACN:water ASE 50 °C	9.1	0.95	2.7	0.24	5.0	1.35	3.5	0.01
Prothioconazole	0.86	0.09	(extract combined with initial ACN:water extract)		(extract combined with initial ACN:water extract)			
JAU6746-desthio	0.27	0.03						
JAU6476-3-OH-desthio	0.09	0.01						
JAU6476-4-OH-desthio	0.06	0.01						
JAU6476-6-OH-desthio								
JAU6476-triazolinone	0.14	0.01						
JAU6746 sulfonic acid	0.27	0.03						
JAU6746 disulfide [2]	0.17	0.02						
TLC origin	6.31	0.66						
Unassigned [6]	0.95	0.10						
ACN:water ASE 100 °C	3.8	0.40	2.6	0.23	4.6	1.22	4.4	0.01
Prothioconazole	0.17	0.02	(extract combined with initial ACN:water extract)		(extract combined with initial ACN:water extract)			
JAU6476-desthio	0.78	0.08						
JAU6476-3-OH-desthio	0.07	0.01						
JAU6476-4-OH-desthio								
JAU6476-6-OH-desthio								
JAU6476-triazolinone	0.11	0.01						
JAU6746 sulfonic acid	0.05	0.01						
TLC origin	2.39	0.25						
Unassigned [7]	0.32	0.03						
Non-extractable	3.6	0.38	10.2	0.90	12.1	3.23	31.6	0.02
① Dioxane:HCl			7.2	0.64	8.2	2.18	21.6	0.02
Prothioconazole			1.44	0.13	1.6	0.42		
JAU6476-desthio			1.03	0.09	1.4	0.37		
JAU6476-3-OH-desthio			0.38	0.03	0.6	0.15		
JAU6476-4-OH-desthio								
JAU6476-triazolinone			0.41	0.04	0.5	0.13		
JAU6476-α-acetoxy-desthio			0.23	0.02	0.5	0.15		
Benzylpropyldiol			--	--				
JAU6476-disulfide [2]			0.48	0.04	0.3	0.09		
TLC origin			3.23	0.29	3.3	0.88		
Water dissolvable slurry			1.00	0.08	0.9	0.23	Not reported	
JAU6476-desthio			0.14	0.01	0.11	0.03		
JAU6476-3-OH-desthio			0.10	0.01	0.07	0.02		

TABLE C.2.2.	**Distribution of the Parent and the Metabolites in Wheat Matrices Following Foliar Application of [Phenyl-UL-^{14}C]-Prothioconazole at 0.371 lb a.i./A (415 g a.i./ha).** [1]							
Metabolite Fraction	Forage		Hay		Straw		Grain	
	TRR = 10.45 ppm		TRR = 8.90 ppm		TRR = 26.74 ppm		TRR = 0.08 ppm	
	%TRR	ppm	%TRR	ppm	%TRR	ppm	%TRR	ppm
JAU6476-4-OH-desthio								
TLC origin			0.77	0.06	0.72	0.19		
Non-extractable			2.0	0.18	3.1	0.83		
② Diastase hydrolysate							14.7	0.0110
Non-extractable							17.5	0.0130

[1] Shading indicates that the extraction step and/or characterization analysis was not conducted for the matrix in question.
[2] Tentative identification.
[3] Consisting of: 1 component in forage; 3 components in hay, each ≤2.1% of the TRRs (≤0.19 ppm); and 2 components in straw, each ≤1.8% of the TRRs (≤0.48 ppm).
[4] Characterized by hydrolysis.
[5] Consisting of: 2 components in forage, each ≤1.7% of the TRRs (≤0.18 ppm); 2 components in hay, each ≤3.0% of the TRRs (≤0.27 ppm); 2 components in straw, each ≤4.0% of the TRRs (1.08 ppm); and 1 component in grain.
[6] Consisting of: 5 components, each ≤0.56% of the TRRs (≤0.06 ppm).
[7] Consisting of: 5 components, each ≤0.18% of the TRRs (≤0.02 ppm).

TABLE C.2.3. Distribution of the Parent and the Metabolites in Wheat Matrices Following Foliar Application of [Triazole-3,5-^{14}C]-Prothioconazole at 0.420 lb a.i./A (470 g a.i./ha). [1]

Metabolite Fraction	Forage		Hay		Straw		Grain	
	TRR = 7.956 ppm		TRR = 11.175		TRR = 7.942 ppm		TRR = 4.973 ppm	
	%TRR	ppm	%TRR	ppm	%TRR	ppm	%TRR	ppm
MeOH and/or ACN:water	80.8	6.426	70.1	7.837	64.7	5.141	68.8	3.422
Prothioconazole	5.0	0.397	2.3	0.256	5.3	0.418	--	--
JAU6476-desthio	17.2	1.370	7.3	0.814	5.9	0.471	--	--
JAU6476-α-OH-desthio	7.9	0.630	6.2	0.688	5.6	0.442	--	--
JAU6476-OH-desthio [2]	2.0	0.162	-- [3]	--	5.5	0.435	--	--
JAU6476-triazolinone	1.6	0.131	-- [3]	--	-- [3]	--	--	--
JAU6476-α-acetoxy-desthio	2.9	0.233	-- [3]	--	2.1	0.167	--	--
Triazolylalanine (TA)	10.8	0.857	19.1	2.130	3.4	0.267	49.4	2.456
Triazolylacetic acid (TAA)	1.4	0.108	4.5	0.499	4.6	0.365	15.3	0.761
Triazolylhydroxypropionic acid (THPA)	2.8	0.220	7.6	0.849	7.7	0.609	0.4	0.022
Triazolyl-ethanol	--	--	--	--	1.6	0.124	--	--
Triazolyl-ethanol-glucoside	1.0	0.081	2.0	0.227	2.1	0.168	--	--
JAU6476-OH-desthio-glucoside isomers [4]	0.9	0.073	1.1	0.119	0.6	0.051	--	--
JAU6476-OH-desthio-glucoside + JAU6476-OH-sulfonic acid glucoside	0.6	0.052	--	--	--	--	--	--
JAU6476-OH-sulfonic acid glucoside + JAU6476-desthio-malonyl-glucoside	--	--	0.5	0.057	--	--	--	
JAU6476-OH-desthio-malonyl-glucoside isomers [5]	0.7	0.056	2.6	0.290	3.8	0.299	--	--
JAU6476-OH-desthio-malonyl-glucoside + JAU6476-OH-sulfonic acid glucoside	--	--	1.5	0.165	--	--	--	--
JAU6476-desthio-malonyl-glucoside	0.6	0.049	--	--	--	--	--	--
JAU6476-desthio-phenyl-cysteine isomers [6]	2.2	0.177	--	--	--	--	--	--
JAU6476-OH-desthio-malonyl-glucoside isomers [7] + JAU6476-OH-desthio-glucoside	1.2	0.095	2.6	0.295	--	--	--	--
JAU6476-diOH-desthio-malonyl-glucoside	1.9	0.148	--	--	--	--	--	--
Unknowns [8]	10.6	0.860	13.7	1.527	11.2	0.888	3.7	0.184
Non-extractable	18.4	1.461	27.2	3.044	33.6	2.665	27.8	1.380
ACN:water ASE	7.6	0.606	14.1	1.579	14.2	1.128	23.9	1.186
Prothioconazole	--	--	0.6	0.070	0.8	0.063	--	--
JAU6476-desthio	1.6	0.126	0.9	0.102	1.1	0.086	--	--
JAU6476-α-OH-desthio	0.6	0.047	0.7	0.076	0.7	0.053	--	--
JAU6476-triazolinone	0.2	0.014	--	--	--	--	--	--

TABLE C.2.3. Distribution of the Parent and the Metabolites in Wheat Matrices Following Foliar Application of [Triazole-3,5-^{14}C]-Prothioconazole at 0.420 lb a.i./A (470 g a.i./ha). [1]

Metabolite Fraction	Forage		Hay		Straw		Grain	
	TRR = 7.956 ppm		TRR = 11.175		TRR = 7.942 ppm		TRR = 4.973 ppm	
	%TRR	ppm	%TRR	ppm	%TRR	ppm	%TRR	ppm
Triazolylalanine (TA)	1.2	0.094	5.7	0.639	0.7	0.054	17.9	0.892
Triazolylacetic acid (TAA)	0.4	0.030	1.7	0.186	0.7	0.059	3.7	0.185
Triazolylhydroxypropionic acid (THPA)							--	--
Unknowns [2]	--	--	2.0	0.219	7.9	0.622	1.9	0.093
Non-extractable	11.3	0.898	16.3	1.822	21.0	1.668	7.3	0.361
Water ASE			2.8	0.316	6.7	0.529	6.4	0.319
Prothioconazole			0.5	0.052	0.6	0.048	--	--
JAU6476-desthio			0.5	0.053	0.5	0.044	--	--
JAU6476-α-OH-desthio			0.1	0.010	0.2	0.014	--	--
JAU6476-triazolinone			0.3	0.029	0.4	0.036	--	--
Triazolylalanine			0.4	0.044	0.4	0.029	3.8	0.189
Triazolylacetic acid							--	--
Triazolylhydroxypropionic acid (THPA)							--	--
Unknowns [10]			0.6	0.065	2.8	0.221	0.7	0.033
Non-extractable			N/R	N/R	N/R	N/R	N/R	N/R
2N HCl:methanol	2.2	0.172						
Non-extractable	N/R	N/R						
2N HCl:dioxane	3.9	0.312	9.4	1.054	10.7	0.850	0.8	0.041
JAU6476-desthio			3.2	0.363	1.8	0.143		
JAU6476-triazolinone			1.0	0.107	0.6	0.047		
JAU6476-OH-desthio			--	--	0.6	0.050		
Unknown			0.8	0.088	0.4	0.031		
Non-extractable	5.5	0.441	3.5	0.388	3.7	0.294	0.1	0.004

[1] Shading indicates that the extraction step and/or characterization analysis was not conducted for the matrix in question.
[2] JAU6476-3-OH-desthio and/or JAU6476-4-OH-desthio; 2 isomers in forage (relative distribution not specified); and 2 isomers in straw, each ≤3.5% of the TRRs (≤0.275 ppm).
[3] The applicant noted that this compound was not observed in the initial HPLC analyses which were used for metabolite quantitation; however, a compound eluting with a retention time similar to that of this compound was observed during metabolite isolation and purification. In the case of wheat straw, LC/MS analyses confirmed the presence of JAU6476-triazolinone.
[4] Two isomers in forage, each ≤0.6% of the TRRs (≤0.049 ppm); 2 isomers in hay, each ≤0.6% of the TRRs (≤0.068 ppm); and 1 isomer in straw.
[5] One isomer in forage; 3 isomers in hay, each ≤1.3% of the TRRs (≤0.149 ppm); and 3 isomers in straw, each ≤1.5% of the TRRs (≤0.117 ppm).
[6] Two isomers in forage, each ≤1.1% of the TRRs (≤0.090 ppm).
[7] Three isomers in forage and one isomer in hay; relative distributions were not specified.
[8] Consisting of: 14 components in forage, each ≤2.8% of the TRRs (≤0.223 ppm); 7 components in hay, each ≤3.2% of the TRRs (≤0.358 ppm); 9 components in straw, each ≤2.6% of the TRRs (≤0.206 ppm); and 1 component in grain.
[9] Consisting of: 3 components in hay, each ≤0.8% of the TRRs (≤0.085 ppm); 3 components in straw, each ≤6.9% of the TRRs (≤0.549 ppm); and 2 components in grain, each ≤1.0% of the TRRs (≤0.050 ppm).
[10] Consisting of: 5 components in hay, each ≤0.2% of the TRRs (≤0.021 ppm); 2 components in straw, each ≤2.4 TRR (≤0.191 ppm); and 2 components in grain, each ≤0.4% of the TRRs (≤0.019 ppm).

TABLE C.2.4. Summary of Characterization and Identification of Radioactive Residues in Wheat Matrices Following Foliar Application of [Phenyl-UL-^{14}C]-Prothioconazole at 0.371 lb a.i./A (415 g a.i./ha).

Compound	Forage		Hay		Straw		Grain	
	TRR = 10.45 ppm		TRR = 8.90 ppm		TRR = 26.74 ppm		TRR = 0.08 ppm	
	% of the TRRs	ppm	% of the TRRs	ppm	% of the TRRs	ppm	% of the TRRs	ppm
Identified								
Prothioconazole	3.3	0.35	2.6	0.24	3.7	0.98	1.0	0.0009
JAU6476-desthio	35.4	3.70	18.5	1.64	22.3	5.95	15.9	0.0139
JAU6476-α-OH-desthio	4.5	0.47	9.4	0.83	5.8	1.54	2.8	0.0024
JAU6476-3-OH-desthio	2.5	0.26	8.5	0.75	2.9	0.76	--	--
JAU6476-4-OH-desthio	1.2	0.13	6.7	0.60	2.7	0.72	--	--
JAU6476-6-OH-desthio	1.1	0.12	1.2	0.11	1.2	0.32	--	--
JAU6476-triazolinone	6.9	0.71	5.1	0.46	6.1	1.64	1.3	0.0012
JAU6746 sulfonic acid	7.1	0.75	3.3	0.29	8.4	2.24	--	--
JAU6476-α-acetoxy-desthio	--	--	4.6	0.41	2.0	0.55	0.4	0.0003
Benzylpropyldiol	--	--	--	--			--	--
Benzylpropyldiol glucoside	--	--	0.7	0.06	1.8	0.47	1.5	0.0013
Tentatively identified								
JAU6476-OH-desthio [1]	0.2	0.02	0.5	0.04	0.7	0.17	1.1	0.0010
JAU6746 disulfide	2.5	0.26	0.9	0.08	1.6	0.45	1.3	0.0011
JAU6476-OH-desthio glucoside (Isomer 1)	2.4	0.25	1.7	0.16	4.0	1.08	5.6	0.0042
JAU6476-OH-desthio glucoside (Isomer 2)	3.8	0.40			2.0	0.53	2.8	0.0020
JAU6476-OH-desthio glucoside (Isomer 3)	2.4	0.26	0.9	0.08	1.3	0.35	--	--
JAU6476-desthio glucoside	--	--	2.2	0.19	6.7	1.79	--	--
Characterized								
Unknowns	5.6	0.58	1.6	0.15	3.1	0.83	2.6	0.0020
Polar activity (aqueous)	--	--	--	--	--	--	6.3	0.0048
ASE extracts	--	--	--	--	--	--	7.9	0.0063
Diastase hydrolysate	--	--	--	--	--	--	14.7	0.0110
TLC Origins	12.3	1.29	20.1	1.78	12.3	3.28	14.2	0.0118
Unassigned	6.2	0.65	9.6	0.85	8.6	2.29	2.5	0.0019

TABLE C.2.4. Summary of Characterization and Identification of Radioactive Residues in Wheat Matrices Following Foliar Application of [Phenyl-UL-^{14}C]-Prothioconazole at 0.371 lb a.i./A (415 g a.i./ha).

Compound	Forage		Hay		Straw		Grain	
	TRR = 10.45 ppm		TRR = 8.90 ppm		TRR = 26.74 ppm		TRR = 0.08 ppm	
	% of the TRRs	ppm	% of the TRRs	ppm	% of the TRRs	ppm	% of the TRRs	ppm
Total identified	73.3	7.68	66.8	5.94	73.2	19.54	33.7	0.0283
Total characterized	24.1	2.52	31.3	2.78	24.0	6.40	48.2	0.0378
Total extractable	96.4	10.07	98.0	8.72	97.0	25.93	83.1	0.0661
Unextractable (PES) [2]	3.6	0.38	2.0	0.18	3.1	0.83	17.5	0.0130
Accountability [3]	100		100		100.1		98.9	

[1] Comprised of 3-OH, 4-OH and/or 6-OH isomers.
[2] Residues remaining after exhaustive extractions.
[3] Accountability = (Total extractable + Total unextractable)/(absolute values of the TRRs (ppm)) * 100.

TABLE C.2.5. Summary of Characterization and Identification of Radioactive Residues in Wheat Matrices Following Foliar Application of [Triazole-3,5-^{14}C]-Prothioconazole at 0.357 lb a.i./A (420 g a.i./ha).

Compound	Forage		Hay		Straw		Grain	
	TRR = 7.956 ppm		TRR = 11.175		TRR = 7.942 ppm		TRR = 4.973 ppm	
	% of the TRRs	ppm	% of the TRRs	ppm	% of the TRRs	ppm	% of the TRRs	ppm
Identified								
Prothioconazole	5.0	0.397	3.4	0.378	6.7	0.529	--	--
JAU6476-desthio	18.8	1.496	11.9	1.332	9.3	0.744	--	--
JAU6476-α-OH-desthio	8.5	0.677	7.0	0.774	6.5	0.509	--	--
JAU6476-OH-desthio	2.0	0.162	-- [1]	--	6.1	0.485	--	--
JAU6476-triazolinone	1.8	0.145	1.3	0.136	1.0	0.083	--	--
JAU6476-α-acetoxy-desthio	2.9	0.233	-- [1]	--	2.1	0.167	--	--
Triazolylalanine (TA)	12.0	0.951	24.8	2.769	4.1	0.321	71.1	3.537
Triazolylacetic acid (TAA)	1.4	0.108	4.5	0.499	4.6	0.365	19.0	0.946
Triazolylhydroxypropionic acid (THPA)	2.8	0.220	7.6	0.849	7.7	0.609	0.4	0.022
Unresolved TA, TAA, and/or THPA	0.4	0.030	2.1	0.230	1.1	0.088	--	--
Triazolyl-ethanol-glucoside	1.0	0.081	2.0	0.227	2.1	0.168	--	--
JAU6476-OH-desthio-glucoside isomers	0.9	0.073	1.1	0.119	0.6	0.051	--	--
JAU6476-OH-desthio-malonyl-glucoside isomers	0.7	0.056	2.6	0.290	3.8	0.299	--	--
JAU6476-desthio-malonyl-glucoside	0.6	0.049	--	--	--	--	--	--
JAU6476-desthio-phenyl-cysteine isomers	2.2	0.177	--	--	--	--	--	--
Tentatively identified								
Triazolyl-ethanol	--	--	--	--	1.6	0.124	--	--

TABLE C.2.5. Summary of Characterization and Identification of Radioactive Residues in Wheat Matrices Following Foliar Application of [Triazole-3,5-^{14}C]-Prothioconazole at 0.357 lb a.i./A (420 g a.i./ha).

Compound	Forage		Hay		Straw		Grain	
	TRR = 7.956 ppm		TRR = 11.175		TRR = 7.942 ppm		TRR = 4.973 ppm	
	% of the TRRs	ppm	% of the TRRs	ppm	% of the TRRs	ppm	% of the TRRs	ppm
JAU6476-diOH-desthio-malonyl-glucoside	1.9	0.148	--	--	--	--	--	--
Unresolved glucoside isomers [2]	1.8	0.147	4.6	0.517	--	--	--	--
Characterized								
Unknowns [3]	10.6	0.860	17.1	1.899	22.3	1.762	6.3	0.310
HCl hydrolysates	6.1	0.484	--	--	--	--	0.8	0.041
Total identified	64.7	5.150	68.9	8.120	57.3	4.542	90.5	4.505
Total characterized	16.7	1.344	17.1	1.899	22.3	1.762	7.1	0.351
Total extractable	94.5	7.516	96.4	10.786	96.3	7.648	99.9	4.968
Unextractable (PES) [4]	5.5	0.441	3.5	0.388	3.7	0.294	0.1	0.004
Accountability [5]	100.0		100.0		100.0		100.0	

[1] This compound was not observed in the initial HPLC analyses which were used for quantitation; however, the compound was tentatively identified during metabolite isolation and purification.

[2] Including assorted pairs of the following: JAU6476-OH-desthio-glucoside, JAU6476-OH-sulfonic acid glucoside, JAU6476-desthio-malonyl-glucoside, and JAU6476-OH-desthio-malonyl-glucoside.

[3] Refer to TABLE C.2.2 for relative distribution of unknowns.

[4] Residues remaining after exhaustive extractions.

[5] Accountability = (Total extractable + Total unextractable)/(absolute values of the TRRs (ppm)) * 100. Extraction results were normalized.

C.3. Proposed Metabolic Profile

Based on the results of the wheat metabolism study, the applicant concluded that prothioconazole is initially metabolized in wheat by oxidation and loss of sulfur to form JAU6476-desthio, after which two major metabolic processes occur: (1) hydroxylation of the phenyl ring and or benzylic carbon to form isomers of JAU6476-OH-desthio, JAU6476-diOH desthio, and JAU6476-α-OH-desthio, followed by conjugation to form the corresponding glucosides, malonyl-glucosides and acetate; and (2) release of the triazole moiety to form TA and THPA and further metabolism of the triazole conjugates to form TAA. The applicant noted that the absence of 1,2,4-triazole in any wheat matrix suggested that immediate or very rapid conjugation of released triazole occurred. The following minor metabolic pathways were reported: formation of JAU6476-triazolinone and JAU6476-desthio-phenyl-cysteine; conjugation of JAU6476-desthio with glucose and malonic acid; oxidation of the sulfur atom of prothioconazole to form JAU6746 sulfonic acid; cleavage of the benzylic group to form triazolyl ethanol and its glucoside; and conjugation of the benzylpropyldiol portion of the remaining molecule. The pathway is represented in FIGURE C.3.1.

FIGURE C.3.1. Proposed Metabolic Profile of Prothioconazole in Wheat

This figure was copied without alteration from MRID 46246141.

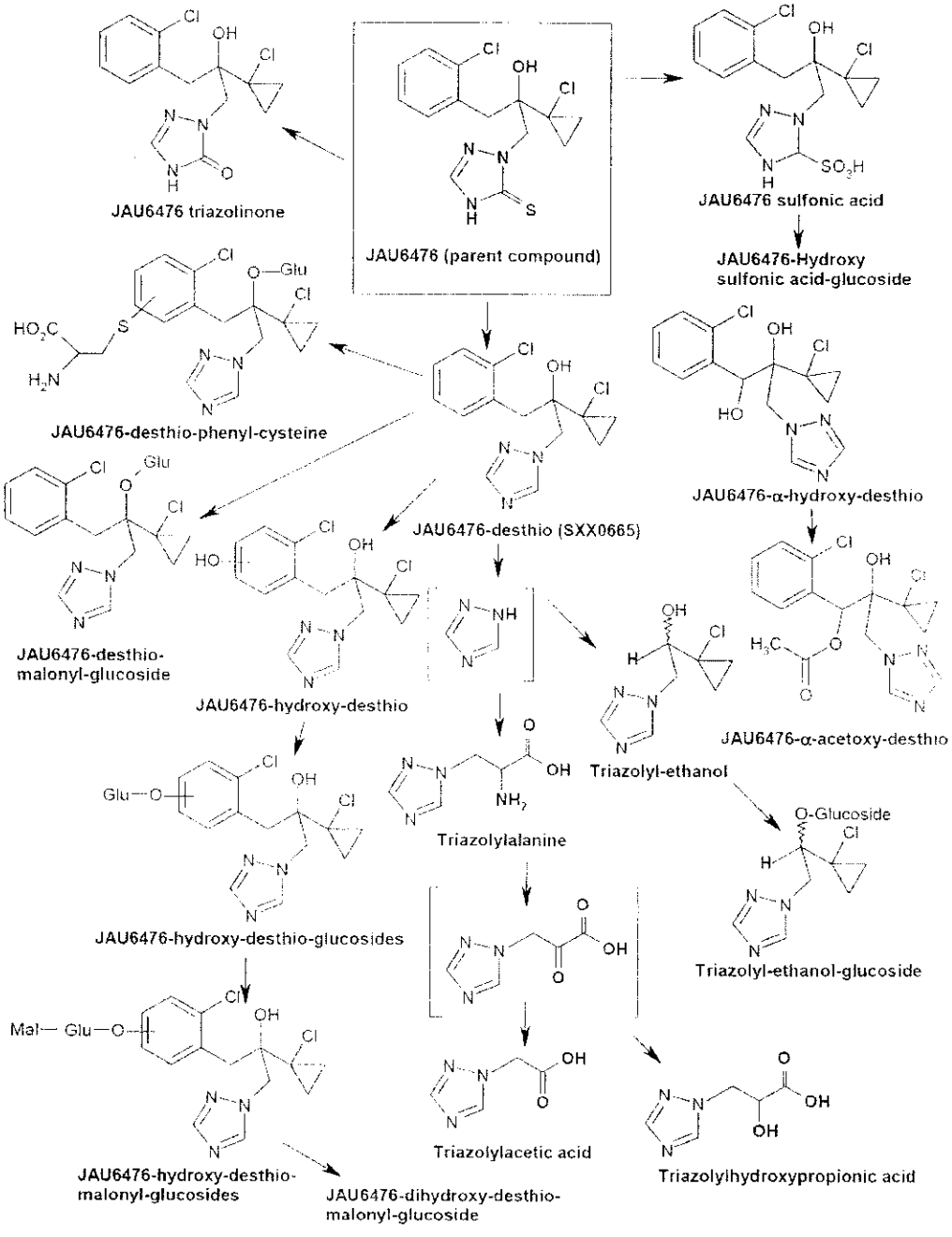


TABLE C.3.1. Identification of Compounds from the Phenyl-Label Metabolism Study.

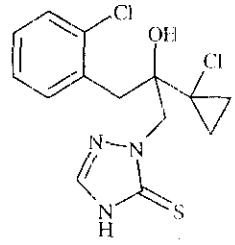
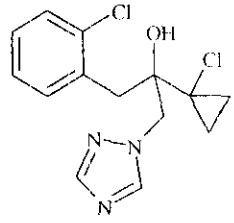
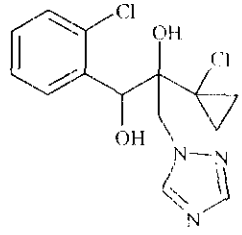
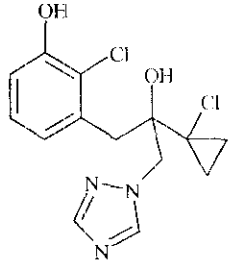
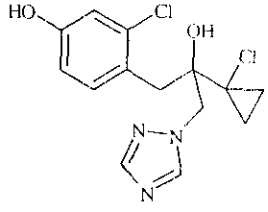
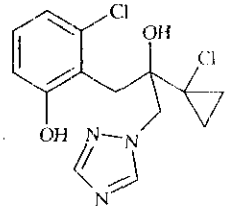
Common name/code Figure C.3.1 ID No.	Chemical name	Chemical structure
Prothioconazole	2-[2-(1-chlorocyclopropyl)-3-(2-*chlorophenyl*)-2-hydroxypropyl]-1,2-dihydro-3*H*-1,2,4-triazole-3-thione	
JAU6476-desthio	α-(1-chlorocyclopropyl)-α-[(2-chlorophenyl)methyl]-1*H*-1,2,4-triazole-1-ethanol	
JAU6476-α-OH-desthio	2-(1-chlorocyclopropyl)-1-(2-chlorophenyl)-3-(1*H*-1,2,4-triazol-1-yl)-1,2-propanediol	
JAU6476-3-OH-desthio	α-(1-chlorocyclopropyl)-α-[(2-chloro-3-hydroxyphenyl)methyl]-1*H*-1,2,4-triazole-1-ethanol	
JAU6476-4-OH-desthio	α-(1-chlorocyclopropyl)-α-[(2-chloro-4-hydroxyphenyl)methyl]-1*H*-1,2,4-triazole-1-ethanol	
JAU6476-6-OH-desthio	α-(1-chlorocyclopropyl)-α-[(2-chloro-6-hydroxyphenyl)methyl]-1*H*-1,2,4-triazole-1-ethanol	

TABLE C.3.1. Identification of Compounds from the Phenyl-Label Metabolism Study.

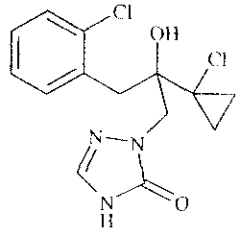
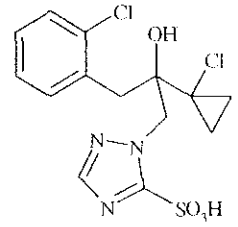
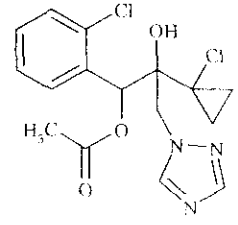
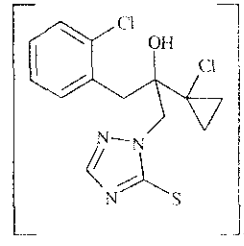
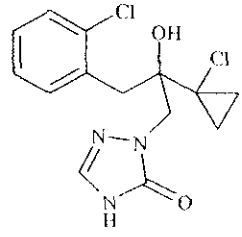
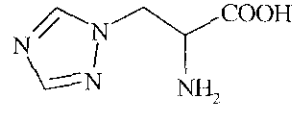
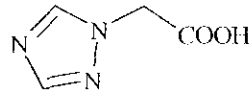
Common name/code Figure C.3.1 ID No.	Chemical name	Chemical structure
JAU6476-triazolinone	2-[2-(1-chlorocyclopropyl)-3-(2-chlorophenyl)-2-hydroxypropyl]-2,4-dihydro-3*H*-1,2,4-triazole-3-one	
JAU6476 sulfonic acid	1-[2-(1-chlorocyclopropyl)-3-(2-chlorophenyl)-2-hydroxypropyl]-1*H*-1,2,4-triazole-5-sulfonic acid	
JAU6476-α-acetoxy-desthio	2-(1-chlorocyclopropyl)-1-(2-chlorophenyl)-2-hydroxy-3-(1*H*-1,2,4-triazol-1-yl)propyl acetate	
JAU6476-disulfide		
JAU6476-triazolinone	2-[2-(1-chlorocyclopropyl)-3-(2-chlorophenyl)-2-hydroxypropyl]-2,4-dihydro-3*H*-1,2,4-triazole-3-one	
Triazolylalanine (TA)	α-amino-1*H*-1,2,4-triazole-1-propanoic acid	
Triazolylacetic acid (TAA)	1*H*-1,2,4-triazole-1-acetic acid	

TABLE C.3.1. Identification of Compounds from the Phenyl-Label Metabolism Study.

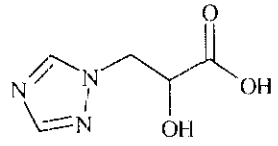
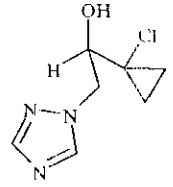
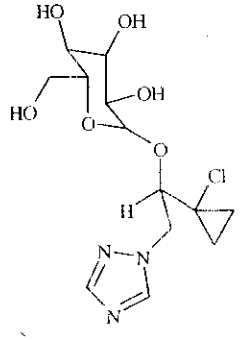
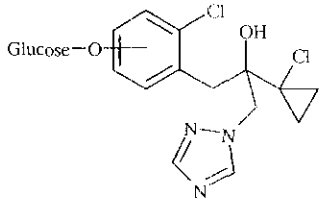
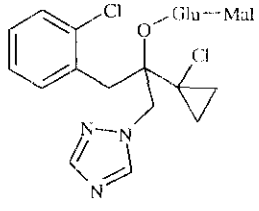
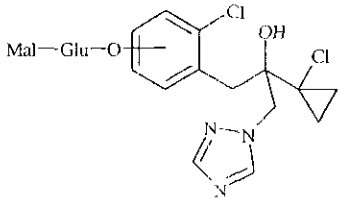
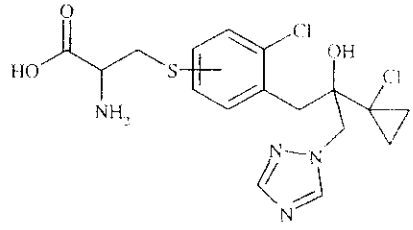
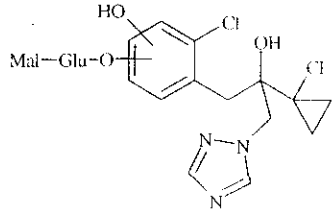
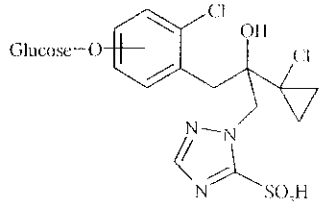
Common name/code *Figure C.3.1 ID No.*	Chemical name	Chemical structure
Triazolylhydroxypropionic acid (THPA)	α-hydroxy-1*H*-1,2,4-triazole-1-propanoic acid	
Triazolyl-ethanol	1-(1-chlorocyclopropyl)-2-(1*H*-1,2,4-triazol-1-yl)ethanol	
Triazolyl-ethanol-glucoside		
JAU6476-OH-desthio-glucoside		
JAU6476-desthio-malonyl-glucoside		
JAU6476-OH-desthio-malonyl-glucoside		

TABLE C.3.1. Identification of Compounds from the Phenyl-Label Metabolism Study.		
Common name/code Figure C.3.1 ID No.	Chemical name	Chemical structure
JAU6476-desthio-phenyl-cysteine		
JAU6476-dihydroxy-desthio-malonyl-glucoside		
JAU6476-OH-sulfonic acid-glucoside		

D. CONCLUSION

Total radioactive residues (TRRs) in wheat matrices harvested following two foliar applications of [phenyl-UL-^{14}C]-prothioconazole or [triazole-3,5-^{14}C]-prothioconazole at seasonal application rates of 0.371-0.420 lb a.i./A (415-470 g a.i./ha) ranged from 7.956-10.45 ppm in forage, 8.90-11.175 ppm in hay, 7.942-26.74 ppm in straw and 0.08-4.973 ppm in grain.

Prothioconazole was identified at low levels (<7% of the TRRs) in all wheat matrices except in the triazole-label study grain, in which neither prothioconazole nor any structurally related metabolites unique to prothioconazole were identified. Metabolite JAU6476-desthio, accounting for ~9-35% of the TRRs, was the major identified residue in all wheat matrices except triazole-label study hay and grain, in which trialolylalanine (TA) was the major identified residue at 25% of the TRRs and 71% of the TRRs, respectively. TA was also identified in triazole-label study forage at 12% of the TRRs. In triazole-label study grain, triazolylacetic acid (TAA) accounted for 19% of the TRRs, and triazolylhydroxypropionic acid (THPA) constituted the remaining identified residue at 0.4% of the TRRs.

The first step in the extensive metabolism of JAU6476 was the desulfuration to JAU6476-desthio. This process was followed by the hydroxylation of the phenyl ring and /or benzylic carbon to form multiple isomers of JAU6476-OH-desthio, JAU6476-diOH-desthio, and JAU6476-α-OH-desthio, followed by conjugation to form the corresponding glucosides or malonyl-glucosides. Another major pathway involved the cleavage of the H_2C-N-bond to release

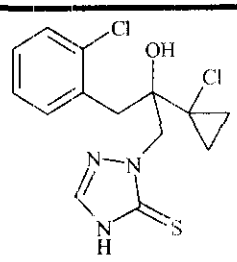
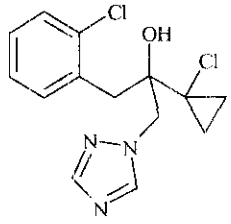
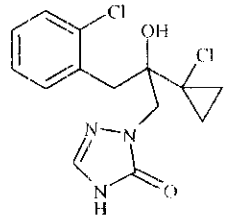
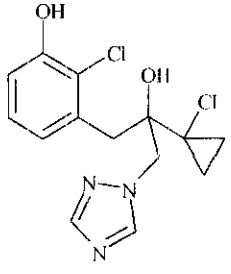
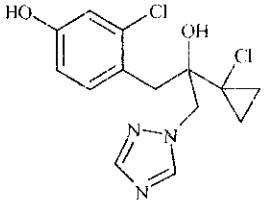
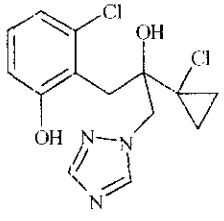
the triazole moiety (and benzylpropyldiol) leading to the formation of TA and THPA and further metabolism of the triazole conjugates to TAA. Minor metabolic processes involved the successive reductions of the phenyl ring to form dienes, olefins, and hydroxy-dienyl-cysteine; formation of JAU6476-triazolinone; oxidation of the sulfur atom on JAU6476 to form JAU6476 sulfonic acid; and cleavage of the chlorobenzylic group to form triazolyl-ethanol and its glucoside.

E. REFERENCES

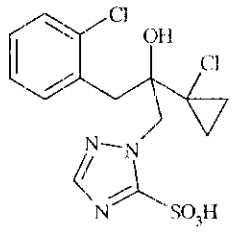
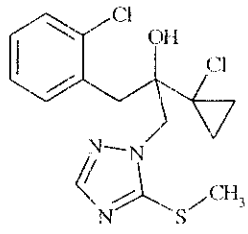
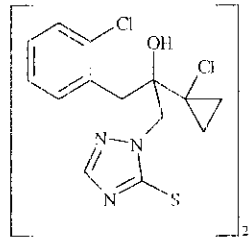
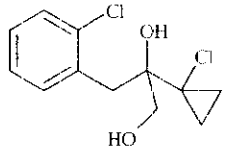
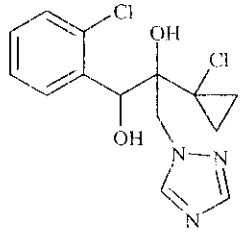
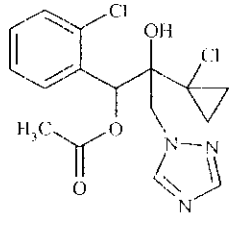
None.

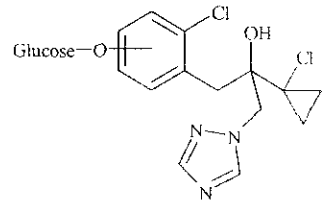
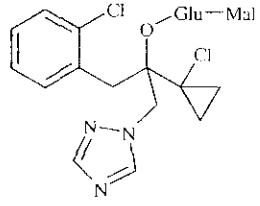
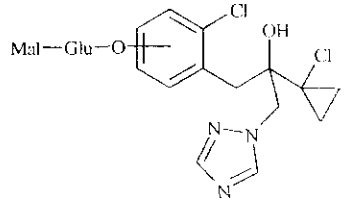
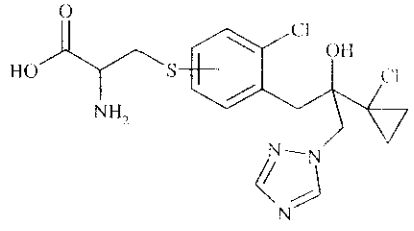
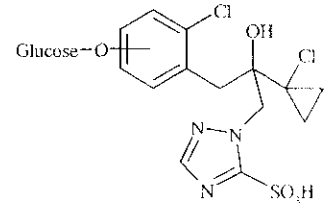
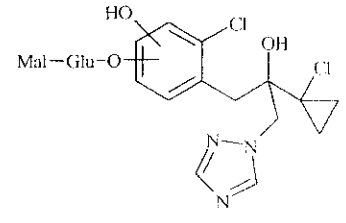
F. DOCUMENT TRACKING

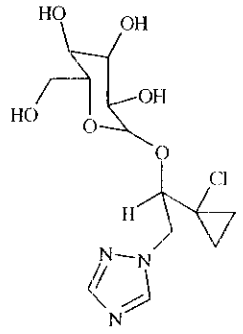
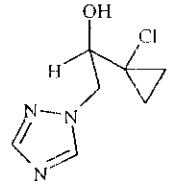
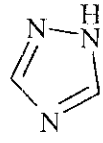
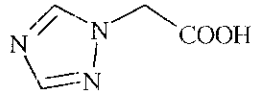
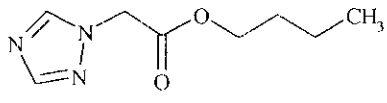
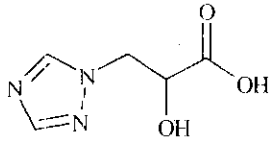
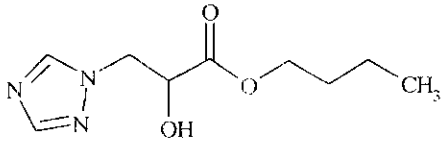
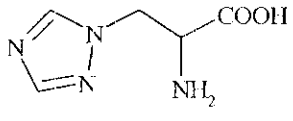
RDI: Louise Croteau, Suzan Mathew (PMRA) 23/01/2006; Henri Bietlot (PMRA) 24/01/2006; Stephen Funk (IO)13/03/2006; Leung Cheng (RAB3)
Petition Number: PP#4F6830
DP Barcode: D303508
PC Code: 113961

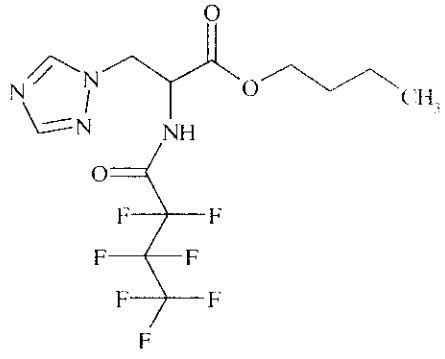
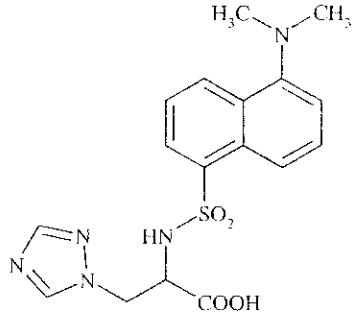
APPENDIX I.	**Chemical Names and Structures of Reference Standards Used in the Foliar Wheat Metabolism Study.**	
Common name; Company code	Chemical name	Chemical structure
Prothioconazole JAU6476	2-[2-(1-chlorocyclopropyl)-3-(2-chlorophenyl)-2-hydroxypropyl]-1,2-dihydro-3*H*-1,2,4-triazole-3-thione	
JAU6476-desthio (a phenyl-labelled standard also used)	α-(1-chlorocyclopropyl)-α-[(2-chlorophenyl)methyl]-1*H*-1,2,4-triazole-1-ethanol	
JAU6746 triazolinone	2-[2-(1-chlorocyclopropyl)-3-(2-chlorophenyl)-2-hydroxypropyl]-2,4-dihydro-3*H*-1,2,4-triazole-3-one	
JAU6476-3-hydroxy-desthio	α-(1-chlorocyclopropyl)-α-[(2-chloro-3-hydroxyphenyl)methyl]-1*H*-1,2,4-triazole-1-ethanol	
JAU6476-4-hydroxy-desthio	α-(1-chlorocyclopropyl)-α-[(2-chloro-4-hydroxyphenyl)methyl]-1*H*-1,2,4-triazole-1-ethanol	
JAU6476-6-hydroxy-desthio	α-(1-chlorocyclopropyl)-α-[(2-chloro-6-hydroxyphenyl)methyl]-1*H*-1,2,4-triazole-1-ethanol	

APPENDIX I. Chemical Names and Structures of Reference Standards Used in the Foliar Wheat Metabolism Study.

Common name; Company code	Chemical name	Chemical structure
JAU6476-sulfonic acid	1-[2-(1-chlorocyclopropyl)-3-(2-chlorophenyl)-2-hydroxypropyl]-1*H*-1,2,4-triazole-5-sulfonic acid	
JAU6476-S-methyl	α-(1-chlorocyclopropyl)-α-[(2-chlorophenyl)methyl]-3-(methylthio)-1*H*-1,2,4-triazole-1-ethanol	
JAU6476-disulfide; dimer of JAU6476		
Benzylpropyldiol	2-(1-chlorocyclopropyl)-3-(2-chlorophenyl)propane-1,2-diol	
JAU6476-α-OH-desthio	2-(1-chlorocyclopropyl)-1-(2-chlorophenyl)-3-(*1H*-1,2,4-triazol-1-yl)-1,2-propanediol	
JAU6476-α-acetoxy-desthio	2-(1-chlorocyclopropyl)-1-(2-chlorophenyl)-2-hydroxy-3-(1*H*-1,2,4-triazol-1-yl)propyl acetate	

APPENDIX I.	**Chemical Names and Structures of Reference Standards Used in the Foliar Wheat Metabolism Study.**	
Common name; Company code	Chemical name	Chemical structure
JAU6476-OH-desthio-glucoside		
JAU6476-desthio-malonyl-glucoside		
JAU6476-OH-desthio-malonyl-glucoside		
JAU6476-desthio-phenyl-cysteine		
JAU6476-OH-sulfonic acid-glucoside		
JAU6476-dihydroxy-desthio-malonyl-glucoside		

APPENDIX I.	**Chemical Names and Structures of Reference Standards Used in the Foliar Wheat Metabolism Study.**	
Common name; Company code	Chemical name	Chemical structure
Triazolyl-ethanol-glucoside		
Triazolyl-ethanol	1-(1-chlorocyclopropyl)-2-(1*H*-1,2,4-triazol-1-yl)ethanol	
1H-1,2,4-Triazole; free triazole		
Triazolylacetic acid (TAA)	1*H*-1,2,4-triazole-1-acetic acid	
Triazolylacetic acid n-butyl ester (TAA n-butyl ester)	butyl 1*H*-1,2,4-triazole-1-acetate	
Triazolylhydroxypropionic acid (THPA)	α-hydroxy-1*H*-1,2,4-triazole-1-propanoic acid	
Triazolylhydroxypropionic acid n-butyl ester (THPA n-butyl ester)	butyl α-hydroxy-1*H*-1,2,4-triazole-1-propanoate	
Triazolylalanine (TA)	α-amino-1*H*-1,2,4-triazole-1-propanoic acid	

APPENDIX I.	**Chemical Names and Structures of Reference Standards Used in the Foliar Wheat Metabolism Study.**	
Common name; Company code	Chemical name	Chemical structure
Triazolylalanine butyl ester heptafluorobutyric anhydride (TA BEHFBA)	butyl α-[(2,2,3,4,4,4,4-heptafluoro-1-oxobutyl)amino]-1*H*-1,2,4-triazole-1-propanoate	
Dansyl-1,2,4-triazole (dansyl TA)		

Primary Evaluators

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Immediate Office

Date: Mar 13 2006

Louise G Croteau

Louise G Croteau, Senior Evaluation Officer
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Date: 23/01/06

Suzan Mathew

Suzan Mathew, Evaluation Officer
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Date: January 23/06

Approved by

L Cheng

Leung Cheng, Ph. D., Team Leader
HED/RAB3

Date:

Henri P Bietlot, Acting Section Head
FREAS, HED

Date: JAN 24/06

In the absence of signatures, this document is considered to be a draft with deliberative material for internal use only.

This DER was originally prepared under contract by Dynamac Corporation (20440 Century Boulevard, Suite 100; Germantown, MD 20874; submitted 05/20/2005). The DER has been reviewed by the Health Effects Division (HED) and Health Canada's Pest Management Regulatory Agency (PMRA). The DER has been revised to reflect current Office of Pesticide Programs (OPP) policies and Directive 98-02.

STUDY REPORT:

46246220 Lenz, C. (2004) JAU6476 480 SC - Magnitude of the Residue In/On Barley. Project Number: J619BA01, 200806, RCJAY005. Unpublished study prepared by Bayer Corp., Battelle and Agvise Inc. 797 p.

EXECUTIVE SUMMARY:

Bayer CropScience has submitted field trial data on barley from field trials conducted in the U.S. and Canada. A total of 25 five trials were conducted in Regions 1 (PA; 1 trial), 5 (ND; 2 trials, ON; 1 trial), 5B (QC; 1 trial), 7 (ND; 3 trials, and SK; 1 trial), 9 (AZ; 1 trial), 10 (AZ; 1 trial), 11 (ID and OR; 2 trials) and 14 (AB; 4 trials, MB; 4 trials, and SK; 4 trials) during the 2000-2001

growing season. The number and locations of field trials are in accordance with OPPTS Guideline 860.1500 and Directive 98-02; Section 9.

At each test location, two broadcast foliar applications of the 4 lb/gal (480 g/L) suspension concentrate formulation (flowable concentrate; FlC) were made to barley at ~0.11-0.18 lb a.i./A (~0.123-0.202 kg a.i./ha) at an average 12-day retreatment interval, for a total seasonal application rate of ~0.29 lb a.i./A (~0.33 kg a.i./ha). Applications were made in ~5-43 gal/A (~45-407 L/ha) of water using ground equipment. An adjuvant was not added to the spray mixture for any applications. Barley hay was cut at 23 test sites, 12-16 days after treatment, and was left in the field for 1-14 days prior to collection of barley hay. Samples of barley grain and straw were harvested at 23 test sites 30-71 days after the last application. At two locations, additional samples were collected to determine residue decline. In the decline trial performed in Region 7 (Northwood, ND), samples were harvested 8, 13, 22, and 28 days after treatment for barley hay and 32, 37, 44, and 47 days after treatment for barley grain and straw. In the decline trial performed in Region 5 (Branchton, ON), samples were harvested 9, 14, 21, and 29 days after treatment for barley hay and 36, 39, 45, and 49 days after treatment for barley grain and straw.

Samples were analyzed for total prothioconazole-derived residues (prothioconazole and the metabolite prothioconazole-desthio) using LC-MS/MS method RPA JA/03/01. The validated LOQs for the total prothioconazole-derived residues were 0.02 ppm for barley grain and 0.05 ppm for barley hay and straw. The method is adequate for data collection for barley grain, hay and straw based on acceptable concurrent method recovery data and method validation data. Samples were analyzed for residues of 1*H*-1,2,4-triazole and the triazole conjugates triazolylalanine and triazolylacetic acid using an LC-MS/MS method (Bayer Report No. G200598). The validated LOQ for 1*H*-1,2,4-triazole was 0.01 ppm for barley grain, hay and straw, and the validated LOQs for the triazole conjugates were 0.10 ppm for barley grain and straw, and 0.05 ppm for hay. The methods are adequate for data collection for barley matrices based on acceptable concurrent method recovery data.

In barley matrices harvested 30-71 days (12-16 days for hay), total prothioconazole-derived residues were 0.158 ppm, 6.59 ppm and 1.87 ppm, respectively, in/on barley grain, hay, and straw. Residues of 1*H*-1,2,4-triazole were less than the LOQ (<0.01 ppm) in/on barley grain, hay, and straw; and 0.915 ppm, 0.547 ppm and 0.385 ppm, respectively, in/on barley grain, hay, and straw for the triazole conjugates. Total prothioconazole-derived residues did not increase with increasing sampling intervals in barley grain, hay, and straw, and residues of the triazole conjugates did not increase in grain, but increased slightly with increasing sampling intervals in samples from one trial each for hay and straw.

The maximum storage intervals of crop samples from harvest to analysis for total prothioconazole-derived residues were 1234 days (40.6 months) for barley grain and 1269 days (41.7 months) for barley hay and straw. The degree of loss of prothioconazole-derived residues and prothioconazole-desthio residues is not expected to exceed 30% after 41.7 months in barley grain, hay and straw.

STUDY/WAIVER ACCEPTABILITY/DEFICIENCIES/CLARIFICATIONS:

Under the conditions and parameters used in the study, the field trial residue data are tentatively classified as scientifically acceptable. The final reports of the ongoing storage stability studies with prothioconazole, prothioconazole-desthio, 1*H*-1,2,4-triazole, triazolylalanine, and triazolylacetic acid will be submitted as confirmatory data.

The acceptability of this study for regulatory purposes is addressed in the forthcoming U.S. EPA Residue Chemistry Summary Document, DP Barcode D303508, and in Canada's Regulatory Decision Document.

COMPLIANCE:

Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided. No deviations from regulatory requirements were reported which would have an impact on the validity of the study.

A. BACKGROUND INFORMATION

Prothioconazole is a broad-spectrum systemic fungicide intended for the control of many diseases in different crops such as cereals, oilseed rape or peanuts. The biochemical mode of action is the inhibition of the demethylation of lanosterol or 24-methylene-dihydrolanosterol, which are precursors of sterols in fungi. The chemical has been submitted for joint EPA and PMRA review by Bayer CropScience. The crops proposed for joint review include barley, the dried shell and bean subgroup, the oilseed crop group, and wheat. Uses on peanuts and rice are being proposed for the U.S. only.

TABLE A.1. Test Compound Nomenclature.

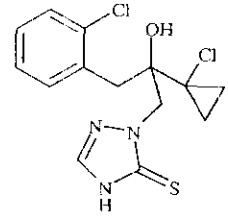
Chemical structure	
Common name	Prothioconazole (ISO accepted)
Company experimental name	JAU6476
IUPAC name	2-[(2*RS*)-2-(1-chlorocyclopropyl)-3-(2-chlorophenyl)-2-hydroxypropyl]-2*H*-1,2,4-triazole-3(4*H*)-thione
CAS name	2-[2-(1-chlorocyclopropyl)-3-(2-chlorophenyl)-2-hydroxypropyl]-1,2-dihydro-3*H*-1,2,4-triazole-3-thione
CAS registry number	178928-70-6
PC Code	113961
End-use product (EP)	Proline® 480 SC (4 lb/gal suspension concentrate), EPA File Symbol 264-IEL

TABLE A.2. Physicochemical Properties of the Technical Grade Test Compound.

Parameter	Value	References
Melting range	139.1 to 144.5°C	MRID 46246003/CES
pH	5.8 (1% solution)	MRID 46246003/CES
Density at 20°C	1.36 g/mL	MRID 46246003/CES
Water solubility at 20°C	pH: mg/L 4: 5 8: 300 9: 2000	MRID 46246003/CES
Solvent solubility at 20°C	Solvent: g/L Acetone: >250 Acetonitrile: 69 Dichloromethane: 88 Dimethysulfoxide: 126 Ethyl acetate: >250 n-Heptane: <0.1 1-Octanol: 58 Polyethylene glycol: >250 2-Propanol: 87 Xylene: 8	MRID 46246003/CES
Vapor pressure at 20 or 25°C	$<4 \times 10^{-7}$ Pa (calculated from determinations at 70°C)	MRID 46246003/CES
Dissociation constant, pK_a	6.9 (calculated from K_{OW})	MRID 46246003/CES
Octanol/water partition coefficient, at 20°C	pH: $Log(K_{OW})$ unbuffered water: 4.05 4: 4.16 7: 3.82 9: 2.00	MRID 46246003/CES
UV/visible absorption spectrum	Peak maxima at 257 nm. No absorption at > 300 nm.	MRID 46246003/CES

CES - Chemistry Evaluation Section of PMRA

B. EXPERIMENTAL DESIGN

B.1. Study Site Information

TABLE B.1.1. Trial Site Conditions The table below was copied from the data report (MRID 46246220) without alteration.

Study location (city, state)	Trial Number	Year	Soil Characteristics[a]				Meterological Data[b]	
			Type	% OM*	pH*	CEC*	Total Rainfall (in)	Temp. range (°F)
Northwood, North Dakota	J6001-00D	2000	NA	NA	NA	NA	4.69	48-92
Hermiston, Oregon	J6002-00H	2000	NA	NA	NA	NA	17.19[c]	45-102
Maricopa, Arizona	J6002-00H	2000	NA	NA	NA	NA	1.07	41-104
Wilcox, Arizona	J6004-00H	2000	NA	NA	NA	NA	1.86	28-103
Velva, North Dakota	J6005-00H	2000	NA	NA	NA	NA	4.20	40-88
New Rockford, N. Dakota	J6006-00H	2000	NA	NA	NA	NA	3.98	45-96
Ellendale, North Dakota	J6007-00H	2000	NA	NA	NA	NA	7.38	48-95
Branchton, Ontario	J6008-00D	2000	NA	NA	NA	NA	7.97	42-83
Jerome, Idaho	J6009-00H	2000	NA	NA	NA	NA	16.4[c]	37-103
Northwood, North Dakota	J6010-00H	2000	NA	NA	NA	NA	7.48	48-92
Germansville, Pennsylvania	J6013-00H	2000	NA	NA	NA	NA	11.65	45-92
Minto, Manitoba	J6078-00H	2000	NA	NA	NA	NA	2.94	39-91
Minto, Manitoba	J6079-00H	2000	NA	NA	NA	NA	2.97	39-91
Brookdale, Manitoba	J6080-00H	2000	NA	NA	NA	NA	2.67	41-92
Clanwilliam, Manitoba	J6081-00H	2000	NA	NA	NA	NA	4.99	32-87
Marcelin, Saskatchewan	J6082-00H	2000	NA	NA	NA	NA	5.11	34-91
Rosthern, Saskatchewan	J6083-00H	2000	NA	NA	NA	NA	3.47	35-91
Wakaw, Saskatchewan	J6084-00H	2000	NA	NA	NA	NA	1.91	36-87
Lacombe, Alberta	J6085-00HA	2000	NA	NA	NA	NA	6.10	30-89
Penhold, Alberta	J6086-00HA	2000	NA	NA	NA	NA	7.24	28-91
Rosthern, Saskatchewan	J6087-00H	2000	NA	NA	NA	NA	2.73	-9-97
Kipp, Alberta	J6088-00H	2000	NA	NA	NA	NA	4.68[c]	37-99
Leduc, Alberta	J6089-00H	2000	NA	NA	NA	NA	2.77	37-84
Delisle, Saskatchewan	J6090-00H	2000	NA	NA	NA	NA	4.38	42-97
St-Paul-d'Abbotsford, Quebec	J6091-00H	2000	NA	NA	NA	NA	5.45	DNF[d]

* These parameters are optional except in cases where their value affects the use pattern for this chemical.

[a] NA = Not applicable since these parameters do not affect the use pattern of the chemical.
[b] The data is for the interval from the first application to the last sampling unless noted otherwise.
[c] Overhead irrigation values added to rainfall value.
[d] DNF = Data not found.

The actual temperature and rainfall recordings were within average historical values for the residue study period with the exception of trial J6091-00H (QC) for which no temperature range

was reported. The applicant indicated that irrigation was used to supplement as needed at three trial sites.

TABLE B.1.2. Study Use Pattern.

Location (City, State; Year)	EP[1]	Application				
		Method; Timing	Vol. (GPA[2]) [L/ha]	Rate (lb a.i./A) [g a.i./ha]	RTI[3] (days)	Total Rate (lb a.i./A) [g a.i./ha]
Northwood, ND; 2000 J6001-00D	480 SC	1: Broadcast foliar; Flag leaf sheath opening	30 [281]	0.117 [0.132]	---	0.294 [0.330]
		2: Broadcast foliar; End of flowering	30 [283]	0.177 [0.198]	11	
Hermiston, OR; 2000 J6002-00H	480 SC	1: Broadcast foliar; Flag leaf just visible, still rolled	29 [270]	0.111 [0.124]	---	0.295 [0.331]
		2: Broadcast foliar; End of flowering	32 [295]	0.184 [0.207]	14	
Maricopa, AZ; 2000 J6003-00H	480 SC	1: Broadcast foliar; Late boot stage: flag leaf sheath swollen	30 [285]	0.117 [0.131]	---	0.301 [0.337]
		2: Broadcast foliar; Full flowering: 50% of anthers mature	30 [282]	0.184 [0.206]	19	
Wilcox, AZ; 2000 J6004-00H	480 SC	1: Broadcast foliar; Flag leaf sheath opening	30 [278]	0.112 [0.126]	---	0.286 [0.321]
		2: Broadcast foliar; Full flowering: 50% of anthers mature	29 [270]	0.174 [0.195]	8	
Velva, ND; 2000 J6005-00H	480 SC	1: Broadcast foliar; Late boot stage: flag leaf sheath swollen	30 [281]	0.114 [0.127]	---	0.295 [0.330]
		2: Broadcast foliar; Full flowering: 50% of anthers mature	30 [281]	0.181 [0.203]	13	
New Rockford, ND; 2000 J6006-00H	480 SC	1: Broadcast foliar; Flag leaf sheath opening	30 [282]	0.112 [0.125]	---	0.301 [0.337]
		2: Broadcast foliar; End of flowering	30 [283]	0.189 [0.212]	14	
Ellendale, ND; 2000 J6007-00H	480 SC	1: Broadcast foliar; Flag leaf sheath opening	20 [188]	0.114 [0.127]	---	0.294 [0.329]
		2: Broadcast foliar; Full flowering: 50% of anthers mature	20 [188]	0.180 [0.202]	5	
Branchton, ON; 2000 J6008-00D	480 SC	1: Broadcast foliar; Late boot stage: flag leaf sheath swollen	22 [206]	0.114 [0.128]	---	0.294 [0.330]
		2: Broadcast foliar; End of flowering	22 [208]	0.180 [0.202]	17	
Jerome, ID; 2000 J6009-00H	480 SC	1: Broadcast foliar; Late boot stage: flag leaf sheath swollen	30 [278]	0.112 [0.126]	---	0.294 [0.330]
		2: Broadcast foliar; First grains have reached half their final size	30 [281]	0.182 [0.204]	27	

TABLE B.1.2. Study Use Pattern.

Location (City, State; Year)	EP[1]	Application				
		Method; Timing	Vol. (GPA[2]) [L/ha]	Rate (lb a.i./A) [g a.i./ha]	RTI[3] (days)	Total Rate (lb a.i./A) [g a.i./ha]
Northwood, ND 2000 J6010-00H	480 SC	1: Broadcast foliar; Flag leaf sheath opening	30 [279]	0.112 [0.126]	---	0.291 [0.327]
		2: Broadcast foliar; End of flowering	30 [281]	0.179 [0.201]	11	
Germansville, PA; 2000 J6013-00H	480 SC	1: Broadcast foliar; Late boot stage: flag leaf sheath swollen	37 [342]	0.117 [0.131]	--	0.293 [0.328]
		2: Broadcast foliar; Full flowering: 50% of anthers mature	32 [302]	0.176 [0.107]	14	
Minto, MB; 2000 J6078-00H	480 SC	1: Broadcast foliar; First awns visible (in awned forms only)	42 [395]	0.112 [0.126]	---	0.296 [0.332]
		2: Broadcast foliar; End of flowering	43 [407]	0.184 [0.206]	8	
Minto, MB; 2000 J6079-00H	480 SC	1: Broadcast foliar; First awns visible (in awned forms only)	43 [399]	0.114 [0.128]	---	0.287 [0.322]
		2: Broadcast foliar; End of flowering	41 [382]	0.173 [0.194]	8	
Brookdale, MB; 2000 J6080-00H	480 SC	1: Broadcast foliar; Flag leaf sheath opening	12 [114]	0.117 [0.131]	---	0.297 [0.333]
		2: Broadcast foliar; Full flowering: 50% of anthers mature	12 [110]	0.180 [0.202]	6	
Clanwilliam, MB; 2000 J6081-00H	480 SC	1: Broadcast foliar; Late boot stage: flag leaf sheath swollen	12 [110]	0.113 [0.127]	---	0.295 [0.331]
		2: Broadcast foliar; Full flowering: 50% of anthers mature	12 [112]	0.182 [0.204]	9	
Marcelin, SK; 2000 J6082-00H	480 SC	1: Broadcast foliar; Mild boot stage: flag leaf sheath just vis. swollen	42 [393]	0.111 [0.124]	---	0.291 [0.325]
		2: Broadcast foliar; Full flowering: 50% of anthers mature	42 [395]	0.179 [0.201]	13	
Rosthern, SK; 2000 J6083-00H	480 SC	1: Broadcast foliar; Flag leaf sheath opening	43 [403]	0.113 [0.127]	---	0.292 [0.328]
		2: Broadcast foliar; Full flowering: 50% of anthers mature	43 [401]	0.179 [0.201]	7	
Wakaw, SK; 2000 J6084-00H	480 SC	1: Broadcast foliar; Flag leaf sheath opening	12 [109]	0.113 [0.127]	---	0.291 [0.327]
		2: Broadcast foliar; Full flowering: 50% of anthers mature	12 [109]	0.178 [0.200]	11	
Lancombe, AB; 2000 J6085-00HA	480 SC	1: Broadcast foliar; Late boot stage: flag leaf sheath swollen	11 [100]	0.113 [0.127]	---	0.293 [0.329]
		2: Broadcast foliar; Full flowering: 50% of anthers mature	11 [100]	0.180 [0.202]	13	

TABLE B.1.2. Study Use Pattern.

Location (City, State; Year)	EP[1]	Application				
		Method: Timing	Vol. (GPA[2]) [L/ha]	Rate (lb a.i./A) [g a.i./ha]	RTI[3] (days)	Total Rate (lb a.i./A) [g a.i./ha]
Penhold, AB; 2000 J6086-00HA	480 SC	1: Broadcast foliar; Late boot stage: flag leaf sheath swollen	11 [100]	0.112 [0.126]	---	0.294 [0.330]
		2: Broadcast foliar; Full flowering: 50% of anthers mature	11 [101]	0.182 [0.204]	13	
Rosthern, SK; 2000 J6087-00H	480 SC	1: Broadcast foliar; Flag leaf sheath opening	12 [108]	0.111 [0.124]	---	0.294 [0.330]
		2: Broadcast foliar; End of heading	12 [112]	0.183 [0.205]	11	
Kipp, AB; 2000 J6088-00H	480 SC	1: Broadcast foliar; Late boot stage: flag leaf sheath swollen	21 [201]	0.113 [0.127]	---	0.299 [0.336]
		2: Broadcast foliar; Full flowering: 50% of anthers mature	22 [206]	0.186 [0.209]	7	
Leduc, AB; 2000 J6089-00H	480 SC	1: Broadcast foliar; Flag leaf sheath opening	12 [114]	0.115 [0.129]	---	0.301 [0.338]
		2: Broadcast foliar; End of heading	12 [114]	0.186 [0.209]	8	
Delisle, SK; 2000 J6090-00H	480 SC	1: Broadcast foliar; First awns visible (in awned forms only)	42 [397]	0.113 [0.127]	---	0.292 [0.328]
		2: Broadcast foliar; Full flowering: 50% of anthers mature	42 [393]	0.179 [0.201]	12	
St-Paul-d'Abbotsford, QC; 2000 J6091-00H	480 SC	1: Broadcast foliar; Flag leaf sheath opening	5 [50]	0.123 [0.139]	---	0.309 [0.348]
		2: Broadcast foliar; End of flowering	5 [45]	0.186 [0.209]	17	

[1] EP = End-use Product; 480 SC = 4 lb/gal (480 g/L) suspension concentrate (flowable concentrate; FIC) formulation
[2] GPA = Gallons per acre
[3] RTI = Retreatment Interval
No tank mix adjuvant was used.

TABLE B.1.3 Trial Numbers and Geographical Locations.

NAFTA Growing Region	Barley		
	Submitted	Requested	
		Canada	US
1	1		1
1A			
2			
3			
4			
5	3	1	2
5A			
5B	1	1	
6			
7	4	1	3
7A			
8			
9	1		1
10	1		1
11	2		2
12			
13			
14	12	12	
15			
16			
17			
18			
19			
20			
21			
Total	25	15	10

B.2. Sample Handling and Preparation

Barley hay was cut from each site 12-16 days (average = 14 days) after the last application and was left in the field to dry. Samples of barley hay were collected 1-14 days after the hay was cut. Samples of barley grain and straw were collected from each site 30-71 days (average = 45 days) after the last application. Samples were frozen within 4 hours of collection and were shipped frozen to Battelle-AgriFood Laboratories (Columbus, OH) for homogenization. At Battelle, the samples were homogenized with dry ice and stored frozen until shipment to Bayer Research Park (Stilwell, KS) for analysis.

B.3. Analytical Methodology

Samples of barley grain, hay, and straw were analyzed for total prothioconazole-derived residues (prothioconazole and the metabolite prothioconazole-desthio) using LC-MS/MS method RPA JA/03/01 with modifications. Samples were extracted with a mixture of methanol, 30% hydrogen peroxide, and 0.05M 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 was spiked 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. Residue levels were quantitated from a calibration curve of mixed reference standards of prothioconazole, as the sulfonic acid, and prothioconazole-desthio. The method was modified to use a different solvent for preparation of the spiking solutions and to use slightly different m/z values for the quantitation ions. The validated LOQs for total prothioconazole-derived residues were 0.02 ppm for barley grain and 0.05 ppm for barley hay and straw.

Samples were analyzed for residues of 1*H*-1,2,4-triazole and the triazole conjugates (triazolylalanine and triazolylacetic acid) using an LC-MS/MS method (Bayer Report No. G200598) with modifications. The applicant referred to the method as a "modified" method, but did not identify any modifications that had been made to the method. Briefly, crop matrices were extracted with aqueous methanol, and three separate aliquots of the extract were removed for determination of each of the three analytes. Isotopically labeled internal standard was added to each aliquot. For 1*H*-1,2,4-triazole, the aliquot was mixed with dansyl chloride to form the dansyl derivative of 1*H*-1,2,4-triazole, which was partitioned into ethyl acetate and then redissolved in acetonitrile (ACN)/water for LC-MS/MS analysis. For triazolylalanine, the aliquot was cleaned up by SPE, derivatized to the butyl ester using butanolic HCl, and then further derivatized using heptafluorobutyric anhydride (HFBA). The mixture was redissolved in ACN/water for LC-MS/MS analysis. For determination of triazolylacetic acid, the aliquot was cleaned up by SPE, derivatized to the butyl ester using butanolic HCl, and then redissolved in ACN/water for LC-MS/MS analysis. Residue levels were quantitated from a calibration curve of reference standards of dansyl-1,2,4-triazole, triazolylalanine butyl ester HFBA, and triazolylacetic acid butyl ester (generated by derivatizing reference standard solutions of triazolylacetic acid). The validated LOQ for 1*H*-1,2,4-triazole was 0.01 ppm for barley grain, hay, and straw, and the validated LOQs for the triazole conjugates were 0.10 ppm for barley grain and straw, and 0.05 ppm for hay.

C. RESULTS AND DISCUSSION

Concurrent method recovery data are presented in TABLE C.1. Samples were analyzed for total prothioconazole-derived residues (prothioconazole and the metabolite prothioconazole-desthio) using LC-MS/MS method RPA JA/03/01. The validated LOQs for the total prothioconazole-derived residues were 0.02 ppm for barley grain and 0.05 ppm for barley hay and straw. The method is adequate for data collection for barley grain, hay and straw based on acceptable

concurrent method recovery data and method validation data submitted separately in support of method RPA JA/03/01. Samples were analyzed for residues of 1*H*-1,2,4-triazole and the triazole conjugates triazolylalanine and triazolylacetic acid using an LC-MS/MS method (Bayer Report No. G200598). The validated LOQ for 1*H*-1,2,4-triazole was 0.01 ppm for barley grain, hay and straw, and the validated LOQs for the triazole conjugates were 0.10 ppm for barley grain and straw, and 0.05 ppm for hay. The methods are adequate for data collection for barley matrices based on acceptable concurrent method recovery data.

Apparent residues of total prothioconazole-derived residues were below the method LOQs (<0.02 ppm for barley grain and <0.05 ppm for hay and straw) in/on all samples of untreated barley grain and hay, and in/on 24 samples of untreated barley straw; quantifiable residues of 0.06 ppm were observed in/on one sample of straw. Apparent residues of 1*H*-1,2,4-triazole were below the method LOQ (<0.01 ppm) in/on all samples of untreated barley grain, hay, and straw. Apparent residues of the triazole conjugates were below the method LOQs (<0.10 ppm for grain and straw and <0.05 ppm for hay) in/on samples of untreated grain (n = 34), hay (n = 28), and straw (n = 40); quantifiable apparent residues were observed in 6 samples of untreated grain (residue range of 0.16-0.25 ppm), 10 samples of untreated hay (residue range of 0.06-0.28 ppm), and 3 samples of untreated straw (residue range of 0.10-0.15 ppm). The measurable residues of the triazole conjugates in the control matrices may have resulted from the use of triazole fungicides from previous seasons or from naturally occurring triazole conjugates.

Sample storage conditions and intervals are summarized in TABLE C.2. The maximum storage intervals of crop samples from harvest to analysis for total prothioconazole-derived residues were 1234 days (40.6 months) for barley grain and 1269 days (41.7 months) for barley hay and straw. Prothioconazole-derived residues are stable for up to 1 year based on an interim report. The need to correct the results of the current study for decline of prothioconazole-derived residues will not be necessary taking into account the absolute (ppm) and relative % residue levels in plant matrices. The degree of loss of prothioconazole-desthio residues is not expected to exceed 30% after 41.7 months.

The maximum storage intervals of crop samples from harvest to analysis for 1*H*-1,2,4-triazole and triazole conjugate residues were 1396 days (45.9 months) for barley grain and 1245 days (40.9 months) for barley hay and straw.

Residue data from the barley field trials are reported in TABLE C.3. A summary of prothioconazole residue data for barley grain, hay, and straw is presented in TABLE C.4. In barley harvested 30-71 days (12-16 days for hay) following the last of two broadcast foliar applications at a total seasonal rate of 0.286-0.309 lb a.i./A (0.321-0.348 kg a.i./ha), total prothioconazole-derived residues were <0.02-0.158 ppm in/on barley grain, 0.317-6.59 ppm in/on hay, and <0.05-1.87 ppm in/on straw. Residues of 1*H*-1,2,4-triazole were less than the LOQ (<0.01 ppm) in/on barley grain, hay, and straw, and residues of the triazole conjugates were <0.010-0.915 ppm in/on barley grain, <0.05-0.547 ppm in/on hay, and <0.010-0.385 ppm in/on straw.

In the residue decline trials, residues of 1*H*-1,2,4-triazole at all sampling intervals were less than the method LOQ (<0.01 ppm) in barley grain, hay, and straw. Total prothioconazole-derived residues did not increase with increasing sampling intervals in barley grain, hay, and straw, and residues of the triazole conjugates did not increase in grain, but increased slightly with increasing sampling intervals in samples from one trial each for hay and straw.

TABLE C.1. Summary of Concurrent Recoveries of Prothioconazole from Barley.

Matrix	Analyte	Spike level (ppm)	Sample size (n)	Recoveries (%)	Mean ± std dev
Grain	Prothioconazole	0.02	3	78, 82, 85	83 ± 4
	Prothioconazole-desthio	0.02	3	96, 100, 105	100 ± 5
	1*H*-1,2,4-triazole	0.03	6	71, 71, 71, 73, 74, 83	74 ± 5
	Triazolylalanine	0.25	3	77, 82, 93	84 ± 9
		0.75	6	72, 80, 80, 86, 88, 93	83 ± 7
	Triazolylacetic acid	0.25	3	72, 76, 92	80 ± 11
		0.75	6	77, 84, 87, 88, 90, 91	86 ± 5
Hay	Prothioconazole	0.10	5	75, 79, 81, 83, 106	85 ± 12
	Prothioconazole-desthio	0.10	5	94, 98, 99, 104, 112	101 ± 7
	Prothioconazole	7.00	3	82, 83, 86	84 ± 2
	Prothioconazole-desthio	7.00	3	99, 97, 98	98 ± 1
	1*H*-1,2,4-triazole	0.03	6	74, 75, 75, 76, 77, 81	76 ± 3
		0.20	1	82	82
	Triazolylalanine	0.05	3	92, 95, 115	101 ± 13
		0.2	1	86	86
		0.25	3	93, 96, 97	95 ± 2
		0.75	5	87, 91, 95, 97, 98	94 ± 5
	Triazolylacetic acid	0.05	3	73, 97, 113	94 ± 20
		0.2	1	74	74
		0.25	3	87, 90, 104	94 ± 9
		0.75	5	80, 89, 91, 94, 97	90 ± 7
Straw	Prothioconazole	0.10	3	84, 88, 93	88 ± 5
	Prothioconazole-desthio	0.10	3	97, 97, 114	103 ± 10
	1*H*-1,2,4-triazole	0.03	5	73, 78, 79, 79, 93	80 ± 8
		0.20	1	94	94
	Triazolylalanine	0.20	1	96	96
		0.25	3	90, 95, 99	95 ± 5
		0.75	5	84, 86, 95, 103, 105	95 ± 10
	Triazolylacetic acid	0.20	1	73	73
		0.25	3	94, 97, 108	100 ± 7
		0.75	5	68, 86, 90, 94, 96	87 ± 11

TABLE C.2. Summary of Storage Conditions.			
Matrix (RAC or Extract)	Storage Temp. (°C)	Actual Storage Duration [1]	Interval of Demonstrated Storage Stability
Total Prothioconazole-Derived Residues			
Barley, grain	-4.8 to -30.0	824-1234 days (27.1-40.6 months)	Stability demonstrated for up to 12.7 months (interim report). Awaiting final report as confirmatory data.
Barley, hay	-4.8 to -30.0	859-1269 days (28.2-41.7 months)	
Barley, straw	-4.8 to -30.0	825-1240 days (27.1-40.8 months	
1*H*-1,2,4-triazole and Triazole Conjugate Residues			
Barley, grain	-4.8 to -30.0	621-1396 days (20.4-45.9 months)	Not applicable at this time. Awaiting final report.
Barley, hay	-4.8 to -30.0	662-1245 days (21.8-40.9 months)	
Barley, straw	-4.8 to -30.0	600-1240 days (19.7-40.8 months)	

[1] Actual storage duration from collection to analysis; hay samples were collected 1-14 days after plants were cut and left in the field. All samples were analyzed within 12 days of extraction.

TABLE C.3 Residue Data from Crop Field Trials with Prothioconazole.								
Trial ID (City, State; Year)	Region	Barley Variety	Commodity or Matrix	Total Rate (lb a.i./A) [kg a.i./ha]	PHI (days)	Total Prothioconazole-Derived Residues (ppm)	1*H*-1,2,4-triazole Residues[1] (ppm)	Triazole Conjugate Residues[1] (ppm)
Northwood, ND 2000 J6001-00D	5	Robust	Grain	0.294 [0.330]	32	0.036, 0.044	<0.01, <0.01	0.269, 0.265
					37	0.041, 0.047	<0.01, <0.01	0.270, 0.267
					44	0.044, 0.049	<0.01, <0.01	0.272, 0.247
					47	<0.02, 0.028	<0.01, <0.01	0.218, 0.188
			Hay	0.294 [0.330]	8	0.741, 0.831	<0.01, <0.01	0.121, 0.067
					13	0.647, 0.731	<0.01, <0.01	0.154, 0.146
					22	0.523, 0.610	<0.01, <0.01	0.176, 0.167
					28	0.561, 0.782	<0.01, <0.01	0.222, 0.177
			Straw	0.294 [0.330]	32	0.180, 0.193	<0.01, <0.01	<0.10, <0.10
					37	0.188, 0.225	<0.01, <0.01	<0.10, <0.10
					44	0.075, 0.096	<0.01, <0.01	<0.10, <0.10
					47	0.102, 0.112	<0.01, <0.01	<0.10, <0.10
Hermiston, OR; 2000 J6002-00H	11	Steptoe	Grain	0.295 [0.331]	42	<0.02, <0.02	<0.01, <0.01	0.597, 0.602
			Hay		13	0.318, 0.358	<0.01, <0.01	0.320, 0.235
			Straw		42	0.051, 1.011	<0.01, <0.01	0.331, 0.337
Maricopa, AZ; 2000 J6003-00H	10	Baretta	Grain	0.301 [0.337]	48	0.082, 0.088	<0.01, <0.01	<0.10, <0.10
			Hay		14	1.741, 1.814	<0.01, <0.01	<0.05, <0.05
			Straw		48	1.321, 1.336	<0.01, <0.01	<0.10, <0.10

TABLE C.3. Residue Data from Crop Field Trials with Prothioconazole.

Trial ID (City, State; Year)	Region	Barley Variety	Commodity or Matrix	Total Rate (lb a.i./A) [kg a.i./ha]	PHI (days)	Total Prothioconazole-Derived Residues (ppm)	*1H*-1,2,4-triazole Residues[1] (ppm)	Triazole Conjugate Residues[1] (ppm)
Wilcox, AZ; 2000 J6004-00H	9	Baretta	Grain	0.286 [0.321]	71	0.059, 0.083	<0.01, <0.01	0.900, 0.915
			Hay		15	1.669, 2.577	<0.01, <0.01	0.230, 0.199
			Straw		71	1.269, 1.324	<0.01, <0.01	0.383, 0.335
Velva, ND; 2000 J6005-00H	7	Robust	Grain	0.295 [0.330]	33	<0.02, <0.02	<0.01, <0.01	<0.10, 0.107
			Hay		13	0.668, 0.758	<0.01, <0.01	<0.05, <0.05
			Straw		33	0.083, 0.100	<0.01, <0.01	<0.10, <0.10
New Rockford, ND; 2000 J6006-00H	7	Robust	Grain	0.301 [0.337]	36	0.031, 0.035	<0.01, <0.01	0.321, 0.344
			Hay		14	0.656, 0.829	<0.01, <0.01	0.068, 0.071
			Straw		36	0.209, 0.313	<0.01, <0.01	0.385, 0.150
Ellendale, ND; 2000 J6007-00H	7	Robust	Grain	0.294 [0.329]	43	<0.02, <0.02	<0.01, <0.01	0.119, 0.221
			Hay		14	0.551, 0.578	<0.01, <0.01	0.052, 0.060
			Straw		43	<0.05, 0.053	<0.01, <0.01	<0.10, 0.127
Branchton, ON; 2000 J6008-00D	5	Chapais	Grain	0.294 [0.330]	36	0.020, 0.033	<0.01, <0.01	0.276, 0.240
					39	0.036, 0.045	<0.01, <0.01	0.319, 0.229
					45	0.028, 0.028	<0.01, <0.01	0.239, 0.276
					49	0.022, 0.037	<0.01, <0.01	0.193, 0.207
			Hay	0.294 [0.330]	9	0.627, 0.966	<0.01, <0.01	0.109, 0.147
					14	0.317, 0.734	<0.01, <0.01	0.172, 0.148
					21	0.167, 0.211	<0.01, <0.01	0.186, 0.180
					29	0.147, 0.199	<0.01, <0.01	0.080, 0.186
			Straw	0.294 [0.330]	36	0.128, 0.211	<0.01, <0.01	<0.10, 0.136
					39	0.177, 0.217	<0.01, <0.01	<0.10, 0.126
					45	0.151, 0.155	<0.01, <0.01	0.388, <0.10
					49	0.147, 0.159	<0.01, <0.01	0.161, 0.139
Jerome, ID; 2000 J6009-00H	11	Morex	Grain	0.294 [0.330]	43	<0.02, <0.02	<0.01, <0.01	0.111, 0.118
			Hay		12	0.438, 0.462	<0.01, <0.01	<0.05, 0.053
			Straw		43	0.052, 0.065	<0.01, <0.01	<0.10, <0.10
Northwood, ND; 2000 J6010-00H	5	Robust	Grain	0.291 [0.327]	44	0.027, 0.031	<0.01, <0.01	0.689, 0.803
			Hay		14	0.556, 0.767	<0.01, <0.01	0.342, 0.547
			Straw		44	0.085, 0.109	<0.01, <0.01	<0.10, 0.101
Germansville, PA; 2000 J6013-00H	1	AC Stephen	Grain	0.293 [0.328]	57	<0.02, 0.022	<0.01, <0.01	0.514, 0.532
			Hay		13	1.191, 1.235	<0.01, <0.01	0.192, 0.231
			Straw		57	0.185, 0.199	<0.01, <0.01	<0.10, <0.10
Minto, MB; 2000 J6078-00H	14	Robust	Grain	0.296 [0.332]	36	0.132, 0.138	<0.01, <0.01	0.292, 0.277
			Hay		13	5.305, 6.590	<0.01, <0.01	0.163, 0.135
			Straw		36	0.711, 0.932	<0.01, <0.01	<0.10, <0.10

TABLE C.3. Residue Data from Crop Field Trials with Prothioconazole.

Trial ID (City, State; Year)	Region	Barley Variety	Commodity or Matrix	Total Rate (lb a.i./A) [kg a.i./ha]	PHI (days)	Total Prothioconazole-Derived Residues (ppm)	1*H*-1,2,4-triazole Residues[1] (ppm)	Triazole Conjugate Residues[1] (ppm)
Minto, MB; 2000 J6079-00H	14	Robust	Grain	0.287 [0.322]	32	0.144, 0.158	<0.01, <0.01	0.354, 0.350
			Hay		16	1.796, 3.895	<0.01, <0.01	0.100, 0.250
			Straw		32	1.282, 1.828	<0.01, <0.01	<0.10, <0.10
Brookdale, MB; 2000 J6080-00H	14	AC Rosser	Grain	0.297 [0.333]	43	0.052, 0.061	<0.01, <0.01	0.329, 0.343
			Hay		14	2.933, 3.445	<0.01, <0.01	0.142, 0.152
			Straw		43	0.603, 0.606	<0.01, <0.01	<0.10, <0.10
Clanwilliam, MB; 2000 J6081-00H	14	Bedford	Grain	0.295 [0.331]	65	0.022, 0.030	<0.01, <0.01	0.177, 0.249
			Hay		14	2.718	<0.01	0.223
			Straw		65	0.294, 0.396	<0.01, <0.01	<0.10, 0.102
Marcelin, SK; 2000 J6082-00H	14	AC Metcalf	Grain	0.291 [0.325]	48	<0.02, <0.02	<0.01, <0.01	0.200, 0.207
			Hay		12	1.133, 1.220	<0.01, <0.01	0.123, 0.067
			Straw		48	0.348, 0.349	<0.01, <0.01	0.111, <0.10
Rosthern, SK; 2000 J6083-00H	14	Harrington	Grain	0.292 [0.328]	43	<0.02, <0.02	<0.01, <0.01	0.210, 0.262
			Hay		12	2.246, 2.534	<0.01, <0.01	0.098, 0.092
			Straw		43	0.751, 0.774	<0.01, <0.01	<0.10, <0.10
Wakaw, SK; 2000 J6084-00H	14	Harrington	Grain	0.291 [0.327]	34	<0.02, <0.02	<0.01, <0.01	0.480, 0.585
			Hay		15	3.366, 3.715	<0.01, <0.01	0.200, 0.164
			Straw		34	0.911, 1.008	<0.01, <0.01	<0.10, <0.10
Lacombe, AB; 2000 J6085-00HA	14		Grain	0.293 [0.329]	71	<0.02	<0.01, <0.01	<0.10, 0.133
			Hay		13	1.178, 1.375	<0.01, <0.01	<0.05, <0.05
			Straw		71	0.136, 0.161	<0.01, <0.01	<0.10, <0.10
Penhold, AB; 2000 J6086-00HA	14		Grain	0.294 [0.330]	71	<0.02, <0.02	<0.01, <0.01	0.138, 0.162
			Hay		13	1.151, 1.514	<0.01, <0.01	<0.05, <0.05
			Straw		71	0.178, 0.258	<0.01, <0.01	0.123, <0.10
Rosthern, SK; 2000 J6087-00H	14	Stein	Grain	0.294 [0.330]	52	<0.02, <0.02	<0.01, <0.01	0.394, 0.405
			Hay		13	1.865, 2.021	<0.01, <0.01	0.148, 0.150
			Straw		52	0.750, 0.766	<0.01, <0.01	<0.10, <0.10
Kipp, AB; 2000 J6088-00H	14	AC Harper	Grain	0.299 [0.336]	47	<0.02, <0.02	<0.01, <0.01	0.123, 0.130
			Hay		14	0.512, 0.697	<0.01, <0.01	0.149, 0.065
			Straw		47	0.063, 0.076	<0.01, <0.01	<0.10, <0.10
Leduc, AB; 2000 J6089-00H	14		Grain	0.301 [0.338]	33	<0.02, 0.022	<0.01, <0.01	0.148, 0.153
			Hay		15	0.890, 0.941	<0.01, <0.01	0.185, 0.280
			Straw		33	0.251, 0.278	<0.01, <0.01	<0.10, <0.10
Delisle, SK; 2000 J6090-00H	7	Excel	Grain	0.292 [0.328]	30	0.050, 0.094	<0.01, <0.01	0.164, 0.186
			Hay		15	1.747, 1.843	<0.01, <0.01	<0.05, <0.05
			Straw		30	1.003, 1.056	<0.01, <0.01	<0.10, <0.10

TABLE C.3. Residue Data from Crop Field Trials with Prothioconazole.

Trial ID (City, State; Year)	Region	Barley Variety	Commodity or Matrix	Total Rate (lb a.i./A) [kg a.i./ha]	PHI (days)	Total Prothioconazole-Derived Residues (ppm)	1*H*-1,2,4-triazole Residues[1] (ppm)	Triazole Conjugate Residues[1] (ppm)
St-Paul-d'Abbotsford, QC; 2000 J6091-00H	5B	Chapais	Grain	0.309 [0.348]	36	0.102, 0.109	<0.01, <0.01	0.144, 0.216
			Hay		15	3.931, 4.197	<0.01, <0.01	0.118, 0.053
			Straw		36	1.439, 1.871	<0.01, <0.01	<0.10, <0.10

[1] Residue values are listed respectively for 1*H*-1,2,4-triazole and the triazole conjugates.

TABLE C.4. Summary of Residue Data from Crop Field Trials with Prothioconazole.

Commodity	Total Applic. Rate (lb a.i./A) [kg a.i./ha]	PHI (days)	Residue Levels (ppm) [1]						
			n	Min.	Max.	HAFT[2]	Median (STMdR[3])	Mean (STMR[4])	Std. Dev.
Total Prothioconazole-Derived Residues									
Barley, hay	0.286-0.309 [0.321-0.348]	12-16	49	0.317	6.59	5.95	1.22	1.72	1.39
Barley, grain	0.286-0.309 [0.321-0.348]	30-71	49	<0.02	0.158	0.151	0.022	0.040	0.041
Barley, straw	0.286-0.309 [0.321-0.348]	30-71	50	<0.05	1.871	1.65	0.304	0.554	0.510
1*H*-1,2,4-triazole Residues									
Barley, hay	0.286-0.309 [0.321-0.348]	12-16	49	<0.01	<0.01	<0.01	0.005	0.005	0.0
Barley, grain	0.286-0.309 [0.321-0.348]	30-71	50	<0.01	<0.01	<0.01	0.005	0.005	0.0
Barley, straw	0.286-0.309 [0.321-0.348]	30-71	50	<0.01	<0.01	<0.01	0.005	0.005	0.0
Triazole Conjugate Residues									
Barley, hay	0.286-0.309 [0.321-0.348]	12-16	49	<0.05	0.547	0.445	0.135	0.134	0.104
Barley, grain	0.286-0.309 [0.321-0.348]	30-71	50	<0.10	0.915	0.909	0.239	0.300	0.215
Barley, straw	0.286-0.309 [0.321-0.348]	30-71	50	<0.1	0.385	0.359	0.05	0.090	0.093

[1] For the calculation of minimum, maximum, and HAFT values, the LOQ value was used for residues reported as below the LOQ in TABLE C.3. For calculation of the median, mean and standard deviation, ½LOQ was used for residues reported as below the LOQ. Residue values from the appropriate harvest intervals from the residue decline trials (13- to 14-day PHI for hay and 37- to 39-day PHI for grain and straw) were included in the summary table.

[2] HAFT = Highest Average Field Trial.

[3] STMdR = Supervised Trial Median Residue.

[4] STMR = Supervised Trial Mean Residue.

D. CONCLUSION

The study use pattern was two foliar applications of the 4 lb/gal FlC formulation for a total seasonal rate of 0.286-0.309 lb a.i./A (0.321-0.348 kg a.i./ha), with an average of a 12-day re-treatment interval. Barley grain and straw were harvested 30-71 days (12-16 days for hay) after the last application. The maximum total prothioconazole-derived residues were 6.6 ppm (barley hay), 0.16 ppm (barley grain), and 1.9 ppm (barley straw). Residues of 1,2,4-triazole were less than the LOQ (<0.01 ppm) in/on barley grain, hay, and straw. Maximum residues of the triazole conjugates were 0.55 ppm, 0.92 ppm and 0.39 ppm in/on barley hay, grain and straw, respectively. Acceptable methods were used for quantitation of residues in/on barley grain, hay, and straw.

E. REFERENCES

Gould T. and Timberlake B. (2005) Storage stability of JAU6476 and desthio JAU6476 in canola, wheat, mustard greens, turnip root, and tomato fruit, processed products, and solutions, Bayer CropScience Interim Report.

F. DOCUMENT TRACKING

RDI: Louise Croteau, Suzan Mathew (PMRA) 23/01/2006; Henri Bietlot (PMRA) 24/01/2006; Stephen Funk (IO)13/03/2006; Leung Cheng (RAB3).
Petition Number: PP#4F6830
DP Barcode: D303508
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Template Version September 2003

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This DER was originally prepared under contract by Dynamac Corporation (20440 Century Boulevard, Suite 100; Germantown, MD 20874; submitted 05/20/2005). The DER has been reviewed by the Health Effects Division (HED) and Health Canada's Pest Management Regulatory Agency (PMRA). The DER has been revised to reflect current Office of Pesticide Programs (OPP) policies and Directive 98-02.

STUDY REPORT:

46246219 Lemke, V.; Murphy, J. (2004) JAU6476 480 SC - Magnitude of the Residue In/On Wheat. Project Number: J619WH01, RCJAY009, 200524. Unpublished study prepared by Bayer Corp , Battelle and Vaughn Agricultural Research Serv., Ltd. 1270 p.

EXECUTIVE SUMMARY:

Bayer CropScience has submitted field trial data on wheat from trials conducted in the U.S. and Canada. A total of 54 trials were conducted in Regions 2 (GA and NC; 2 trials), 4 (MS; 2 trials), 5 (IN; 1 trial, KS; 2 trials, NE; 2 trials, and ON; 2 trials), 6 (TX; 2 trials), 7 (AB; 1 trial, ND; 5 trials, SD; 2 trials, and SK; 3 trials), 7A (AB; 2 trials), 8 (OK; 3 trials and TX; 7 trials), 11 (OR; 2 trials), and 14 (AB; 6 trials, MB; 6 trials, and SK; 4 trials) during the 2000 growing season.

The number and locations of field trials are in accordance with OPPTS Guideline 860.1500 and Directive 98-02; Section 9.

At each test location, two broadcast foliar applications of the 4 lb/gal (480 g/L) suspension concentrate formulation (flowable concentrate; FlC) were made to wheat. The first application was made at 0.108-0.120 lb a.i./A (0.122-0.135 kg a.i./ha) followed by a second application at 0.170-0.199 lb a.i./A (0.190-0.223 kg a.i./ha) with a 5- to 18-day retreatment interval, for a total seasonal application rate of ~0.29 lb a.i./A (~0.33 kg a.i./ha). In one field trial conducted in IN, the first application was made at 0.185 lb a.i./A (0.207 kg a.i./ha) followed by a second application at 0.190 lb a.i./A (0.213 kg a.i./ha) with a 14-day retreatment interval, for a total seasonal application rate of 0.375 lb a.i./A (0.420 kg a.i./ha). Applications were made in 11-45 gal/A of water using ground equipment. An adjuvant was not added to the spray mixture for any applications.

For 33 trials, including two decline trials, two treatment plots (designated as FORAG and HGRST) were used. The timing of the application varied for the two treatment plots. In the FORAG plot the second application was made 1 day prior to the first cutting of forage and in the HGRST plot the second application was made at full flowering. Wheat forage from the FORAG plots was harvested one day after treatment, but these samples were never analyzed or reported. Wheat hay from the HGRST plots was cut 12-17 days after treatment and was left in the field for 0-14 days prior to collection of wheat hay. Samples of wheat grain and straw from the HGRST plots were harvested at earliest commercial harvest, 30-57 days after the last application, except in one trial in which samples were harvested 10 days after second application.

For 21 trials, one treatment plot (designated as TRTD) was used; the second application was made 7 days prior to the first cutting of the forage. Only wheat forage was harvested from these trials.

At two locations (ND and NE), additional samples were collected to determine residue decline. The samples were harvested at both locations 0, 1, 7, and 14 days after treatment for wheat forage at 6 or 7, 14, 20 or 21, and 28 days after treatment for wheat hay, and at 35 or 36, 39 or 40, 44 or 46, and 49 or 50 days after treatment for wheat grain and straw.

Samples were analyzed for total prothioconazole-derived residues (prothioconazole and the metabolite prothioconazole-desthio) using LC-MS/MS method RPA JA/03/01. The validated LOQs for the total prothioconazole-derived residues were 0.02 ppm for wheat grain and 0.05 ppm for wheat forage, hay, and straw. The method is adequate for data collection for wheat grain hay, forage and straw based on acceptable concurrent method recovery data and method validation data. Samples were analyzed for residues of 1*H*-1,2,4-triazole and the triazole conjugates triazolylalanine and triazolylacetic acid using an LC-MS/MS method (Bayer Report No. G200598). The validated LOQ for 1*H*-1,2,4-triazole and triazolylalanine was 0.01 ppm for wheat forage, hay, grain, and straw; and the validated LOQs for triazolylacetic acid were 0.01 ppm for wheat forage, hay, and grain and 0.025 ppm for wheat straw. The method is adequate for data collection in wheat matrices based on acceptable concurrent method recovery data.

The results from the wheat field trials show that in wheat matrices harvested 10-57 days (12-17 days for hay) following the last of two broadcast foliar applications at a total seasonal rate of 0.281-0.375 lb a.i./A (0.315-0.420 kg a.i./ha), the maximum residues of prothioconazole were 0.061 ppm, 1.96 ppm, and 3.571 ppm, respectively, in/on wheat grain, straw, and hay for the total prothioconazole-derived residues; less than the LOQ (<0.01 ppm) in/on wheat grain, straw, and hay for 1*H*-1,2,4-triazole; and 0.495 ppm, 0.665 ppm, and 1.76 ppm, respectively, in/on wheat straw, hay, and grain for the triazole conjugates. In wheat forage harvested 7 days following the last of two broadcast foliar applications at a total seasonal rate of 0.286-0.299 lb a.i./A (0.320-0.336 kg a.i./ha), the maximum residues were 6.987 ppm for the total prothioconazole-derived residues, less than the LOQ (<0.01 ppm) for 1*H*-1,2,4-triazole, and 0.175 ppm for the triazole conjugates.

In the residue decline trials, residues of 1*H*-1,2,4-triazole at all sampling intervals were less than the method LOQ (<0.01 ppm) in/on wheat hay, grain, straw, and forage for both trials (NE and ND). Total prothioconazole-derived residues did not increase in any wheat matrix with increasing sampling intervals, and residues of the triazole conjugates increased slightly in samples of wheat forage from both trials and in wheat straw from one trial but did not increase in wheat hay or grain with increasing sampling intervals.

The maximum storage intervals of crop samples from harvest to analysis for total prothioconazole-derived residues were 469 days (15.4 months) for wheat forage, 1214 days (39.9 months) for wheat grain, 1221 days (40.1 months) for wheat hay, and 1203 days (39.5 months) for wheat straw. Prothioconazole-derived residues are relatively stable up to 1 year (interim report) in wheat matrices. Corrections due to apparent dissipation of prothioconazole-derived residues in samples stored beyond a year are not necessary due to the low absolute (ppm) and % residue levels in wheat matrices. Residues of prothioconazole-desthio are stable for up to 1 year and the degree of loss is not expected to exceed 30% after 40.1 months.

STUDY/WAIVER ACCEPTABILITY/DEFICIENCIES/CLARIFICATIONS:

The number of field trials conducted for forage were not in accordance with the guidelines (OPPTS 860.1500 and Dir98-02). However, this is not a concern as the number of field trials for grain, hay and straw were in accordance to the guidelines.

Under the conditions and parameters used in the study, the field trial residue data are tentatively classified as scientifically acceptable. The final reports of the ongoing storage stability studies with prothioconazole, prothioconazole-desthio, 1*H*-1,2,4-triazole, triazolylalanine, and triazolylacetic acid will be submitted as confirmatory data.

The acceptability of this study for regulatory purposes is addressed in the forthcoming U.S. EPA Residue Chemistry Summary Document, DP Barcode D303508, and in Canada's Regulatory Decision Document.

COMPLIANCE:

Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided. No deviations from regulatory requirements were reported which would have an impact on the validity of the study.

A. BACKGROUND INFORMATION

Prothioconazole is a broad-spectrum systemic fungicide intended for the control of many diseases in different crops such as cereals, oilseed rape or peanuts. The biochemical mode of action is the inhibition of the demethylation of lanosterol or 24-methylene-dihydrolanosterol, which are precursors of sterols in fungi. The chemical has been submitted for joint EPA and PMRA review by Bayer CropScience. The crops proposed for joint review include barley, the dried shell and bean subgroup, the oilseed crop group, and wheat. Uses on peanuts and rice are being proposed for the U.S. only.

TABLE A.1. Test Compound Nomenclature.

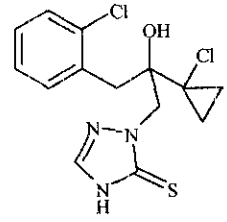
Chemical structure	
Common name	Prothioconazole (ISO accepted)
Company experimental name	JAU6476
IUPAC name	(*RS*)-2-[2-(1-chlorocyclopropyl)-3-(2-chlorophenyl)-2-hydroxypropyl]-2,4-dihydro-1,2,4-triazole-3-thione
CAS name	2-[2-(1-chlorocyclopropyl)-3-(2-chlorophenyl)-2-hydroxypropyl]-1,2-dihydro-3*H*-1,2,4-triazole-3-thione
CAS registry number	178928-70-6
PC Code	113961
End-use product (EP)	Proline® 480 SC (4 lb/gal suspension concentrate), EPA File Symbol 264-JEL

TABLE A.2. Physicochemical Properties of the Technical Grade Test Compound.		
Parameter	Value	References
Melting range	139.1 to 144.5°C	MRID 46246003/CES
pH	5.8 (1% solution)	MRID 46246003/CES
Density at 20°C	1.36 g/mL	MRID 46246003/CES
Water solubility at 20°C	pH: mg/L 4: 5 8: 300 9: 2000	MRID 46246003/CES
Solvent solubility at 20°C	Solvent: g/L Acetone: >250 Acetonitrile: 69 Dichloromethane: 88 Dimethysulfoxide: 126 Ethyl acetate: >250 n-Heptane: <0.1 1-Octanol: 58 Polyethylene glycol: >250 2-Propanol: 87 Xylene: 8	MRID 46246003/CES
Vapor pressure at 20 or 25°C	$<4 \times 10^{-7}$ Pa (calculated from determinations at 70°C)	MRID 46246003/CES
Dissociation constant, pK_a	6.9 (calculated from K_{OW})	MRID 46246003/CES
Octanol/water partition coefficient, at 20°C	pH: $Log(K_{OW})$ unbuffered water: 4.05 4: 4.16 7: 3.82 9: 2.00	MRID 46246003/CES
UV/visible absorption spectrum	Peak maxima at 257 nm. No absorption at > 300 nm.	MRID 46246003/CES

CES - Chemistry Evaluation Section of PMRA.

B. EXPERIMENTAL DESIGN

B.1. Study Site Information

TABLE B.1.1. Trial Site Conditions.						
Location (City, State/Province; Year) Trial Number	Soil characteristics				Meteorological data	
	Type	%OM[1]	pH[1]	CEC[1]	Total rainfall (inches)[2]	Overall temperature range (°C)
St. George, ON; 2000 J6044-00H	Not Provided	Not Applicable			10.69	0.6-30
Louisville, NE; 2000 J6045-00D	Not Provided	Not Applicable			12.82(I)	-3.3-38
New Rockford, ND; 2000 J6046-00D	Not Provided	Not Applicable			12.73	5-36

TABLE B.1.1. Trial Site Conditions.

Location (City, State/Province; Year) Trial Number	Soil characteristics				Meteorological data	
	Type	%OM[1]	pH[1]	CEC[1]	Total rainfall (inches)[2]	Overall temperature range (°C)
Hermiston, OR; 2000 J6047-00H	Not Provided	Not Applicable			0.36(I)	5.6-41
Uvalde, TX; 2000 J6048-00H	Not Provided	Not Applicable			7.25(I)	0.6-37
Claude, TX; 2000 J6049-00H	Not Provided	Not Applicable			0.60	-1.7-38
Cordell, OK; 2000 J6050-00H	Not Provided	Not Applicable			8.60	-2.2-35
Frederick, OK; 2000 J6051-00H	Not Provided	Not Applicable			4.02	3.3-37
Hart, TX; 2000 J6052-00H	Not Provided	Not Applicable			6.18(I)	-5-40
Velva, ND; 2000 J6053-00H	Not Provided	Not Applicable			6.41	-1.7-31
Levelland, TX; 2000 J6054-00H	Not Provided	Not Applicable			7.83	-5.6-38
Ellendale, ND; 2000 J6055-00H	Not Provided	Not Applicable			7.83	5-35
Lake Andes, SD; 2000 J6056-00H	Not Provided	Not Applicable			5.45	1.7-39
Paris, ON; 2000 J6057-00H	Not Provided	Not Applicable			10.69	0.6-30
East Bernard, TX; 2000 J6058-00H	Not Provided	Not Applicable			12.73	5-33
Stilwell, KS; 2000 J6059-00H	Not Provided	Not Applicable			18.21	2.2-36
Oxford, IN; 2000 J6060-00H	Not Provided	Not Applicable			9.47	-3.9-33
Red Deer, AB; 2000 J6061-00H	Not Provided	Not Applicable			10.05	-5-30
Monarch, AB; 2000 J6062-00H	Not Provided	Not Applicable			1.57	1.1-44
Benoit, MS; 2000 J6063-00H	Not Provided	Not Applicable			7.18	1.7-33
Knightdale, NC; 2000 J6064-00H	Not Provided	Not Applicable			9.03	-5-32
Minto, MB; 2000 J6066-00H	Not Provided	Not Applicable			7.60	2.2-34
Minto, MB; 2000 J6067-00H	Not Provided	Not Applicable			6.42	2.2-34
Wakaw, SK; 2000 J6068-00H	Not Provided	Not Applicable			7.89	-1.1-32

TABLE B.1.1. Trial Site Conditions.

Location (City, State/Province; Year) Trial Number	Soil characteristics				Meteorological data	
	Type	%OM[1]	pH[1]	CEC[1]	Total rainfall (inches)[2]	Overall temperature range (°C)
Leask, SK; 2000 J6069-00H	Not Provided	Not Applicable			6.70	1.1-32
Rostern, SK; 2000 J6070-00H	Not Provided	Not Applicable			7.07	1.1-32
Brookdale, MB; 2000 J6071-00H	Not Provided	Not Applicable			6.28	2.8-33
Lancombe, AB; 2000 J6072-00H	Not Provided	Not Applicable			10.66	-6.1-28
Delisle, SK; 2000 J6073-00H	Not Provided	Not Applicable			6.09	1.7-34
Delisle, SK; 2000 J6074-00H	Not Provided	Not Applicable			6.24	1.7-34
Warner, AB; 2000 J6075-00H	Not Provided	Not Applicable			2.47	0-42
Coaldale, AB; 2000 J6076-00H	Not Provided	Not Applicable			1.59(I)	1.1-42
Kipp, AB; 2000 J6077-00H	Not Provided	Not Applicable			1.57(I)	1.1-44
Tifton, GA; 2000 J6169-00H	Not Provided	Not Applicable			9.54	6.1-27
Leland, MS; 2000 J6170-00H	Not Provided	Not Applicable			2.92	1.7-31
Stilwell, KS; 2000 J6171-00H	Not Provided	Not Applicable			12.17	-5-33
Louisville, NE; 2000 J6172-00H	Not Provided	Not Applicable			1.11	-7.2-31
Uvalde, TX; 2000 J6173-00H	Not Provided	Not Applicable			0.13	-2.2-26
New Rockford. ND; 2000 J6174-00HA	Not Provided	Not Applicable			0.98	4.4-32
Eldridge, ND; 2000 J6175-00HA	Not Provided	Not Applicable			2.22	3.3-33
Britton, SD; 2000 J6176-00H	Not Provided	Not Applicable			3.16	8.9-36
Dundurn, SK; 2000 J6177-00H	Not Provided	Not Applicable			1.06	0-34
Taber, AB; 2000 J6178-00H	Not Provided	Not Applicable			0.59	6.1-37
Levelland, TX; 2000 J6179-00H	Not Provided	Not Applicable			1.87	-8.8-39
Hart, TX; 2000 J6180-00H	Not Provided	Not Applicable			2.55	-3.9-28

TABLE B.1.1. Trial Site Conditions.

Location (City, State/Province; Year) Trial Number	Soil characteristics				Meteorological data	
	Type	%OM[1]	pH[1]	CEC[1]	Total rainfall (inches)[2]	Overall temperature range (°C)
Wolforth, TX ; 2000 J6181-00H	Not Provided	Not Applicable			3.42(I)	-5-39
Colony, OK; 2000 J6182-00H	Not Provided	Not Applicable			15.6	-5-41
Hood River, OR; 2000 J6183-00H	Not Provided	Not Applicable			0.0(I)	6.1-37
Minot, MB; 2000 J6184-00H	Not Provided	Not Applicable			3.92	5-32
Rosthern, SK, 2000 J6185-00HA	Not Provided	Not Applicable			2.87(I)	-2.8-32
Leduc, AB; 2000 J6186-00H	Not Provided	Not Applicable			0.57	2.2-30
Spruce Grove, AB; 2000 J6187-00H	Not Provided	Not Applicable			0.57	2.2-30
Brookdale, MB 2000 J6188-00H	Not Provided	Not Applicable			0.31	3.3-34
Bethany, MB; 2000 J6189-00H	Not Provided	Not Applicable			0.49	5-34

[1] These parameters are not applicable since they do not affect the prosed use pattern for this chemical.
[2] (I) indicates that supplemental irrigation was received.

The actual temperature and rainfall recordings were within average historical values for the residue study period. The applicant indicated that irrigation was used to supplement as needed at nine trial sites.

TABLE B.1.2. *Study Use Pattern.*

Location (City, State/Province; Year) Trial Number	EP[1]	Plot Name[2]	Application				
			Method; Timing	Vol. (GPA[3]) [L/ha]	Rate (lb a.i./A) [kg a.i./ha]	RTI[4] (days)	Total Rate (lb a.i./A) [kg a.i./ha]
St. George, ON; 2000 J6044-00H	480 SC	FORAG	1: Broadcast foliar; Beginning of stem elongation	24 [220]	0.120 [0.135]	0	0.308 [0.346]
			2: Broadcast foliar; Midboot stage; flag leaf sheath just vis. swollen	22 [210]	0.188 [0.211]	13	
		HGRST	1: Broadcast foliar; Midboot stage; flag leaf sheath just vis. swollen	23 [211]	0.117 [0.131]	0	0.303 [0.339]
			2: Broadcast foliar; Full flowering: 50% of anthers mature	23 [212]	0.186 [0.208]	15	

TABLE B.1.2. Study Use Pattern.

Location (City, State/Province, Year) Trial Number	EP[1]	Plot Name[2]	Application				
			Method; Timing	Vol. (GPA[3]) [L/ha]	Rate (lb a.i./A) [kg a.i./ha]	RTI[4] (days)	Total Rate (lb a.i./A) [kg a.i./ha]
Louisville, NE; 2000 J6045-00D (decline study)	480 SC	FORAG	1: Broadcast foliar; Beginning of stem elongation	18 [165]	0.113 [0.127]	0	0.293 [0.329]
			2: Broadcast foliar; First node at least 1 cm above tillering node	18 [164]	0.180 [0.202]	15	
		HGRST	1: Broadcast foliar; Late boot stage: flag leaf sheath swollen	17 [163]	0.113 [0.127]	0	0.293 [0.329]
			2: Broadcast foliar; Full flowering: 50% of anthers mature	17 [159]	0.180 [0.202]	14	
New Rockford, ND; 2000 J6046-00D (decline study)	480 SC	FORAG	1: Broadcast foliar; 5 leaves unfolded	30 [280]	0.111 [0.124]	0	0.288 [0.322]
			2: Broadcast foliar; First node at least 1 cm above tillering node	29 [270]	0.177 [0.198]	14	
		HGRST	1: Broadcast foliar; First awns visible (in awned forms only)	30 [282]	0.110 [0.123]	0	0.291 [0.326]
			2: Broadcast foliar; Full flowering: 50% of anthers mature	30 [281]	0.181 [0.203]	14	
Hermiston, OR; 2000 J6047-00H	480 SC	FORAG	1: Broadcast foliar; 3 tillers detectable	31 [289]	0.115 [0.128]	0	0.299 [0.334]
			2: Broadcast foliar; Node 6 at least 2 cm above node 5	31 [292]	0.184 [0.206]	13	
		HGRST	1: Broadcast foliar; First awns visible (in awned forms only)	29 [272]	0.116 [0.130]	0	0.299 [0.335]
			2: Broadcast foliar; Full flowering: 50% of anthers mature	31 [292]	0.183 [0.205]	14	
Uvalde, TX; 2000 J6048-00H	480 SC	FORAG	1: Broadcast foliar; End of tillering: 9 or more tillers detectable	20 [188]	0.116 [0.129]	0	0.291 [0.325]
			2: Broadcast foliar; Node 3 at least 2 cm above node 2	18 [169]	0.175 [0.196]	14	
		HGRST	1: Broadcast foliar; Late boot stage: flag leaf sheath swollen	18 [169]	0.112 [0.125]	0	0.293 [0.328]
			2: Broadcast foliar; Full flowering: 50% of anthers mature	20 [186]	0.181 [0.203]	14	

TABLE B.1.2. Study Use Pattern.

Location (City, State/Province; Year) Trial Number	EP[1]	Plot Name[2]	Application				
			Method; Timing	Vol. (GPA[3]) [L/ha]	Rate (lb a.i./A) [kg a.i./ha]	RTI[4] (days)	Total Rate (lb a.i./A) [kg a.i./ha]
Claude, TX; 2000 J6049-00H	480 SC	FORAG	1: Broadcast foliar; Beginning of stem elongation	21 [198]	0.114 [0.128]	0	0.299 [0.336]
			2: Broadcast foliar; Node 4 at least 2 cm above node 3	22 [201]	0.185 [0.208]	15	
		HGRST	1: Broadcast foliar; Node 4 at least 2 cm above node 3	21 [197]	0.113 [0.127]	0	0.295 [0.331]
			2: Broadcast foliar; Beginning of flowering: first anthers visible	21 [199]	0.182 [0.204]	15	
Cordell, OK; 2000 J6050-00H	480 SC	FORAG	1: Broadcast foliar; End of tillering: 9 or more tillers detectable	13 [125]	0.110 [0.124]	0	0.291 [0.327]
			2: Broadcast foliar; First node at least 1 cm above tillering node	14 [128]	0.181 [0.203]	15	
		HGRST	1: Broadcast foliar; Late boot stage: flag leaf sheath swollen	13 [124]	0.114 [0.127]	0	0.313 [0.351]
			2: Broadcast foliar; Full flowering: 50% of anthers mature	14 [127]	0.199 [0.223]	14	
Frederick, OK; 2000 J6051-00H	480 SC	FORAG	1: Broadcast foliar; 20% of inflorescence emerged	20 [186]	0.107 [0.120]	0	0.284 [0.319]
			2: Broadcast foliar; 30% of inflorescence emerged	21 [195]	0.177 [0.199]	13	
		HGRST	1: Broadcast foliar; 30% of inflorescence emerged	20 [185]	0.111 [0.124]	0	0.281 [0.315]
			2: Broadcast foliar; End of flowering	21 [194]	0.170 [0.190]	14	
Hart, TX; 2000 J6052-00H	480 SC	FORAG	1: Broadcast foliar; Beginning of stem elongation	16 [151]	0.113 [0.126]	0	0.292 [0.326]
			2: Broadcast foliar; First node at least 1 cm above tillering node	16 [149]	0.179 [0.200]	12	
		HGRST	1: Broadcast foliar; Midboot stage; flag leaf sheath just vis. swollen	16 [151]	0.113 [0.127]	0	0.291 [0.327]
			2: Broadcast foliar; Full flowering: 50% of anthers mature	16 [153]	0.178 [0.200]	18	

TABLE B.1.2. Study Use Pattern.

Location (City, State/Province; Year) Trial Number	EP[1]	Plot Name[2]	Application				
			Method; Timing	Vol. (GPA[3]) [L/ha]	Rate (lb a.i./A) [kg a.i./ha]	RTI[4] (days)	Total Rate (lb a.i./A) [kg a.i./ha]
Velva, ND; 2000 J6053-00H	480 SC	FORAG	1: Broadcast foliar; 3 leaves unfolded	30 [282]	0.114 [0.128]	0	0.293 [0.329]
			2: Broadcast foliar; First node at least 1 cm above tillering node	30 [279]	0.179 [0.201]	15	
		HGRST	1: Broadcast foliar; Early boot stage; flag leaf sheath extending	30 [281]	0.114 [0.127]	0	0.294 [0.328]
			2: Broadcast foliar; Full flowering: 50% of anthers mature	30 [279]	0.180 [0.201]	13	
Levelland, TX; 2000 J6054-00H	480 SC	FORAG	1: Broadcast foliar; Beginning of stem elongation	20 [189]	0.113 [0.126]	0	0.293 [0.327]
			2: Broadcast foliar; First node at least 1 cm above tillering node	20 [188]	0.180 [0.201]	13	
		HGRST	1: Broadcast foliar; Flag leaf sheath opening	20 [189]	0.112 [0.126]	0	0.294 [0.330]
			2: Broadcast foliar; Beginning of flowering; first anthers vis.	20 [189]	0.182 [0.204]	15	
Ellendale, ND; 2000 J6055-00H	480 SC	FORAG	1: Broadcast foliar; 4 leaves unfolded	20 [186]	0.112 [0.125]	0	0.292 [0.326]
			2: Broadcast foliar; First node at least 1 cm above tillering node	20 [187]	0.180 [0.201]	13	
		HGRST	1: Broadcast foliar; Node 4 at least 2 cm above node 3	20 [187]	0.113 [0.127]	0	0.290 [0.326]
			2: Broadcast foliar; Full flowering: 50% of anthers mature	20 [185]	0.177 [0.199]	14	
Lake Andes, SD; 2000 J6056-00H	480 SC	FORAG	1: Broadcast foliar; Beginning of stem elongation	19 [176]	0.112 [0.125]	0	0.291 [0.325]
			2: Broadcast foliar; First node at least 1 cm above tillering node	19 [176]	0.179 [0.200]	14	
		HGRST	1: Broadcast foliar; Flag leaf stage	24 [226]	0.114 [0.128]	0	0.290 [0.325]
			2: Broadcast foliar; Full flowering: 50% of anthers mature	20 [192]	0.176 [0.197]	16	

TABLE B.1.2. Study Use Pattern.

Location (City, State/Province; Year) Trial Number	EP[1]	Plot Name[2]	Application				
			Method; Timing	Vol. (GPA[3]) [L/ha]	Rate (lb a.i./A) [kg a.i./ha]	RTI[4] (days)	Total Rate (lb a.i./A) [kg a.i./ha]
Paris, ON; 2000 J6057-00H	480 SC	FORAG	1: Broadcast foliar; Beginning of stem elongation	25 [235]	0.128 [0.144]	0	0.306 [0.344]
			2: Broadcast foliar; Midboot stage; flag leaf sheath just vis. swollen	21 [199]	0.178 [0.200]	13	
		HGRST	1: Broadcast foliar; Midboot stage; flag leaf sheath just vis. swollen	23 [215]	0.120 [0.134]	0	0.291 [0.326]
			2: Broadcast foliar; Full flowering: 50% of anthers mature	21 [196]	0.171 [0.192]	15	
East Bernard, TX; 2000 J6058-00H	480 SC	FORAG	1: Broadcast foliar; Beginning of stem elongation	15 [140]	0.112 [0.126]	0	0.287 [0.322]
			2: Broadcast foliar; First node at least 1 cm above tillering node	15 [142]	0.175 [0.196]	14	
		HGRST	1: Broadcast foliar; Beginning of flowering; first anthers vis.	15 [136]	0.113 [0.126]	0	0.292 [0.327]
			2: Broadcast foliar; End of flowering	15 [139]	0.179 [0.208]	14	
Stilwell, KS; 2000 J6059-00H	480 SC	FORAG	1: Broadcast foliar; End of tillering: 9 or more tillers detectable	20 [190]	0.115 [0.129]	0	0.295 [0.331]
			2: Broadcast foliar; First node at least 1 cm above tillering node	20 [191]	0.180 [0.202]	14	
		HGRST	1: Broadcast foliar; Flag leaf sheath opening	20 [188]	0.113 [0.127]	0	0.293 [0.329]
			2: Broadcast foliar; First grains have reached half their final size	20 [187]	0.180 [0.202]	14	
Oxford, IN; 2000 J6060-00H	480 SC	FORAG	1: Broadcast foliar; 5 tillers visible	15 [139]	0.116 [0.130]	0	0.297 [0.333]
			2: Broadcast foliar; First node at least 1 cm above tillering node	15 [138]	0.181 [0.203]	14	
		HGRST	1: Broadcast foliar; Beginning of heading	14 [133]	0.185 [0.207]	0	0.375 [0.420]
			2: Broadcast foliar; Beginning of flowering; first anthers vis.	15 [142]	0.190 [0.213]	14	

TABLE B.1.2. Study Use Pattern.

Location (City, State/Province; Year) Trial Number	EP[1]	Plot Name[2]	Application				
			Method; Timing	Vol. (GPA[3]) [L/ha]	Rate (lb a.i./A) [kg a.i./ha]	RTI[4] (days)	Total Rate (lb a.i./A) [kg a.i./ha]
Red Deer, AB; 2000 J6061-00H	480 SC	FORAG	1: Broadcast foliar; 5 leaves unfolded	31 [292]	0.112 [0.126]	0	0.300 [0.337]
			2: Broadcast foliar; 8 leaves unfolded	32 [300]	0.188 [0.211]	15	
		HGRST	1: Broadcast foliar; End of heading	32 [303]	0.112 [0.126]	0	0.296 [0.332]
			2: Broadcast foliar; end of flowering	33 [306]	0.184 [0.206]	12	
Monarch, AB; 2000 J6062-00H	480 SC	FORAG	1: Broadcast foliar; 5 leaves unfolded	43 [403]	0.113 [0.127]	0	0.293 [0.329]
			2: Broadcast foliar; First node at least 1 cm above tillering node	42 [393]	0.180 [0.202]	14	
		HGRST	1: Broadcast foliar; Full flowering; 50% of anthers mature	42 [394]	0.111 [0.124]	0	0.291 [0.326]
			2: Broadcast foliar; End of heading	42 [396]	0.180 [0.202]	14	
Benoit, MS; 2000 J6063-00H	480 SC	FORAG	1: Broadcast foliar; 6 leaves unfolded	17 [156]	0.110 [0.124]	0	0.293 [0.329]
			2: Broadcast foliar; Node 3 at least 2 cm above node 2	18 [171]	0.183 [0.205]	14	
		HGRST	1: Broadcast foliar; Flag leaf stage	18 [168]	0.108 [0.122]	0	0.298 [0.335]
			2: Broadcast foliar; End of heading	19 [175]	0.190 [0.213]	12	
Knightdale, NC; 2000 J6064-00H	480 SC	FORAG	1: Broadcast foliar; Beginning of stem elongation	34 [318]	0.112 [0.125]	0	0.290 [0.325]
			2: Broadcast foliar; First node at least 1 cm above tillering node	34 [320]	0.178 [0.200]	13	
		HGRST	1: Broadcast foliar; Early boot stage; flag leaf sheath extending	35 [327]	0.113 [0.127]	0	0.297 [0.333]
			2: Broadcast foliar; Full flowering: 50% of anthers mature	35 [330]	0.184 [0.206]	13	
Minto, MB; 2000 J6066-00H	480 SC	FORAG	1: Broadcast foliar; 4 leaves unfolded	45 [420]	0.119 [0.133]	0	0.306 [0.343]
			2: Broadcast foliar; Node 2 at least 2 cm above node 1	44 [415]	0.187 [0.210]	13	
		HGRST	1: Broadcast foliar; Flag leaf stage	43 [398]	0.113 [0.127]	0	0.299 [0.335]
			2: Broadcast foliar; Full flowering: 50% of anther mature	44 [410]	0.188 [0.208]	14	

TABLE B.1.2. Study Use Pattern.

Location (City, State/Province; Year) Trial Number	EP[1]	Plot Name[2]	Application				
			Method; Timing	Vol. (GPA[3]) [L/ha]	Rate (lb a.i./A) [kg a.i./ha]	RTI[4] (days)	Total Rate (lb a.i./A) [kg a.i./ha]
Minto, MB; 2000 J6067-00H	480 SC	FORAG	1: Broadcast foliar; 5 leaves unfolded	44 [413]	0.117 [0.132]	0	0.302 [0.339]
			2: Broadcast foliar; Node 2 at least 2 cm above node 1	44 [411]	0.185 [0.207]	12	
		HGRST	1: Broadcast foliar; Late boot stage; flag leaf sheath swollen	43 [400]	0.115 [0.129]	0	0.298 [0.334]
			2: Broadcast foliar; Full flowering: 50% of anther mature	43 [402]	0.183 [0.205]	12	
Wakaw, SK; 2000 J6068-00H	480 SC	FORAG	1: Broadcast foliar; Beginning of tillering; first tiller detectable	12 [109]	0.115 [0.129]	0	0.291 [0.326]
			2: Broadcast foliar; First node at least 1 cm above tillering node	12 [108]	0.176 [0.197]	13	
		HGRST	1: Broadcast foliar; Middle of heading	12 [109]	0.114 [0.128]	0	0.295 [0.331]
			2: Broadcast foliar; Full flowering: 50% of anther mature	12 [110]	0.181 [0.203]	9	
Leask, SK; 2000 J6069-00H	480 SC	FORAG	1: Broadcast foliar; 2 tillers detectable	42 [395]	0.112 [0.125]	0	0.291 [0.326]
			2: Broadcast foliar; Early boot stage; flag leaf sheath extending	42 [396]	0.179 [0.201]	15	
		HGRST	1: Broadcast foliar; Early boot stage; flag leaf sheath extending	42 [395]	0.112 [0.125]	0	0.287 [0.321]
			2: Broadcast foliar; Full flowering: 50% of anthers mature	42 [389]	0.175 [0.196]	13	
Rostern, SK; 2000 J6070-00H	480 SC	FORAG	1: Broadcast foliar; 3 tillers detectable	43 [398]	0.112 [0.126]	0	0.286 [0.321]
			2: Broadcast foliar; Flag leaf stage	41 [387]	0.174 [0.195]	13	
		HGRST	1: Broadcast foliar; Flag leaf stage	42 [392]	0.111 [0.124]	0	0.288 [0.323]
			2: Broadcast foliar; Beginning of flowering: first anthers vis.	42 [392]	0.177 [0.198]	14	
Brookdale, MB; 2000 J6071-00H	480 SC	FORAG	1: Broadcast foliar; First node at least 1 cm above node 2	12 [112]	0.114 [0.128]	0	0.296 [0.332]
			2: Broadcast foliar; Node 3 at least 2 cm above node 2	12 [111]	0.182 [0.204]	12	
		HGRST	1: Broadcast foliar; 30% of inflorescence emerged	12 [116]	0.119 [0.133]	0	0.300 [0.336]
			2: Broadcast foliar; Full flowering: 50% of anthers mature	12 [111]	0.181 [0.203]	5	

TABLE B.1.2. Study Use Pattern.

Location (City, State/Province, Year) Trial Number	EP[1]	Plot Name[2]	Application				
			Method; Timing	Vol. (GPA[3]) [L/ha]	Rate (lb a.i./A) [kg a.i./ha]	RTI[4] (days)	Total Rate (lb a.i./A) [kg a.i./ha]
Lancombe, AB; 2000 J6072-00H	480 SC	FORAG	1: Broadcast foliar; 5 leaves unfolded	32 [297]	0.112 [0.126]	0	0.291 [0.327]
			2: Broadcast foliar; 7 leaves unfolded	32 [298]	0.179 [0.201]	14	
		HGRST	1: Broadcast foliar; End of heading	32 [297]	0.112 [0.125]	0	0.290 [0.325]
			2: Broadcast foliar; End of flowering	32 [298]	0.178 [0.201]	12	
Delisle, SK; 2000 J6073-00H	480 SC	FORAG	1: Broadcast foliar; 4 tillers detectable	43 [399]	0.113 [0.127]	0	0.291 [0.327]
			2: Broadcast foliar; 20% of inflorescence emerged	42 [397]	0.178 [0.200]	13	
		HGRST	1: Broadcast foliar; End of heading	43 [399]	0.114 [0.128]	0	0.293 [0.329]
			2: Broadcast foliar; End of flowering	42 [397]	0.179 [0.201]	13	
Delisle, SK; 2000 J6074-00H	480 SC	FORAG	1: Broadcast foliar; 3 tillers detectable	43 [398]	0.112 [0.126]	0	0.290 [0.326]
			2: Broadcast foliar; 20% of inflorescence emerged	42 [392]	0.178 [0.200]	14	
		HGRST	1: Broadcast foliar; 20% of inflorescence emerged	42 [391]	0.112 [0.126]	0	0.290 [0.326]
			2: Broadcast foliar; Full flowering: 50% of anthers mature	42 [394]	0.178 [0.200]	12	
Warner, AB; 2000 J6075-00H	480 SC	FORAG	1: Broadcast foliar; 5 leaves unfolded	42 [393]	0.111 [0.124]	0	0.294 [0.329]
			2: Broadcast foliar; First node at least 1 cm above tillering node	43 [406]	0.183 [0.205]	14	
		HGRST	1: Broadcast foliar; 80% of inflorescence emerged	42 [393]	0.112 [0.125]	0	0.290 [0.324]
			2: Broadcast foliar; First grains have reached half their final size	42 [391]	0.178 [0.199]	14	
Coaldale, AB; 2000 J6076-00H	480 SC	FORAG	1: Broadcast foliar; 5 leaves unfolded	42 [393]	0.112 [0.125]	0	0.289 [0.323]
			2: Broadcast foliar; 3 tillers detectable	43 [398]	0.177 [0.198]	14	
		HGRST	1: Broadcast foliar; Full flowering: 50% of anthers mature	43 [400]	0.114 [0.128]	0	0.295 [0.331]
			2: Broadcast foliar; End of flowering	43 [399]	0.181 [0.203]	14	

TABLE B.1.2. Study Use Pattern.

Location (City, State/Province; Year) Trial Number	EP[1]	Plot Name[2]	Application				
			Method; Timing	Vol. (GPA[3]) [L/ha]	Rate (lb a.i./A) [kg a.i./ha]	RTI[4] (days)	Total Rate (lb a.i./A) [kg a.i./ha]
Kipp, AB; 2000 J6077-00H	480 SC	FORAG	1: Broadcast foliar; 5 leaves unfolded	43 [400]	0.112 [0.126]	0	0.290 [0.326]
			2: Broadcast foliar; First node at least 1 cm above tillering node	42 [394]	0.178 [0.200]	14	
		HGRST	1: Broadcast foliar; Full flowering: 50% of anthers mature	44 [408]	0.115 [0.129]	0	0.295 [0.331]
			2: Broadcast foliar; End of flowering	42 [397]	0.180 [0.202]	14	
Tifton, GA; 2000 J6169-00H	480 SC	TRTD	1: Broadcast foliar; 6 tillers detectable	11 [105]	0.113 [0.126]	0	0.293 [0.328]
			2: Broadcast foliar; Beginning of stem elongation	14 [135]	0.180 [0.202]	14	
Leland, MS; 2000 J6170-00H	480 SC	TRTD	1: Broadcast foliar; 7 tillers detectable	17 [159]	0.114 [0.128]	0	0.299 [0.336]
			2: Broadcast foliar; Beginning of heading	18 [169]	0.185 [0.208]	14	
Stilwell, KS; 2000 J6171-00H	480 SC	TRTD	1: Broadcast foliar; Beginning of stem elongation	15 [143]	0.111 [0.125]	0	0.288 [0.324]
			2: Broadcast foliar; Node 2 at least 2 cm above node 1	13 [120]	0.177 [0.199]	14	
Louisville, NE; 2000 J6172-00H	480 SC	TRTD	1: Broadcast foliar; 6 tillers detectable	18 [168]	0.113 [0.127]	0	0.293 [0.328]
			2: Broadcast foliar; 8 tillers detectable	16 [147]	0.180 [0.201]	14	
Uvalde, TX; 2000 J6173-00H	480 SC	TRTD	1: Broadcast foliar; 6 tillers detectable	17 [161]	0.111 [0.124]	0	0.293 [0.328]
			2: Broadcast foliar; 7 tillers detectable	23 [218]	0.182 [0.204]	14	
New Rockford, ND; 2000 J6174-00HA	480 SC	TRTD	1: Broadcast foliar; First leaf unfolded	30 [282]	0.114 [0.127]	0	0.293 [0.328]
			2: Broadcast foliar; 4 leaves unfolded	30 [279]	0.179 [0.201]	14	
Eldridge, ND; 2000 J6175-00HA	480 SC	TRTD	1: Broadcast foliar; 5 tillers visible	30 [280]	0.112 [0.125]	0	0.299 [0.334]
			2: Broadcast foliar; First node at least 1 cm above tillering node	31 [290]	0.187 [0.209]	12	
Britton, SD 2000 J6176-00H	480 SC	TRTD	1: Broadcast foliar; 2 leaves unfolded	15 [140]	0.114 [0.128]	0	0.295 [0.330]
			2: Broadcast foliar; 4 tillers detectable	15 [140]	0.181 [0.202]	13	

TABLE B.1.2. Study Use Pattern.

Location (City, State/Province; Year) Trial Number	EP[1]	Plot Name[2]	Application				
			Method; Timing	Vol. (GPA[3]) [L/ha]	Rate (lb a.i./A) [kg a.i./ha]	RTI[4] (days)	Total Rate (lb a.i./A) [kg a.i./ha]
Dundurn, SK; 2000 J6177-00H	480 SC	TRTD	1: Broadcast foliar; 3 leaves unfolded	12 [109]	0.111 [0.125]	0	0.288 [0.323]
			2: Broadcast foliar; Beginning of tillering: first tiller detectable	12 [109]	0.177 [0.198]	13	
Taber, AB; 2000 J6178-00H	480 SC	TRTD	1: Broadcast foliar; 4 leaves unfolded	11 [99]	0.112 [0.126]	0	0.298 [0.335]
			2: Broadcast foliar; End of tillering: 9 or more tillers detectable	11 [103]	0.186 [0.209]	14	
Levelland, TX; 2000 J6179-00H	480 SC	TRTD	1: Broadcast foliar; 5 tillers visible	20 [190]	0.113 [0.127]	0	0.291 [0.327]
			2: Broadcast foliar; First node at least 1 cm above tillering node	20 [185]	0.181 [0.203]	16	
Hart, TX; 2000 J6180-00H	480 SC	TRTD	1: Broadcast foliar; 8 tillers visible	19 [176]	0.113 [0.127]	0	0.294 [0.330]
			2: Broadcast foliar; Beginning of stem elongation	19 [181]	0.181 [0.203]	14	
Wolforth, TX ; 2000 J6181-00H	480 SC	TRTD	1: Broadcast foliar; 8 tillers visible	20 [190]	0.112 [0.126]	0	0.294 [0.330]
			2: Broadcast foliar; First node at least 1 cm above tillering node	20 [187]	0.182 [0.204]	14	
Colony, OK; 2000 J6182-00H	480 SC	TRTD	1: Broadcast foliar; 7 tillers detectable	14 [129]	0.115 [0.128]	0	0.291 [0.325]
			2: Broadcast foliar; Node 2 at least 2 cm above node 1	13 [123]	0.176 [0.197]	13	
Hood River, OR; 2000 J6183-00H	480 SC	TRTD	1: Broadcast foliar; 3 leaves unfolded	21 [195]	0.110 [0.123]	0	0.292 [0.327]
			2: Broadcast foliar; 9 or more tillers detectable	22 [205]	0.182 [0.204]	14	
Minot, MB; 2000 J6184-00H	480 SC	TRTD	1: Broadcast foliar; 3 leaves unfolded	16 [150]	0.111 [0.125]	0	0.290 [0.326]
			2: Broadcast foliar; 2 tillers detectable	16 [151]	0.179 [0.201]	15	
Rosthern, SK; 2000 J6185-00HA	480 SC	TRTD	1: Broadcast foliar; 2 leaves unfolded	12 [108]	0.112 [0.125]	0	0.289 [0.324]
			2: Broadcast foliar; 3 tillers detectable	12 [109]	0.177 [0.199]	14	
Leduc, AB; 2000 J6186-00H	480 SC	TRTD	1: Broadcast foliar; 3 leaves unfolded	11 [105]	0.112 [0.126]	0	0.291 [0.326]
			2: Broadcast foliar; 2 tillers detectable	12 [109]	0.179 [0.200]	14	

TABLE B.1.2. Study Use Pattern.

Location (City, State/Province; Year) Trial Number	EP[1]	Plot Name[2]	Application				
			Method; Timing	Vol. (GPA[3]) [L/ha]	Rate (lb a.i./A) [kg a.i./ha]	RTI[4] (days)	Total Rate (lb a.i./A) [kg a.i./ha]
Spruce Grove, AB; 2000 J6187-00H	480 SC	TRTD	1: Broadcast foliar; 2 leaves unfolded	12 [109]	0.109 [0.123]	0	0.287 [0.322]
			2: Broadcast foliar; Beginning of tillering: first tiller detectable	11 [104]	0.178 [0.199]	13	
Brookdale, MB; 2000 J6188-00H	480 SC	TRTD	1: Broadcast foliar; 2 leaves unfolded	12 [109]	0.110 [0.123]	0	0.288 [0.322]
			2: Broadcast foliar; 5 leaves unfolded	18 [167]	0.178 [0.199]	14	
Bethany, MB; 2000 J6189-00H	480 SC	TRTD	1: Broadcast foliar; 2 leaves unfolded	12 [112]	0.113 [0.126]	0	0.286 [0.320]
			2: Broadcast foliar; 4 leaves unfolded	18 [164]	0.173 [0.194]	14	

[1] EP = End-use Product; 480 SC = 4 lb/gal (480 g/L) suspension concentrate (SC) or flowable concentrate (FlC) formulation.
[2] For 33 trials, two treatment plots (FORAG and HGRST) were used, and for 21 trials, a single treatment plot (TRTD) was used. The timing of the application was different for each plot. For the FORAG plots, the second application was made 1 day prior to the first cutting of forage. For the HGRST plots, the second application occurred at full flowering. For the TRTD plots, the second application was made 7 days prior to the first cutting of forage.
[3] GPA = Gallons per acre
[4] RTI = Re-treatment Interval

TABLE B.1.3. Trial Numbers and Geographical Locations.

NAFTA Growing Zones	WHEAT			
	Submitted		Requested	
	Forage only	Hay, Grain and Straw	Canada	U.S.
1				
1A				
2	1	1		1
3				
4	1	1		1
5	2+1 [a]	5	2	5
5A				
5B				
6	1	1		1
7	4+1 [b]	7	7	5
7A	1	1	1	
8	4	6		6
9				
10				
11	1	1		1
12				
13				
14	6	10	10	
15				
16				
17				
18				
19				
20				
21				
Total	23	33	20	20

[a] Forage from FORAG decline plot (J6046-00D)
[b] Forage from FORAG decline plot (J6047-00D)

B.2. Sample Handling and Preparation

Wheat hay from the HGRST plots was cut 12- 17 days (average = 14 days) after the last application and left in the field to dry. Samples of wheat hay were collected 0-14 days after the hay was cut. Samples of wheat forage from the TRTD plots plus two HGRST plots (decline trials) were collected 7 days after the last application. Samples of wheat grain and straw from the HGRST plots were collected at earliest commercial harvest, 30-57 days (average = 42 days) after the last application, except in one trial in which samples were harvested 10 days after second application. Samples were frozen within 4 hours of collection and were shipped frozen to Battelle-AgriFood Laboratories (Columbus, OH) or directly to Bayer Research Park (BRP; Stilwell, KS). All samples were homogenized with dry ice and stored frozen until shipment or analysis at BRP.

B.3. Analytical Methodology

Samples of wheat hay, grain, straw, and forage were analyzed for total prothioconazole-derived residues (prothioconazole and the metabolite prothioconazole-desthio) using LC-MS/MS method RPA JA/03/01 with modifications. Samples were extracted with a mixture of methanol, 30% hydrogen peroxide, and 0.05 M 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 was spiked 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. Residue levels were quantitated from a calibration curve of mixed reference standards of prothioconazole, as the sulfonic acid, and prothioconazole-desthio. The method was modified to use a different solvent for preparation of the spiking solutions and to use slightly different m/z values for the quantitation ions. The validated LOQs for the total prothioconazole-derived residues were 0.02 ppm for wheat grain and 0.05 ppm for wheat forage, hay, and straw.

Samples were analyzed for residues of 1*H*-1,2,4-triazole and the triazole conjugates (triazolylalanine and triazolylacetic acid) using an LC-MS/MS method (Bayer Report No. G200598) with modifications.Briefly, crop matrices were extracted with aqueous methanol, and three separate aliquots of the extract were removed for determination of each of the three analytes. Isotopically labeled internal standard was added to each aliquot. For 1*H*-1,2,4-triazole, the aliquot was mixed with dansyl chloride to form the dansyl derivative of 1*H*-1,2,4-triazole, which was partitioned into ethyl acetate and then redissolved in acetonitrile (ACN)/water for LC-MS/MS analysis. For triazolylalanine, the aliquot was cleaned up by SPE, derivatized to the butyl ester using butanolic HCl, and then further derivatized using heptafluorobutyric anhydride (HFBA). The mixture was redissolved in ACN/water for LC-MS/MS analysis. For determination of triazolylacetic acid, the aliquot was cleaned up by SPE, derivatized to the butyl ester using butanolic HCl, and then redissolved in ACN/water for LC-MS/MS analysis. Residue levels were quantitated from a calibration curve of reference standards of dansyl-1,2,4-triazole, triazolylalanine butyl ester HFBA, and triazolylacetic acid butyl ester (generated by derivatizing

reference standard solutions of triazolylacetic acid). The validated LOQ for 1*H*-1,2,4-triazole and triazolylalanine was 0.01 ppm for wheat forage, hay, grain, and straw, and the validated LOQs for triazolylacetic acid were 0.01 ppm for wheat forage, hay, and grain and 0.025 ppm for wheat straw.

C. RESULTS AND DISCUSSION

Concurrent method recovery data are presented in TABLE C.1. Samples were analyzed for total prothioconazole-derived residues (prothioconazole and the metabolite prothioconazole-desthio) using LC-MS/MS method RPA JA/03/01. The validated LOQs for the total prothioconazole-derived residues were 0.02 ppm for wheat grain and 0.05 ppm for wheat forage, hay, and straw. The method is adequate for data collection for wheat grain, forage, hay and straw based on acceptable concurrent method recovery data and method validation data. Samples were analyzed for residues of 1*H*-1,2,4-triazole and the triazole conjugates triazolylalanine and triazolylacetic acid using an LC-MS/MS method (Bayer Report No. G200598). The validated LOQ for 1*H*-1,2,4-triazole and triazolylalanine was 0.01 ppm for wheat forage, hay, grain, and straw, and the validated LOQs for triazolylacetic acid were 0.01 ppm for wheat forage, hay, and grain and 0.025 ppm for wheat straw. The methods are adequate for data collection in wheat matrices based on acceptable concurrent method recovery data.

Apparent total prothioconazole-derived residues were below the method LOQs (<0.02 ppm for wheat grain and <0.05 ppm for wheat hay, straw, and forage) in/on all samples of untreated wheat hay, grain, straw, and forage; and residues of 1*H*-1,2,4-triazole were below the method LOQ (<0.01 ppm) in/on all samples of untreated wheat hay, grain, straw, and forage. Apparent residues of triazole conjugates were below the method LOQs (<0.01 ppm for wheat hay, grain, and forage and <0.025 ppm for wheat straw) in/on samples of untreated wheat hay (n=30), grain (n=23), straw (n=32), and forage (n=25), with the following exceptions. Quantifiable apparent residues of triazole conjugates were observed in: (i) 15 samples of untreated wheat hay (residue range of 0.011-0.253 ppm); (ii) 18 samples of untreated wheat grain (residue range of 0.035-0.707 ppm); (iii) 5 samples of untreated wheat straw (residue range of 0.030-0.147 ppm); and (iv) 3 samples of untreated wheat forage (residue range of 0.011-0.077 ppm). The measurable control residues may have resulted from the use of triazole fungicides from previous seasons or from naturally occurring triazole conjugates.

Sample storage conditions and intervals are summarized in TABLE C.2. The maximum storage intervals of crop samples from harvest to analysis for total prothioconazole-derived residues were 469 days (15.4 months) for wheat forage, 1214 days (39.9 months) for wheat grain, 1221 days (40.1 months) for wheat hay, and 1203 days (39.5 months) for wheat straw. Prothioconazole-derived residues and prothioconazole-desthio residues are stable for up to 1 year based on an interim report. The need to correct the results of the current study for decline of prothioconazole-derived residues will not be necessary taking into account the absolute (ppm) and relative % residue levels in wheat matrices. The degree of loss of prothioconazole-desthio residues is not expected to exceed 30% after 40.1 months.

The maximum storage intervals of crop samples from harvest to analysis for 1*H*-1,2,4-triazole and triazole conjugate residues were 400 days (13.1 months) for wheat forage, 1196 days (39.3 months) for wheat grain, 1192 days (39.2 months) for wheat hay, 1221 days (40.1 months) for wheat straw.

Residue data from the wheat field trials are reported in TABLE C.3. A summary of prothioconazole residue data for wheat hay, grain, straw, and forage is presented in TABLE C.4. Residues in/on wheat harvested 10-57 days (12-17 days for hay) following the last of two broadcast foliar applications at a total seasonal rate of 0.281-0.375 lb a.i./A (0.315-0.420 kg a.i./ha) were 0.288-3.571 ppm in/on wheat hay, <0.02-0.061 ppm in/on wheat grain, and 0.106-1.96 ppm in/on wheat straw for the total prothioconazole-derived residues. Residues of 1*H*-1,2,4-triazole were less than the *LOQ* (<0.01 ppm) in/on wheat hay, grain, and straw, and residues of the triazole conjugates were 0.018-0.665 ppm in/on wheat hay, 0.098-1.76 ppm in/on wheat grain, and <0.025-0.495 ppm in/on wheat straw. Residues in/on wheat forage harvested 7 days following the last of two broadcast foliar applications at a total seasonal rate of 0.286-0.299 lb a.i./A (0.320-0.336 kg a.i./ha) were 0.061-6.987 ppm for total prothioconazole-derived residues, less than the LOQ (<0.01 ppm) for 1*H*-1,2,4-triazole, and <0.01-0.175 ppm for the triazole conjugates.

In the residue decline trials, residues of 1*H*-1,2,4-triazole at all sampling intervals were less than the method LOQ (<0.01 ppm) in/on wheat hay, grain, straw, and forage for both trials (NE and ND). Total prothioconazole-derived residues did not increase in any wheat matrix with increasing sampling intervals, and residues of the triazole conjugates increased slightly in samples of wheat forage from both trials and in wheat straw from one trial but did not increase in wheat hay or grain with increasing sampling intervals.

TABLE C.1. Summary of Concurrent Recoveries of Prothioconazole from Wheat.

Matrix	Analyte	Spike level (ppm)	Sample size (n)	Recoveries (%)	Mean ± std dev
Hay	Prothioconazole	0.05	7	76, 76, 78, 75, 80, 81, 79	77.9 ± 2.3
		0.10	4	75, 75, 80, 84	79 ± 4.4
		1.0	7	76, 77, 77, 72, 71, 73, 74	74.3 ± 2.4
		5.0	3	82, 82, 83	82.3 ± 0.6
	Prothioconazole-desthio	0.05	7	98, 96, 93, 98, 94, 95, 98	96 ± 2.1
		0.10	4	87, 92, 97, 103	95 ± 6.8
		1.0	7	99, 102, 105, 102, 102, 102, 105	102.4 ± 2.1
		5.0	3	96, 97, 98	97 ± 1
	1*H*-1,2,4-triazole	0.05	9	72, 73, 73, 74, 75, 76, 77, 89, 92	77.9 ± 7.4
		0.10	1	91	91
	Triazolylalanine	0.025	3	85, 88, 105	92.7 ± 10.8
		0.05	9	77, 80, 82, 83, 85, 86, 87, 87, 97	84.9 ± 5.6
		0.10	1	104	104
	Triazolylacetic acid	0.01	3	72, 74, 88	78 ± 8.7
		0.05	9	71, 76, 76, 77, 85, 86, 92, 100, 110	85.9 ± 12.8
		0.10	1	91	91
Grain	Prothioconazole	0.02	4	66, 72, 73, 77	72 ± 4.5
	Prothioconazole-desthio	0.02	4	93, 94, 100, 102	97 ± 4.4
	1*H*-1,2,4-triazole	0.05	6	73, 78, 82, 84, 85, 89	82 ± 5.6
	Triazolylalanine	0.05	7	73, 74, 75, 77, 79, 99, 101	83 ± 12.1
	Triazolylacetic acid	0.05	7	72, 72, 77, 85, 97, 98, 99	86 ± 12.3
Straw	Prothioconazole	0.10	4	81, 82, 83, 84	83 ± 1.3
	Prothioconazole-desthio	0.10	4	95, 98, 102, 102	99 ± 3.4
	1*H*-1,2,4-triazole	0.05	8	77, 77, 81, 81, 82, 87, 90, 104	85 ± 8.9
	Triazolylalanine	0.05	8	76, 77, 80, 82, 90, 91, 93, 95	86 ± 7.6
	Triazolylacetic acid	0.05	8	77, 79, 80, 82, 85, 87, 88, 100	85 ± 7.3

TABLE C.1. Summary of Concurrent Recoveries of Prothioconazole from Wheat.

Matrix	Analyte	Spike level (ppm)	Sample size (n)	Recoveries (%)	Mean ± std dev
Forage	Prothioconazole	0.05	7	83, 80, 82, 85, 81, 84, 79	82 ± 2.2
		0.10	3	70, 78, 87	78 ± 8.5
		1.0	6	82, 81, 82, 83, 81, 81	81.7 ± 0.8
		15.0	3	68, 74, 74	72 ± 3.5
	Prothioconazole-desthio	0.05	7	96, 95, 98, 96, 94, 94, 92	95 ± 1.9
		0.10	3	91, 94, 102	96 ± 5.7
		1.0	7	99, 101, 100, 97, 100, 100, 106	100.4 ± 2.8
		15.0	3	98, 98, 97	97.7 ± 0.5
	1*H*-1,2,4-triazole	0.01	3	59, 71, 73	67.7 ± 7.6
		0.05	3	78, 93, 96	89 ± 9.6
		0.10	3	71, 78, 89	79.3 ± 9.1
	Triazolylalanine	0.05	3	84, 88, 98	90 ± 7.2
		0.10	3	83, 89, 93	88.3 ± 5.0
	Triazolylacetic acid	0.05	3	72, 74, 87	77.7 ± 8.1
		0.10	3	79, 82, 84	81.7 ± 2.5

TABLE C.2. Summary of Storage Conditions.

Matrix (RAC or Extract)	Storage Temp. (°C)	Actual Storage Duration [1]	Interval of Demonstrated Storage Stability
Total Prothioconazole-Derived Residues			
Wheat, hay	-4.8 to -30.0	871-1221 days (28.6-40.1 months)	Stability demonstrated for up to 12.7 months (interim report). Awaiting final report as confirmatory data.
Wheat, grain		873-1214 days (28.7-39.9 months)	
Wheat, straw		854-1203 days (28.1-39.5 months)	
Wheat, forage		181-469 days (6.0-15.4 months)	
1*H*-1,2,4-triazole and Triazole Conjugate Residues			
Wheat, hay	-4.8 to -30.0	796-1192 days (26.2-39.2 months)	Not applicable at this time. Awaiting final report.
Wheat, grain		764-1196 days (25.1-39.3 months)	
Wheat, straw		755-1221 days (24.8-40.1 months)	
Wheat, forage		133-400 days (4.4-13.1 months)	

[1] Actual storage duration from collection to analysis; hay samples were collected 0-14 days after plants were cut and left in the field. All samples were analyzed within 0-6 days of extraction.

TABLE C.3. Residue Data from Crop Field Trials with Prothioconazole.

Location (City, State/Province, Year) Trial Number	Region	Crop; Variety	Commodity or Matrix	Total Rate (lb a.i./A) [kg a.i./ha]	PHI (days)	Total Prothioconazole-derived Residues (ppm)	1*H*-1,2,4-triazole Residues[1] (ppm)	Triazole Conjugate Residues[1] (ppm)
St. George, ON; 2000 *J6044-00H*	5	Wheat; Tahoe	Hay	0.303 [0.339]	17	0.288, 0.332	<0.01, <0.01	0.386, 0.346
			Grain		42	<0.02, <0.02	<0.01, <0.01	0.960, 1.417
			Straw		42	0.174, 0.231	<0.01, <0.01	0.269, 0.373
Louisville, NE; 2000 J6045-00D (decline study)	5	Wheat; Arapahoe	Hay	0.293 [0.329]	7	0.637, 0.774	<0.01, <0.01	0.117, 0.125
					14	0.417, 0.482	<0.01, <0.01	0.115, 0.173
					21	0.191, 0.219	<0.01, <0.01	0.220, 0.261
					28	<0.10, 0.102	<0.01, <0.01	0.190, 0.216
			Grain		35	<0.02, <0.02	<0.01, <0.01	0.544, 0.584
					39	<0.02, <0.02	<0.01, <0.01	0.526, 0.544
					44	<0.02, <0.02	<0.01, <0.01	0.418, 0.471
					49	<0.02, <0.02	<0.01, <0.01	0.507, 0.561
			Straw		35	0.210, 0.252	<0.01, <0.01	0.098, 0.080
					39	0.182, 0.209	<0.01, <0.01	0.196, 0.142
					44	0.196, 0.253	<0.01, <0.01	0.151, 0.129
					49	0.209, 0.231	<0.01, <0.01	0.127, 0.160
			Forage		0	7.647, 12.295	<0.01, <0.01	0.023, 0.017
					1	8.046, 8.212	<0.01, <0.01	0.029, 0.013
					7	1.383, 1.827	<0.01, <0.01	0.031, 0.031
					14	0.273, 0.325	<0.01, <0.01	0.040, 0.050
New Rockford, ND; 2000 *J6046-00D* (decline study)	7	Wheat; Russ Wheat	Hay	0.291 [0.326]	6	3.088, 3.096	<0.01, <0.01	0.329, 0.293
					14	0.675, 0.898	<0.01, <0.01	0.249, 0.202
					20	0.306, 0.569	<0.01, <0.01	0.128, 0.227
					28	0.325, 0.601	<0.01, <0.01	0.277, 0.299
			Grain		36	<0.02, <0.02	<0.01, <0.01	0.512, 0.728
					40	<0.02, <0.02	<0.01, <0.01	0.521, 0.560
					46	<0.02, <0.02	<0.01, <0.01	0.522, 0.567
					50	<0.02, <0.02	<0.01, <0.01	0.510, 0.589
			Straw		36	0.389, 0.661	<0.01, <0.01	0.286, 0.219
					40	0.327, 0.352	<0.01, <0.01	0.204, 0.211
					46	0.0493	<0.01, <0.01	0.101
					50	0.309, 0.480	<0.01, <0.01	0.082, 0.116
			Forage		0	7.234, 7.465	<0.01, <0.01	0.016, 0.018
					1	3.035, 3.358	<0.01, <0.01	0.034, 0.036
					7	0.233, 0.263	<0.01, <0.01	0.076, 0.115
					14	<0.10, <0.10	<0.01, <0.01	0.066, 0.073

TABLE C.3. Residue Data from Crop Field Trials with Prothioconazole.

Location (City, State/Province, Year) Trial Number	Region	Crop; Variety	Commodity or Matrix	Total Rate (lb a.i./A) [kg a.i./ha]	PHI (days)	Total Prothioconazole-derived Residues (ppm)	1*H*-1,2,4-triazole Residues[1] (ppm)	Triazole Conjugate Residues[1] (ppm)
Hermiston, OR; 2000 *J6047-00H*	11	Wheat; Penawawa	Hay	0.299 [0.335]	13	0.339, 0.374	<0.01, <0.01	0.597, 0.665
			Grain		42	<0.02, <0.02	<0.01, <0.01	1.089, 1.223
			Straw		42	0.113, 0.136	<0.01, <0.01	0.403, 0.495
Uvalde, TX; 2000 J6048-00H	8	Wheat; Ogallala	Hay	0.293 [0.328]	14	2.998, 3.182	<0.01, <0.01	0.298, 0.304
			Grain		42	<0.02, <0.02	<0.01, <0.01	0.514, 0.594
			Straw		42	0.731, 0.846	<0.01, <0.01	0.098, 0.081
Claude, TX; 2000 J6049-00H	8	Wheat; Jagger	Hay	0.295 [0.331]	14	0.612, 0.668	<0.01, <0.01	0.142, 0.135
			Grain		41	<0.02, <0.02	<0.01, <0.01	0.327, 0.354
			Straw		41	0.307, 0.328	<0.01, <0.01	<0.025, <0.025
Cordell, OK; 2000 J6050-00H	8	Wheat; Custer	Hay	0.313 [0.351]	14	1.252, 1.660	<0.01, <0.01	0.248, 0.161
			Grain		38	<0.02, <0.02	<0.01, <0.01	0.392, 0.408
			Straw		38	0.615, 0.767	<0.01, <0.01	0.063, 0.027
Frederick, OK; 2000 J6051-00H	8	Wheat; Custer	Hay	0.281 [0.315]	14	0.432, 0.490	<0.01, <0.01	0.098, 0.095
			Grain		10	<0.02, <0.02	<0.01, <0.01	0.352, 0.411
			Straw		10	0.650	<0.01, <0.01	0.107
Hart, TX; 2000 J6052-00H	8	Wheat; Tam 200	Hay	0.291 [0.327]	13	1.889, 1.928	<0.01, <0.01	0.096, 0.094
			Grain		35	<0.02, <0.02	<0.01, <0.01	0.180, 0.198
			Straw		35	1.359, 1.643	<0.01, <0.01	<0.025, <0.025
Velva, ND; 2000 J6053-00H	7	Wheat; 2375	Hay	0.294 [0.328]	13	0.322, 0.401	<0.01, <0.01	0.171, 0.206
			Grain		33	<0.02, <0.02	<0.01, <0.01	0.182, 0.263
			Straw		33	0.106, 0.151	<0.01, <0.01	0.052, 0.097
Levelland, TX; 2000 J6054-00H	8	Wheat; TAN 202, Lot Star 10	Hay	0.294 [0.330]	13	2.055, 2.568	<0.01, <0.01	0.028, 0.018
			Grain		43	<0.02, <0.02	<0.01, <0.01	0.147, 0.183
			Straw		43	0.305, 1.854	<0.01, <0.01	<0.025
Ellendale, ND; 2000 J6055-00H	7	Wheat; Alsen	Hay	0.290 [0.326]	12	0.545, 0.580	<0.01, <0.01	0.241, 0.273
			Grain		39	<0.02, <0.02	<0.01, <0.01	0.743, 0.752
			Straw		39	0.181, 0.217	<0.01, <0.01	0.154, 0.146
Lake Andes, SD; 2000 J6056-00H	7	Wheat; Forge spring	Hay	0.290 [0.325]	13	2.442, 3.569	<0.01, <0.01	0.167, 0.152
			Grain		46	<0.02, 0.026	<0.01, <0.01	0.334, 0.349
			Straw		46	1.379	<0.01, <0.01	0.073, 0.086
Paris, ON; 2000 J6057-00H	5	Wheat; Tahoe2000	Hay	0.291 [0.326]	17	0.447, 0.463	<0.01, <0.01	0.557, 0.381
			Grain		42	<0.02, <0.02	<0.01, <0.01	1.760, 1.760
			Straw		42	0.261, 0.264	<0.01, <0.01	0.366, 0.313

TABLE C.3. Residue Data from Crop Field Trials with Prothioconazole.

Location (City, State/Province, Year) Trial Number	Region	Crop; Variety	Commodity or Matrix	Total Rate (lb a.i./A) [kg a.i./ha]	PHI (days)	Total Prothioconazole-derived Residues (ppm)	1*H*-1,2,4-triazole Residues[1] (ppm)	Triazole Conjugate Residues[1] (ppm)
East Bernard, TX; 2000 J6058-00H	6	Wheat; Mit	Hay	0.292 [0.327]	12	0.710, 1.063	<0.01, <0.01	0.109, 0.239
			Grain		32	<0.02, <0.02	<0.01, <0.01	0.400, 0.406
			Straw		32	0.930, 1.053	<0.01, <0.01	0.025, 0.029
Stilwell, KS; 2000 J6059-00H	5	Wheat; Karl 92	Hay	0.293 [0.329]	16	0.344, 0.612	<0.01, <0.01	0.351, 0.343
			Grain		42	<0.02, <0.02	<0.01, <0.01	0.620, 0.694
			Straw		42	0.251, 0.286	<0.01, <0.01	0.066, 0.050
Oxford, IN; 2000 J6060-00H	5	Wheat; Becks 107	Hay	0.375 [0.420]	14	0.567, 0.638	<0.01, <0.01	0.215, 0.307
			Grain		43	<0.02, <0.02	<0.01, <0.01	0.515, 0.569
			Straw		43	0.214, 0.217	<0.01, <0.01	0.078, 0.062
Red Deer, AB; 2000 J6061-00H	14	Wheat; Barrie	Hay	0.296 [0.332]	14	0.341, 0.350	<0.01, <0.01	0.305, 0.245
			Grain		57	<0.02, <0.02	<0.01, <0.01	0.438, 0.474
			Straw		57	0.168, 0.178	<0.01, <0.01	0.050, 0.038
Monarch, AB; 2000 J6062-00H	14	Wheat; HRS wheat Prodigy	Hay	0.291 [0.326]	14	1.822, 2.509	<0.01, <0.01	0.188, 0.231
			Grain		30	0.040, 0.051	<0.01, <0.01	0.556, 0.534
			Straw		30	0.393, 0.485	<0.01, <0.01	<0.025, 0.031
Benoit, MS; 2000 J6063-00H	4	Wheat; Pioneer 2684	Hay	0.298 [0.335]	13	1.470, 1.761	<0.01, <0.01	0.206, 0.183
			Grain		42	<0.02, <0.02	<0.01, <0.01	0.338, 0.534
			Straw		42	0.511, 0.629	<0.01, <0.01	<0.025, 0.092
Knightdale, NC; 2000 J6064-00H	2	Wheat; COOKER 107	Hay	0.297 [0.333]	14	1.928, 2.501	<0.01, <0.01	0.121, 0.148
			Grain		37	<0.02, <0.02	<0.01, <0.01	0.435, 0.439
			Straw		37	1.284, 1.548	<0.01, <0.01	0.041, 0.068
Minto, MB; 2000 J6066-00H	14	Wheat; AC Cora	Hay	0.299 [0.335]	14	1.489, 1.500	<0.01, <0.01	0.065, 0.121
			Grain		47	<0.02, <0.02	<0.01, <0.01	0.560, 0.637
			Straw		47	0.313, 0.328	<0.01, <0.01	0.053, 0.032
Minto, MB; 2000 J6067-00H	14	Wheat; AC Cora	Hay	0.298 [0.334]	14	3.515, 3.571	<0.01, <0.01	0.127, 0.093
			Grain		49	<0.02, <0.02	<0.01, <0.01	0.518, 0.634
			Straw		49	0.804, 0.858	<0.01, <0.01	0.113, 0.080
Wakaw, SK; 2000 J6068-00H	14	Wheat; AC Barrie	Hay	0.295 [0.331]	12	2.866, 3.305	<0.01, <0.01	0.158, 0.229
			Grain		55	<0.02, <0.02	<0.01, <0.01	0.419, 0.430
			Straw		55	0.663, 0.789	<0.01, <0.01	<0.025, <0.025
Leask, SK; 2000 J6069-00H	14	Wheat; McKenzie	Hay	0.287 [0.321]	12	1.420, 1.632	<0.01, <0.01	0.351, 0.417
			Grain		48	<0.02, <0.02	<0.01, <0.01	0.442, 0.466
			Straw		48	0.483, 0.495	<0.01, <0.01	0.067, 0.107

TABLE C.3. Residue Data from Crop Field Trials with Prothioconazole.

Location (City, State/Province; Year) Trial Number	Region	Crop; Variety	Commodity or Matrix	Total Rate (lb a.i./A) [kg a.i./ha]	PHI (days)	Total Prothioconazole-derived Residues (ppm)	1*H*-1,2,4-triazole Residues[1] (ppm)	Triazole Conjugate Residues[1] (ppm)
Rostern, SK; 2000 J6070-00H	14	Wheat; AC Cadillac	Hay	0.288 [0.323]	14	0.809, 0.981	<0.01, <0.01	0.198, 0.184
			Grain		53	<0.02, <0.02	<0.01, <0.01	0.393, 0.396
			Straw		53	0.233, 0.244	<0.01, <0.01	<0.025, <0.025
Brookdale, MB; 2000 J6071-00H	14	Wheat; Barrie (certified)	Hay	0.300 [0.336]	14	2.601, 3.149	<0.01, <0.01	0.177, 0.123
			Grain		43	0.025, 0.040	<0.01, <0.01	0.788, 0.794
			Straw		43	0.228, 0.282	<0.01, <0.01	<0.025, <0.025
Lancombe, AB; 2000 J6072-00H	14	Wheat; Barrie	Hay	0.290 [0.325]	14	1.080, 1.142	<0.01, <0.01	0.302, 0.149
			Grain		57	<0.02, <0.02	<0.01, <0.01	0.604, 0.605
			Straw		57	0.322, 0.347	<0.01, <0.01	0.108, 0.153
Delisle, SK; 2000 J6073-00H	7	Wheat; AC Barrie	Hay	0.293 [0.329]	15	0.821, 0.995	<0.01, <0.01	0.235, 0.213
			Grain		38	<0.02, <0.02	<0.01, <0.01	0.327, 0.359
			Straw		38	0.311, 0.311	<0.01, <0.01	0.032, 0.037
Delisle, SK; 2000 J6074-00H	7	Wheat; Prodigy	Hay	0.290 [0.326]	15	1.431, 1.693	<0.01, <0.01	0.242, 0.190
			Grain		43	<0.02, <0.02	<0.01, <0.01	0.414, 0.454
			Straw		43	0.575, 0.633	<0.01, <0.01	0.061, 0.029
Warner, AB; 2000 J6075-00H	7	Wheat; HRS wheat Prodigy	Hay	0.290 [0.324]	14	1.308, 2.287	<0.01, <0.01	0.079, 0.072
			Grain		31	0.029, 0.040	<0.01, <0.01	0.098, 0.115
			Straw		31	1.838, 1.960	<0.01, <0.01	0.053, 0.052
Coaldale, AB; 2000 J6076-00H	7A	Wheat; Prodigy	Hay	0.295 [0.331]	14	1.286, 1.886	<0.01, <0.01	0.253, 0.266
			Grain		35	<0.02, 0.024	<0.01, <0.01	0.488, 0.499
			Straw		35	1.052, 1.058	<0.01, <0.01	0.109, 0.076
Kipp, AB; 2000 J6077-00H	14	Wheat; Prodigy	Hay	0.295 [0.331]	14	1.641, 2.428	<0.01, <0.01	0.291, 0.282
			Grain		30	0.028, 0.061	<0.01, <0.01	0.410, 0.389
			Straw		30	0.443, 0.515	<0.01, <0.01	0.129, 0.052
Tifton, GA; 2000 J6169-00H	2	Winter wheat; Pioneer 2684	Forage	0.293 [0.328]	7	0.383, 0.547	<0.01, <0.01	0.175, 0.171
Leland, MS; 2000 J6170-00H	4	Winter wheat; not specified	Forage	0.299 [0.336]	7	2.528, 2.864	<0.01, <0.01	0.077, 0.057
Stilwell, KS; 2000 J6171-00H	5	Winter wheat; Jagger	Forage	0.288 [0.324]	7	0.307, 0.366	<0.01, <0.01	0.054, 0.049

TABLE C.3. Residue Data from Crop Field Trials with Prothioconazole.

Location (City, State/Province, Year) Trial Number	Region	Crop; Variety	Commodity or Matrix	Total Rate (lb a.i./A) [kg a.i./ha]	PHI (days)	Total Prothioconazole-derived Residues (ppm)	1*H*-1,2,4-triazole Residues[1] (ppm)	Triazole Conjugate Residues[1] (ppm)
Louisville, NE; 2000 J6172-00H	5	Winter wheat; Wahoo 14 W3-43	Forage	0.293 [0.328]	7	1.112, 1.161	<0.01, <0.01	0.019, 0.016
Uvalde, TX; 2000 J6173-00H	6	Winter wheat; Ogallala	Forage	0.293 [0.328]	7	4.696, 6.987	<0.01, <0.01	0.036, 0.028
New Rockford, ND; 2000 J6174-00HA	7	Spring wheat; Alsen	Forage	0.293 [0.328]	7	0.365, 0.425	<0.01, <0.01	0.023, 0.032
Eldridge, ND; 2000 J6175-00HA	7	Spring wheat; Alsen	Forage	0.299 [0.334]	7	0.105, 0.119	<0.01, <0.01	0.022, 0.027
Britton, SD; 2000 J6176-00H	7	Spring wheat; Ingot	Forage	0.295 [0.330]	7	0.061, 0.068	<0.01, <0.01	0.038, 0.034
Dundurn, SK; 2000 J6177-00H	7	Spring wheat; CDC Teal	Forage	0.288 [0.323]	7	2.388, 2.941	<0.01, <0.01	0.017, 0.022
Taber, AB; 2000 J6178-00H	7A	Spring wheat; A.C. Barrie	Forage	0.298 [0.335]	7	1.413, 1.792	<0.01, <0.01	0.026, 0.043
Levelland, TX; 2000 J6179-00H	8	Winter wheat; Tam 105	Forage	0.291 [0.327]	7	1.461, 1.749	<0.01, <0.01	0.011, 0.014
Hart, TX; 2000 J6180-00H	8	Winter wheat; Jagger	Forage	0.294 [0.330]	7	1.325, 1.883	<0.01, <0.01	0.029, 0.037
Wolforth, TX ; 2000 J6181-00H	8	Winter wheat; TAM 105	Forage	0.294 [0.330]	7	2.294, 2.321	<0.01, <0.01	<0.01, <0.01
Colony, OK; 2000 J6182-00H	8	Winter wheat; Coker 9663	Forage	0.291 [0.325]	7	1.296, 1.448	<0.01, <0.01	0.158, 0.132
Hood River, OR; 2000 J6183-00H	11	Spring wheat; Pennewawa	Forage	0.292 [0.327]	7	0.794, 0.948	<0.01, <0.01	0.100, 0.079
Minot, MB; 2000 J6184-00H	14	Spring wheat; AC Barrie	Forage	0.290 [0.326]	7	0.136, 0.143	<0.01, <0.01	0.047, 0.047
Rosthern, SK; 2000 J6185-00HA	14	Spring wheat; Prodigy	Forage	0.289 [0.324]	7	0.727, 0.802	<0.01, <0.01	0.012, <0.01

TABLE C.3. Residue Data from Crop Field Trials with Prothioconazole.

Location (City, State/Province: Year) Trial Number	Region	Crop; Variety	Commodity or Matrix	Total Rate (lb a.i./A) [kg a.i./ha]	PHI (days)	Total Prothioconazole-derived Residues (ppm)	1*H*-1,2,4-triazole Residues[1] (ppm)	Triazole Conjugate Residues[1] (ppm)
Leduc, AB; 2000 J6186-00H	14	Spring wheat; AC Splender	Forage	0.291 [0.326]	7	1.222, 1.529	<0.01, <0.01	0.029, 0.046
Spruce Grove, AB; 2000 J6187-00H	14	Spring wheat; AC Splender	Forage	0.287 [0.322]	7	1.378, 1.420	<0.01, <0.01	0.056, 0.085
Brookdale, MB; 2000 J6188-00H	14	Spring wheat; AC Cora	Forage	0.288 [0.322]	7	1.532, 2.061	<0.01, <0.01	0.052, 0.048
Bethany, MB; 2000 J6189-00H	14	Spring wheat; AC Cora	Forage	0.286 [0.320]	7	1.702, 1.944	<0.01, <0.01	0.053, 0.043

[1] Residue values are listed respectively for 1*H*-1,2,4-triazole and the triazole conjugates.

TABLE C.4. Summary of Residue Data from Crop Field Trials with Prothioconazole.

Commodity	Total Applic. Rate (lb a.i./A) [kg a.i./ha]	PHI (days)	Residue Levels (ppm)[1]						
			n	Min.	Max.	HAFT[2]	Median (STMdR[3])	Mean (STMR[4])	Std. Dev.
Total Prothioconazole-derived Residues									
Wheat, hay	0.281-0.375 [0.315-0.420]	12-17	66	0.288	3.571	3.543	1.269	1.420	0.970
Wheat, grain	0.281-0.375 [0.315-0.420]	10; 30-57	66	<0.02	0.061	0.045	0.010	0.014	0.011
Wheat, straw	0.281-0.375 [0.315-0.420]	10; 30-57	64	0.106	1.96	1.899	0.350	0.577	0.471
Wheat, forage	0.286-0.299 [0.320-0.336]	7	46	0.061	6.987	5.842	1.352	1.401	1.268
1*H*-1,2,4-triazole Residues									
Wheat, hay	0.281-0.375 [0.315-0.420]	12-17	66	<0.01	<0.01	<0.01	0.005	0.005	0.0
Wheat, grain	0.281-0.375 [0.315-0.420]	10; 30-57	66	<0.01	<0.01	<0.01	0.005	0.005	0.0
Wheat, straw	0.281-0.375 [0.315-0.420]	10; 30-57	66	<0.01	<0.01	<0.01	0.005	0.005	0.0
Wheat, forage	0.286-0.299 [0.320-0.336]	7	46	<0.01	<0.01	<0.01	0.005	0.005	0.0
Triazole Conjugate Residues									
Wheat, hay	0.281-0.375 [0.315-0.420]	12-17	66	0.018	0.665	0.631	0.204	0.220	0.124

TABLE C.4. Summary of Residue Data from Crop Field Trials with Prothioconazole.									
Commodity	Total Applic. Rate (lb a.i./A) [kg a.i./ha]	PHI (days)	Residue Levels (ppm)[1]						
			n	Min.	Max.	HAFT[2]	Median (STMdR[3])	Mean (STMR[4])	Std. Dev.
Wheat, grain	0.281-0.375 [0.315-0.420]	10; 30-57	66	0.098	1.76	1.76	0.460	0.534	0.320
Wheat, straw	0.281-0.375 [0.315-0.420]	10; 30-57	64	<0.025	0.495	0.449	0.063	0.095	0.104
Wheat, forage	0.286-0.299 [0.320-0.336]	7	46	<0.01	0.175	0.173	0.038	0.050	0.042

[1] For the calculation of minimum, maximum, and HAFT values, the LOQ value was used for residues reported as below the LOQ in TABLE C.3. For calculation of the median, mean and standard deviation, ½LOQ was used for residues reported as below the LOQ. Residue values from the appropriate harvest intervals from the residue decline trials (7-day PHI for wheat forage, 14-day PHI for wheat hay, and 39- and 40-day PHIs for wheat grain and straw) were included in the summary table.
[2] HAFT = Highest Average Field Trial.
[3] STMdR = Supervised Trial Median Residue.
[4] STMR = Supervised Trial Mean Residue.

D. CONCLUSION

The study use pattern was two foliar applications of the 4 lb/gal FlC formulation for a total seasonal rate of 0.281-0.375 lb a.i./A (0.315-0.420 kg a.i./ha), with a 5- to 18-day re-treatment interval. Wheat forage was harvested 7 days after the last application, and wheat grain and straw between 10 and 57 days. The maximum total prothioconazole-derived residues were 3.571 ppm (hay), 0.061 ppm (grain), 1.96 ppm (straw) and 6.987 ppm (forage). Residues of 1,2,4-triazole were less than the LOQ (<0.01 ppm) in/on wheat hay, grain, straw and forage. Maximum residues of the triazole conjugates were 0.67 ppm (hay), 1.76 ppm (grain), 0.495 ppm (straw) and 0.175 ppm (forage). Acceptable methods were used for quantitation of residues in/on wheat hay, grain, straw, and forage.

E. REFERENCES

Gould T. and Timberlake B. (2005) Storage stability of JAU6476 and desthio JAU6476 in canola, wheat, mustard greens, turnip root, and tomato fruit, processed products, and solutions, Bayer CropScience Interim Report.

F. DOCUMENT TRACKING

RDI: Louise Croteau, Suzan Mathew (PMRA) 23/01/2006; Henri Bietlot (PMRA) 24/01/2006; Stephen Funk (IO)13/03/2006; Leung Cheng (RAB3).
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In the absence of signatures, this document is considered to be a draft with deliberative material for internal use only.

This DER was originally prepared under contract by Dynamac Corporation (20440 Century Boulevard, Suite 100; Germantown, MD 20874; submitted 05/25/2005). The DER has been reviewed by the Health Effects Division (HED) and Health Canada's Pest Management Regulatory Agency (PMRA). The DER has been revised to reflect current Office of Pesticide Programs (OPP) policies, and Directive 98-02.

STUDY REPORT:

46246223 Lenz, C. (2004) JAU6476 480 SC - Magnitude of the Residues in/on Peanuts and Peanut Processed Commodities. Lab Project Number: J619PE02: RCJAY002: 200518. Unpublished study prepared by Bayer CropScience. 447 p.

EXECUTIVE SUMMARY:

Bayer CropScience has submitted a processing study with peanut. In a single test conducted in GA during 2000, the 4 lb/gal (480 g/L) suspension concentrate formulation (flowable concentrate; FlC), was applied to peanut plants during pod development as four broadcast foliar applications with 13- to 15-day re-treatment intervals at 0.899-0.901 lb a.i./A (1.01 kg a.i./ha), for a total application rate of 3.60 lb a.i./A (4.03 kg a.i./ha; ~5 times the field trial application

rate). Peanuts were dug up 14 days after the last treatment and were left to dry in the field for 7 days prior to sample collection. Sub-samples of nutmeat were reserved, and the remaining bulk samples were processed into meal, refined oil, dry roasted peanuts, and peanut butter using simulated commercial procedures.

Samples were analyzed for total prothioconazole-derived residues (prothioconazole and the metabolite prothioconazole-desthio), 1*H*-1,2,4-triazole and the triazole conjugates triazolylalanine and triazolylacetic acid using an LC/MS/MS method. The validated LOQ for total prothioconazole-derived residues was 0.02 ppm for peanuts and processed commodities. The validated LOQs for 1*H*-1,2,4-triazole were 0.01 ppm for peanut nutmeat, meal, and refined oil and 0.05 ppm for dry roasted peanuts and peanut butter. The validated LOQs for the triazole conjugates were 0.01 ppm for refined oil, 0.05 ppm for peanut nutmeat, dry roasted peanuts, and peanut butter, and 1.5 ppm for peanut meal. The methods are adequate for data collection based on acceptable concurrent method recovery data.

The maximum storage intervals from collection to analysis for total prothioconazole-derived residues were 1090 days (36 months) for nutmeat and 911 days (31 months) for processed peanut commodities. Prothioconazole-derived residues and prothioconazole-desthio residues are stable up to 12.7 months (interim report). The degree of loss of prothioconazole-derived residues and prothioconazole-desthio residues is not expected to exceed 30% after 36 months.

Total prothioconazole-derived residues concentrated >7.9-fold (peanut meal). Processing factors could not be calculated for refined oil, dry roasted peanuts, or peanut butter because residues were below the LOQ in these commodities. Residues of 1*H*-1,2,4-triazole concentrated >1.9-fold (meal), >12.5-fold (dry roasted peanuts), and >11.9-fold (peanut butter). A processing factor could not be calculated for refined oil because residues were below the LOQ in this commodity. Residues of the triazole conjugates concentrated 1.9-fold (peanut meal), and did not concentrate in refined oil, dry roasted peanuts or peanut butter (<0.01-fold, 0.5-fold and 0.6-fold, respectively).

STUDY/WAIVER ACCEPTABILITY/DEFICIENCIES/CLARIFICATIONS:

Under the conditions and parameters used in the study, the peanut processing data are tentatively classified as scientifically acceptable. The final reports of the ongoing storage stability studies with prothioconazole, prothioconazole-desthio, 1*H*-1,2,4-triazole, triazolylalanine, and triazolylacetic acid will be submitted as confirmatory data.

The acceptability of this study for regulatory purposes is addressed in the forthcoming U.S. EPA Residue Chemistry Summary Document, DP Barcode D303508, and in Canada's Regulatory Decision Document.

COMPLIANCE:

Signed and dated GLP, quality assurance, and data confidentiality statements were provided. No deviations from regulatory requirements were noted that would impact the study results or their interpretation

A. BACKGROUND INFORMATION

Prothioconazole is a broad-spectrum systemic fungicide intended for the control of many diseases in different crops such as cereals, oilseed rape or peanuts. The biochemical mode of action is the inhibition of the demethylation of lanosterol or 24-methylene-dihydrolanosterol, which are precursors of sterols in fungi. The chemical has been submitted for joint EPA and PMRA review by Bayer CropScience. The crops proposed for joint review include barley, the dried shell and bean subgroup, the oilseed crop group, and wheat. Uses on peanuts and rice are being proposed for the U.S. only.

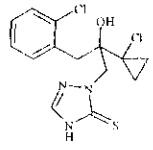
TABLE A.1. Test Compound Nomenclature.	
Chemical structure	
Common name	Prothioconazole (ISO accepted)
Company experimental name	JAU6476
IUPAC name	2-[(2*RS*)-2-(1-chlorocyclopropyl)-3-(2-chlorophenyl)-2-hydroxypropyl]-2*H*-1,2,4-triazole-3(4*H*)-thione
CAS name	2-[2-(1-chlorocyclopropyl)-3-(2-chlorophenyl)-2-hydroxypropyl]-1,2-dihydro-3*H*-1,2,4-triazole-3-thione
CAS registry number	178928-70-6
PC Code	113961
End-use product (EP)	Proline® 480 SC (4 lb/gal suspension concentrate), EPA File Symbol 264-IEL

TABLE A.2. Physicochemical Properties of Prothioconazole

Parameter	Value	Reference
Melting range	139.1 to 144.5°C	MRID 46246003 / CES[1]
pH	5.8 (1% solution)	MRID 46246003 / CES
Density at 20°C	1.36 g/mL	MRID 46246003 / CES
Water solubility at 20°C	pH: mg/L 4: 5 8: 300 9: 2000	MRID 46246003 / CES
Solvent solubility at 20°C	Solvent: g/L Acetone: >250 Acetonitrile: 69 Dichloromethane: 88 Dimethysulfoxide: 126 Ethyl acetate: >250 n-Heptane: <0.1 1-Octanol: 58 Polyethylene glycol: >250 2-Propanol: 87 Xylene: 8	MRID 46246003 / CES
Vapor pressure at 20 or 25°C	$<4 \times 10^{-7}$ Pa (calculated from determinations at 70 °C)	MRID 46246003 / CES
Dissociation constant, pK_a	6.9 (calculated from K_{OW})	MRID 46246003 / CES
Octanol/water partition coefficient, $Log(K_{OW})$, at 20°C	pH: Log Kow unbuffered water: 4.05 pH 4: 4.16 pH 7: 3.82 pH 9: 2.00	MRID 46246003 / CES
UV/visible absorption spectrum	Peak maxima at 257 nm	MRID 46246003 / CES

[1] CES: Chemistry Evaluation Section of PMRA

B. EXPERIMENTAL DESIGN

B.1. Application and Crop Information

TABLE B.1. Study Use Pattern on Peanut

Location (City, State, Year)	Application							
	EP[1]	Method;Timing	Volume (gal/A) [L/ha]	Single Rate (lb a.i./A) [kg a.i./ha]	No. of Appl.	RTI[2] (days)	Total Rate (lb a.i./A) [kg a.i./ha]	Tank Mix Adjuvants
Tifton, GA, 2000	480 SC	1 & 2; Broadcast foliar; Main phase pod developed, continuation of pod filling	15-16 [36-147]	0.899-0.901 [1.008-1.010]	4	13-15	3.6 [4.034]	None
		3: Broadcast foliar; About 70% of pods developed to final size are ripe						
		4: Broadcast foliar; About 80% of pods developed to final size are ripe						

[1] EP = End-use Product; 480 SC = 4 lb/gal (480 g/L) suspension concentrate (flowable concentrate; FlC) formulation
[2] RTI = Retreatment interval

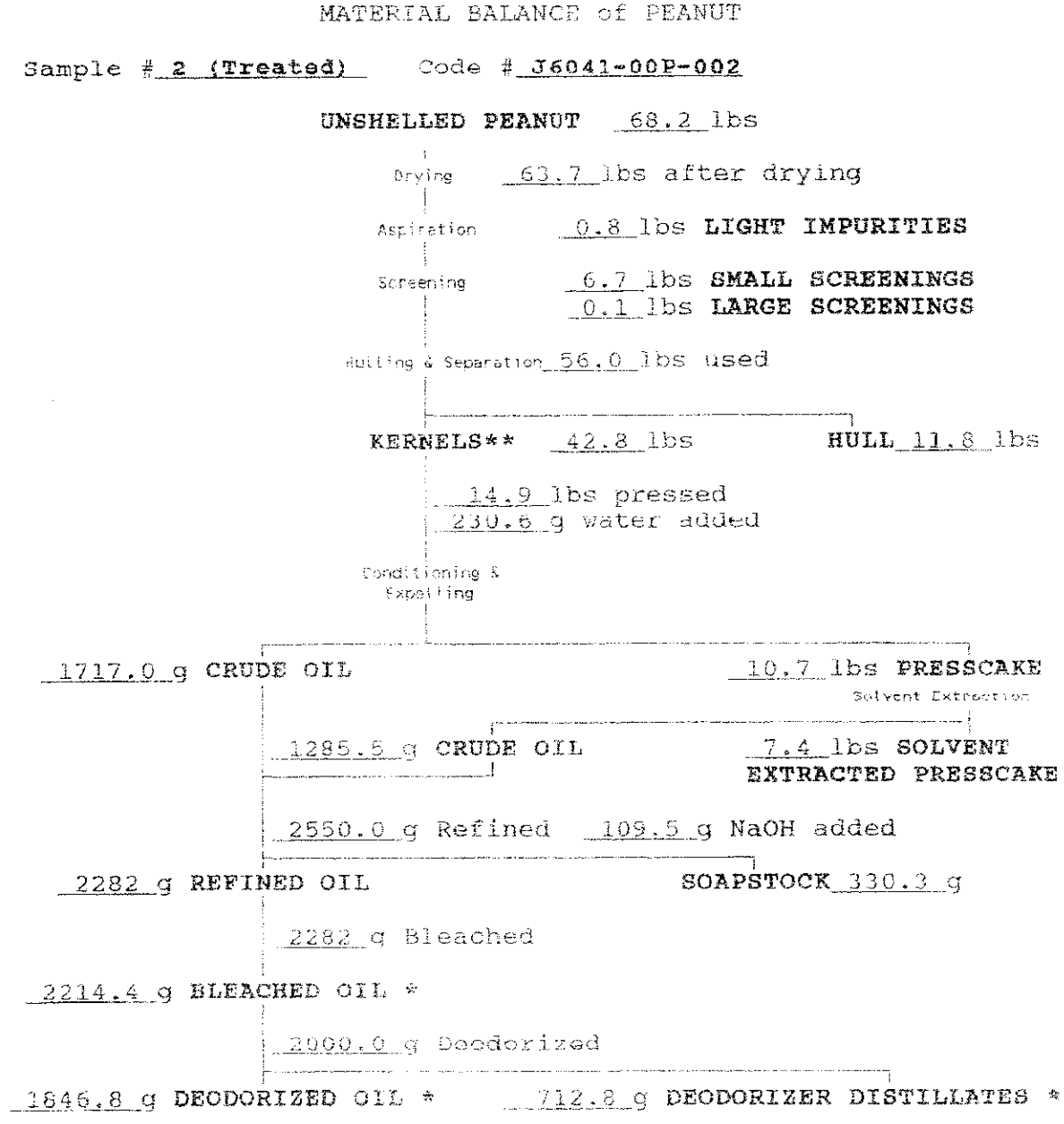
B.2. Processing Procedures

Peanuts were dug up at maturity 14 days after the last treatment and were left in the field to dry for 7 days prior to collection of peanut samples. Peanut samples were placed in frozen storage at the field site within 50 minutes of collection and were stored frozen for 2 days, then shipped frozen via freezer truck to FPRDC at Texas A&M University (Bryan, TX). Whole peanuts were shelled, and nutmeat samples were processed into meal, refined oil, dry roasted peanuts, and peanut butter using simulated commercial procedures. The processed fractions and subsamples of nutmeat were stored frozen, then shipped frozen to Battelle-Agrifood Laboratories (Columbus, OH), where they were stored frozen until shipment to Bayer Research Park (Stilwell, KS) for analysis.

Briefly, whole peanuts were dried to hull moisture of 7-12%, and the kernel (nutmeat) was separated from the shell in a peanut sheller. After shelling, the hull and kernel material were separated in an aspirator, and the kernel material was dried in an oven at 54-71°C to a final moisture of 7-10%. The kernel material was then moisture conditioned to 12%, heated to 93-104°C, and pressed in an expeller to liberate part of the crude oil. The presscake was extracted three times with fresh heated hexane (49-60°C) and dried with warm air to remove residual hexane. The dried presscake forms the peanut meal. The crude oil and hexane collected from the solvent extraction of the presscake were separated, and the crude oil was heated (73-90°C) to remove the remaining hexane. The crude oil fractions that were recovered from the expeller and the solvent extraction were combined and refined. A portion of the nutmeat was dry roasted in an oven at 171-182°C to generate dry roasted peanuts, and a subsample was combined with commercial peanut oil and salt to produce peanut butter.

The peanut processing procedures are summarized in the flow chart below, which was copied without alteration from the data package.

FIGURE B.2.1. Processing Procedures for Peanuts.



* Optional Fractions

** 13.5 lbs of kernels were dry roasted to produce 12.1 lbs of dry roasted peanuts. Six cups of dry roasted peanuts were added to 3 tablespoons of peanut oil and 1½ teaspoons of salt to produce 932 grams of peanut butter.

B.3. Analytical Methodology

Samples of peanut commodities were analyzed for total prothioconazole-derived residues (prothioconazole and the metabolite prothioconazole-desthio) using LC-MS/MS method RPA JA/03/01 with the following modifications: a different solvent was used to prepare the fortification standards, and slightly different quantitation ions were used. Samples were 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 was spiked 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. Residue levels were quantitated from a calibration curve of mixed reference standards of prothioconazole, as the sulfonic acid, and prothioconazole-desthio. The validated LOQ for total prothioconazole-derived residues was 0.02 ppm for peanuts and processed commodities.

Samples were analyzed for residues of 1*H*-1,2,4-triazole and the triazole conjugates (triazolylalanine and triazolylacetic acid) using an LC-MS/MS method (Bayer Report No. G200598) with modifications. Briefly, crop matrices were extracted with aqueous methanol, and three separate aliquots of the extract were removed for determination of each of the three analytes. Isotopically labeled internal standard was added to each aliquot. For 1*H*-1,2,4-triazole, the aliquot was mixed with dansyl chloride to form the dansyl derivative of 1*H*-1,2,4-triazole, which was partitioned into ethyl acetate and then redissolved in acetonitrile (ACN)/water for LC-MS/MS analysis. For triazolylalanine, the aliquot was cleaned up by SPE, derivatized to the butyl ester using butanolic HCl, and then further derivatized using heptafluorobutyric anhydride (HFBA). The mixture was redissolved in ACN/water for LC-MS/MS analysis. For determination of triazolylacetic acid, the aliquot was cleaned up by SPE, derivatized to the butyl ester using butanolic HCl, and then redissolved in ACN/water for LC-MS/MS analysis. Residue levels were quantitated from a calibration curve of reference standards of dansyl-1,2,4-triazole, triazolylalanine butyl ester HFBA, and triazolylacetic acid butyl ester (generated by derivatizing reference standard solutions of triazolylacetic acid). The validated LOQs for 1*H*-1,2,4-triazole were 0.01 ppm for peanut nutmeat, meal, and refined oil and 0.05 ppm for dry roasted peanuts and peanut butter. The validated LOQs for the triazole conjugates were 0.01 ppm for refined oil, 0.05 ppm for peanut nutmeat, dry roasted peanuts, and peanut butter, and 1.5 ppm for peanut meal.

C. RESULTS AND DISCUSSION

Concurrent method recovery data are presented in TABLE C.1. Samples were analyzed for total prothioconazole-derived residues (prothioconazole and the metabolite prothioconazole-desthio) using LC-MS/MS method RPA JA/03/01. The validated LOQ for total prothioconazole-derived residues was 0.02 ppm for peanuts and processed commodities. Samples were analyzed for residues of 1*H*-1,2,4-triazole and the triazole conjugates triazolylalanine and triazolylacetic acid using an LC-MS/MS method (Bayer Report No. G200598) with modifications. The validated

LOQs for 1*H*-1,2,4-triazole were 0.01 ppm for peanut nutmeat, meal, and refined oil and 0.05 ppm for dry roasted peanuts and peanut butter. The validated LOQs for the triazole conjugates were 0.01 ppm for refined oil, 0.05 ppm for peanut nutmeat, dry roasted peanuts, and peanut butter, and 1.5 ppm for peanut meal. The methods are adequate for data collection based on acceptable concurrent method recovery data.

Sample storage intervals and conditions are summarized in TABLE C.2. The maximum storage intervals from collection to analysis for total prothioconazole-derived residues were 1090 days (36 months) for nutmeat and 911 days (31 months) for processed peanut commodities. Based on an interim report, residues of prothioconazole-desthio and prothioconazole-derived residues are stable for up to 12.7 months. The degree of loss of prothioconazole-desthio residues and prothioconazole-derived residues is not expected to exceed 30% after 36 months in peanut and its processed peanut commodities.

The maximum storage intervals from collection to analysis for 1*H*-1,2,4-triazole and triazole conjugate residues were 1098 days (36 months) for nutmeat and 918 days (31 months) for processed peanut commodities.

Residues of prothioconazole (average of triplicate analyses for each analyte) in/on peanut nutmeat (RAC) from the processing study were less than the LOQ (<0.02 ppm) for total prothioconazole-derived residues, less than the LOQ (<0.01 ppm) for 1*H*-1,2,4-triazole, and 2.43 ppm for the triazole conjugates. The processing data for peanuts indicated that total prothioconazole-derived residues concentrated >7.9-fold in meal processed from peanut nutmeat bearing residues below the LOQ; processing factors could not be calculated for refined oil, dry roasted peanuts, or peanut butter because residues were below the LOQ in these commodities. Residues of 1*H*-1,2,4-triazole concentrated >1.9-fold in meal, >12.5-fold in dry roasted peanuts, and >11.9-fold in peanut butter, and residues of the triazole conjugates concentrated 1.9-fold in peanut meal, and did not concentrate in refined oil, dry roasted peanuts or peanut butter (respective processing factors of <0.01-fold, 0.5-fold and 0.6-fold). Because total prothioconazole-derived residues and residues of 1*H*-1,2,4-triazole were each below the respective LOQ (<0.02 ppm and <0.01 ppm) in refined oil, processing factors could not be calculated. However, based on the processing factors that were calculated for peanut meal, and/or dry roasted peanuts and peanut butter, it appears that total prothioconazole-derived residues and residues of 1*H*-1,2,4-triazole did not concentrate significantly in peanut refined oil. We note that residue data for dry roasted peanuts and peanut butter is not required (OPPTS 860.1000, Directive 98-02).

The reported processing factor of 7.9-fold for total prothioconazole-derived residues in peanut meal exceeded the theoretical concentration factor of 2.2-fold for peanut meal and 2.8-fold for peanut refined oil.

TABLE C.1. Summary of Concurrent Recoveries of Prothioconazole from Peanut Commodities.

Matrix	Analyte	Spike level (ppm)	Sample size (n)	Recoveries (%)	Mean ± std dev
Peanut nutmeat	Prothioconazole	0.02	1	78	--
	Prothioconazole-desthio	0.02	1	95	--
	1H-1,2,4-triazole	0.05	2	103, 92	98 ± 7.7
		0.50	1	94	--
	Triazolylalanine	0.05	1	96	--
		1.0	1	92	--
	Triazolylacetic acid	0.05	2	102, 104	103 ± 1.4
		0.5	1	92	--
Peanut meal	Prothioconazole	0.02	3	83, 81, 80	81 ± 1.5
	Prothioconazole-desthio	0.02	3	100, 96, 98	98 ± 2.0
	1*H*-1,2,4-triazole	0.05	1	94	--
		0.5	1	96	--
	Triazolylalanine	0.50	1	96	--
	Triazolylacetic acid	0.05	1	113	--
		0.50	1	96	--
Peanut refined oil	Prothioconazole	0.02	3	74, 78, 80	77 ± 3.0
	Prothioconazole-desthio	0.02	3	99, 100, 95	98 ± 2.6
	1*H*-1,2,4-triazole	0.05	1	97	--
	Triazolylalanine	0.05	1	101	--
	Triazolylacetic acid	0.05	1	102	--
Dry roasted peanuts	Prothioconazole	0.02	3	80, 80, 82	81 ± 1.2
		0.10	3	77, 79, 78	78 ± 1.0
	Prothioconazole-desthio	0.02	3	93, 90, 89	91 ± 2.1
		0.10	3	91, 91, 90	91 ± 0.6
	1H-1,2,4-triazole	0.05	4	103, 111, 106, 97	104 ± 5.9
		0.50	1	92	--
	Triazolylalanine	1.5	3	99, 93, 92	96 ± 3.7
	Triazolylacetic acid	0.05	1	80	--
		0.50	1	94	--
Peanut butter	Prothioconazole	0.02	3	87, 82, 82	84 ± 2.9
		0.10	3	84, 84, 82	83 ± 1.2
	Prothioconazole-desthio	0.02	3	102, 93, 98	98 ± 4.5
		0.10	3	100, 99, 99	99 ± 0.6
	1H-1,2,4-triazole	0.05	4	94, 105, 105, 109	103 ± 6.4
		0.50	1	99	--
	Triazolylalanine	1.5	3	105, 99, 104	103 ± 3.2
	Triazolylacetic acid	0.05	3	102	--
		0.50	4	88	--

TABLE C.2. Summary of Freezer Storage Conditions

Peanut Matrix	Storage Temp. (°C)	Actual Storage Duration [1]	Limit of Demonstrated Storage Stability
Total Prothioconazole-Derived Residues			
Nutmeat	-4.8 to -30.0	1090 days (36 months)	Stability demonstrated for up to 12.7 months (interim report). Awaiting final report as confirmatory data.
Meal, refined oil, dry roasted peanuts, and peanut butter		911 days (31 months)	
1*H*-1,2,4-triazole and Triazole Conjugate Residues			
Nutmeat	-4.8 to -30.0	1098 days (36 months)	Not applicable at this time. Awaiting final report.
Meal, refined oil, dry roasted peanuts, and peanut butter		918 days (31 months)	

[1] Extracts were stored frozen for <3 days prior to analysis.

TABLE C.3. Residue Data from Peanut Processing Study with Prothioconazole.

Trial ID (City, State, Year)	Processed Commodity	Total Rate (lb a.i./A) [kg a.i./ha]	PHI (days)	Residues (ppm) [1]	Processing Factor [2]
Total Prothioconazole-Derived Residues					
Tifton, GA, 2000	Nutmeat (RAC)	3.6 [4.034]	14	<0.02, <0.02, <0.02 (<0.02)	--
	Meal			0.151, 0.159, 0.166 (0.159)	>7.9-fold
	Refined oil			<0.02, <0.02, <0.02	NC
	Dry roasted peanuts			<0.02, <0.02, <0.02	NC
	Peanut butter			<0.02, <0.02, <0.02	NC
1*H*-1,2,4-triazole					
Tifton, GA, 2000	Nutmeat (RAC)	3.6 [4.034]	14	<0.01, <0.01, 0.010 (<0.010)	--
	Meal			<0.01, <0.01, 0.039 (0.019)	>1.9-fold
	Refined oil			<0.01, <0.01, <0.01	NC
	Dry roasted peanuts			0.105, 0.126, 0.144 (0.125)	>12.5-fold
	Peanut butter			0.110, 0.115, 0.132 (0.119)	>11.9-fold
Triazole Conjugates					
Tifton, GA, 2000	Nutmeat (RAC)	3.6 [4.034]	14	2.22, 2.42, 2.64 (2.43)	--
	Meal			4.06, 4.75, 4.83, (4.54)	1.9-fold
	Refined oil			<0.01, <0.01, <0.01	<0.01-fold
	Dry roasted peanuts			1.16, 1.28, 1.46, (1.30)	0.5-fold
	Peanut butter			1.21, 1.45, 1.59, (1.42)	0.6-fold

[1] Samples were analyzed in triplicate; average residues are reported in parentheses.
[2] NC = Not calculated.

D. CONCLUSION

Total prothioconazole-derived residues concentrated >7.9-fold in meal processed from peanut nutmeat. Residues of 1*H*-1,2,4-triazole concentrated >1.9-fold in meal, >12.5-fold in dry roasted peanuts, and >11.9-fold in peanut butter. Residues of the triazole conjugates concentrated 1.9-fold in peanut meal, and did not concentrate in refined oil, dry roasted peanuts, or peanut butter (<0.01-fold, 0.5-fold and 0.6-fold). Because total prothioconazole-derived residues and residues of 1*H*-1,2,4-triazole were each below the respective LOQ (<0.02 ppm and <0.01 ppm) in refined oil, processing factors could not be calculated. However, based on the processing factors that were calculated for peanut meal, and/or dry roasted peanuts and peanut butter, it appears that total prothioconazole-derived residues and residues of 1*H*-1,2,4-triazole did not concentrate significantly in peanut refined oil. Acceptable methods were used for quantitation of residues in/on peanut nutmeat and its processed commodities.

E. REFERENCES

Gould T. and Timberlake B. (2005) Storage stability of JAU6476 and desthio JAU6476 in canola, wheat, mustard greens, turnip root, and tomato fruit, processed products, and solutions, Bayer CropScience Interim Report.

F. DOCUMENT TRACKING

RDI: Louise Croteau, Suzan Mathew (PMRA) 23/01/2006; Henri Bietlot (PMRA) 24/01/2006; Stephen Funk (IO) 13/03/2006; Leung Cheng (RAB3).
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In the absence of signatures, this document is considered to be a draft with deliberative material for internal use only.

This DER was originally prepared under contract by Dynamac Corporation (20440 Century Boulevard, Suite 100; Germantown, MD 20874; submitted 05/05/2005). The DER has been audited and rewritten where necessary by Health Canada's Pest Management Regulatory Agency (PMRA) and reviewed by the Health Effects Division (HED). The DER has been revised to reflect current Office of Pesticide Programs (OPP) policies and PMRA Directive 98-02.

STUDY REPORT:

46246225 Haas, M. (2001) Confined Rotation Crop Study with JAU 6476. Project Number: 110882, M1300891/2. Unpublished study prepared by Bayer Ag Institut fuer Ruckstands-Analytik. 145 p.

46246226 Duah, F.; Kraai, M. (2004) The Accumulation of ((Triazole-3,5-(Carbon 14)) JAU6476 in Confined Rotational Crops. Project Number: J6051601, 200623. Unpublished study prepared by Bayer Corp. 324 p.

EXECUTIVE SUMMARY:

Phenyl-label study

Bayer CropScience has submitted a confined rotational crop study with [phenyl-UL-^{14}C]-prothioconazole (specific activity 3.31 MBq/mg) in rotated crops. The radiolabeled test substance was dissolved in acetonitrile (ACN) and applied to bare sandy loam soil in a single planting container at 0.52 lb a.i./A (582 g a.i./ha). Rotational Swiss chard, turnip, and spring wheat were planted at plantback intervals (PBIs) of 28, 146, and 269 days.

Total radioactive residues (TRRs), expressed as JAU6476 equivalents were determined by combustion and/or liquid scintillation counting (LSC). Aliquots of each raw agricultural commodities (RACs) were extracted using acetonitrile (ACN/water; 4:1), containing 1 mg/mL of cysteine HCl, followed by accelerated solvent extraction (ASE) and/or acidic extraction using dioxane/2N HCl (9:1). As needed, extracts were cleaned up by solid phase extraction (SPE) prior to analysis by thin-layer chromatography (TLC) and/or high performance liquid chromatography (HPLC). Metabolites were identified by co-chromatography with authentic reference compounds as well as by spectroscopic methods (mass spectroscopy or NMR). Samples were stored for a maximum of 57 days between harvest and HPLC analysis.

The TRRs were variable from the first rotation to the second and third rotations, as there was no clear pattern of increasing or decreasing TRRs over time. TRRs from the first rotation to the second and third rotations were as follows: wheat forage (0.021 to 0.062 to 0.040 ppm); wheat hay (0.114 to 0.135 to 0.160 ppm); wheat straw (0.450 to 0.307 to 0.312 ppm); wheat grain (0.007 ppm); Swiss chard (0.039 to 0.053 to 0.021 ppm); turnip tops (0.046 to 0.028 to 0.036 ppm); and turnip roots (0.043 to 0.031 to 0.015 ppm). Because of the low radioactivity levels in wheat grain samples from the 146- and 269-day PBIs were not analyzed.

The majority of the TRRs (61-87% of the TRRs) were released from all rotational crop commodities with ACN/water, with the exception of wheat grain. ACN/water released only 23% of the TRRs from wheat grain. Accelerated solvent extraction with ACN/water released an additional 4 to 8% of the TRRs from wheat hay, straw, and grain. Acid hydrolysis with HCl/dioxane released approximately 9 to 21% of the TRRs from wheat hay and straw. Non-extractable residues remaining following extraction/hydrolysis accounted for less than <39% of the TRRs (< 0.029 ppm) in rotational crop matrices. Total accountabilities ranged from 99.1-143%.

Total identified residues ranged from 34 to 77% of the TRRs (0.011-0.304 ppm) in rotated crop commodities, except for wheat grain. The highest absolute residues identified were in wheat straw (0.179-0.304 ppm) at all PBIs. Only 5% of the TRRs were identified in wheat grain (0.003 ppm). Prothioconazole was detected at very low levels (<1% of the TRRs; <0.005 ppm) only in samples of 146-day PBI Swiss chard, 28-day PBI turnip root, 146-day PBI turnip top, and 28- and 146-day PBI wheat straw.

JAU6476-desthio was detected in all rotational crop commodities at all PBIs analyzed, and was found to be a major metabolite (present at >10% of the TRRs; 0.003 -0.016 ppm) in the following rotational crop commodities: 28- and 146-day PBI Swiss chard, 28-, 146-, and 269-day PBI turnip root, 28- and 269-day PBI turnip top, 28-day PBI wheat forage, and 28- and 146-day PBI wheat hay. JAU6476 sulfonic acid was found to be a major metabolite in 28-day PBI wheat hay (0.013 ppm) and 269-day PBI wheat straw (0.04 ppm). Glucosides of JAU6476-desthio-dihydroxy-olefin (two isomers) were detected in all rotational crop commodities except 28-day PBI wheat grain, and one or both isomers were found to be major metabolites in 28-day PBI Swiss chard (0.005 ppm), 28-day PBI turnip root (0.005 ppm), all rotations of turnip top (0.004-0.006 ppm), 146- and 269-day PBI wheat forage (<0.01 ppm), all rotations of wheat hay (0.012-0.029 ppm), and 269-day PBI wheat straw (0.033 ppm). Up to three isomers of the glucoside of JAU6476-hydroxy-desthio were also detected in all rotational crop commodities, except 28-day PBI wheat grain and 269-day PBI wheat forage, and at least one of the isomers accounted for significant radioactivity in 146-day PBI Swiss chard (0.006 ppm), 28-day PBI turnip root and top (≤0.006 ppm), and 146-day PBI wheat straw (0.031 ppm).

Additional metabolites identified in rotational crops, each at <10% of the TRRs, were JAU6476-triazolinone, JAU6476-3-hydroxy-desthio, JAU6476-4-hydroxy-desthio, JAU6476-6-hydroxy-desthio, JAU6476-α-hydroxy-desthio, JAU6476-α-acetoxy-desthio, JAU6476-benzylpropyldiol and its glucoside, and JAU6476-disulfide.

Triazole-label study

Bayer CropScience has submitted a confined rotational crop study with [triazole-3,5-^{14}C]-prothioconazole (specific activity 18.6 mCi/mmole) in rotated crops. The radiolabeled test substance was mixed with formulation blank and applied to bare sandy loam soil in a single planting container as four applications, with a 14-day retreatment interval, at ~0.18 lb a.i./A/application (~204 g a.i./ha), for a total rate of 0.727 lb a.i./A (815 g a.i./ha). Rotational Swiss chard, turnip, and spring wheat were planted at PBIs of 30, 125, and 366 days.

TRRs, expressed as JAU6476 equivalents were determined by combustion and/or LSC. Aliquots of each RAC were extracted using methanol and/or acetonitrile (ACN/water; 4:1), containing 1 mg/mL of cysteine HCl. If 10% or more of the TRRs in a matrix remained unextracted, ASE followed by reflux with MeOH/2N HCl (1:1) and/or dioxane/2N HCl (4:1) was performed. Identification of metabolites was achieved using reverse phase HPLC. Polar residues from sample extracts with retention times between 11 min and 15 min were further separated by ion-pair chromatography into two peaks (TA and THPA/TAA). The THPA and TAA mixture was separated by esterification followed by analysis using a third reverse phase HPLC system. Samples were stored for a maximum of 47 days between harvest and HPLC analysis.

The TRRs were variable from the first rotation to the second and third rotations, as there was no clear pattern of increasing or decreasing TRRs over time. TRRs from the first rotation to the second and third rotations were as follows: wheat forage (0.251 to 0.575 to 0.439 ppm); wheat hay (2.224 to 2.580 to 2.016 ppm); wheat straw (1.695 to 1.361 to 1.597 ppm); wheat grain (3.806 to 4.136 to 5.875 ppm); swiss chard (0.188 to 0.047 to 0.129 ppm); turnip tops (0.131 to

0.507 to 0.084 ppm); and turnip roots (0.059 to 0.442 to 0.061 ppm). TRRs were highest in wheat grain, hay, and straw.

Extraction with ACN/water (Swiss chard and turnip root and top) or ACN/water and MeOH (wheat forage, hay, straw, and grain) released the majority of the TRRs (70-98% of the TRRs). Accelerated solvent extraction with ACN/water at 50°C and 100°C released an additional ~3 to 26% of the TRRs from all wheat matrices, and subsequent ASE with water released ~1 to 5% of the TRRs in wheat hay, straw, and grain. Acid hydrolysis with HCl/dioxane or HCl released ~1 to 5% of the TRRs in wheat hay, straw, and grain. Non-extractable residues remaining following extraction/hydrolysis accounted for <1 to 6% of the TRRs (0.002-0.076 ppm) in rotational crop matrices. Total accountabilities ranged from 97-102%.

Total identified residues ranged from 72 to 99% of the TRRs (0.034-5.43 ppm) in rotated crop commodities. Prothioconazole was not detected in any rotational crop commodity. Triazolylalanine was the major residue identified in Swiss chard, turnip root and top, and wheat forage and grain, at 44 to 93% of the TRRs (0.023-3.9 ppm) at all PBIs. Triazolylalanine accounted for a major portion of the radioactivity in wheat hay and straw, at 15 to 36% of the TRRs (0.197-0.85 ppm). THPA was a major residue in Swiss chard and wheat forage, hay, and straw, at 18 to 39% of the TRRs (0.008-0.87 ppm). THPA was also found at ≤7% of the TRRs (≤ 0.047 ppm) in rotated turnip root and top and wheat grain from the 30- and 125-day PBIs. THPA was not found in these commodities from the 366-day PBI. Triazolylacetic acid accounted for significant radioactivity in wheat hay, straw, and grain (10-29% of the TRRs; 0.2-1.5 ppm). Triazolylacetic acid was found at ≤6% of the TRRs (≤0.034 ppm) in Swiss chard, turnip root and top, and wheat forage. Additional metabolites identified in rotational crops, each at ≤7% of the TRRs (≤0.063 ppm), were triazolyl-ethanol, triazolyl-ethanol glucoside, JAU6476-desthio, and JAU6476-α-hydroxy-desthio. Free triazole (1*H*-1,2,4-triazole) was not identified in any rotational crop commodity.

Based on the results of the study, it was concluded that the metabolism in rotational crops was qualitatively similar to that in the primary crops peanut, sugar beet and wheat, as the same major metabolites were detected. Additionally, the presence of minor unknown polar compounds indicated that composition of metabolites in rotational crops was influenced by the metabolism of prothioconazole in soil. In addition, it appeared that conjugation was more prevalent in rotational crop metabolism than in primary crop metabolism.

STUDY/WAIVER ACCEPTABILITY/DEFICIENCIES/CLARIFICATIONS:

Under the conditions and parameters used in the study, the confined rotational crop residue data are classified as scientifically acceptable.

The acceptability of this study for regulatory purposes is addressed in the forthcoming U.S. EPA Residue Chemistry Summary Document, DP Barcode D303508, PP#4F6830, and in Canada's Regulatory Decision Document.

COMPLIANCE:

Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided. No deviations from regulatory requirements were reported which would have an impact on the validity of the study.

A. BACKGROUND INFORMATION

Prothioconazole is a broad-spectrum systemic fungicide intended for the control of ascomycetes, basidiomycetes, and deuteromycetes fungi in barley, canola, the dried shell and bean crop subgroup, the oilseed crop group, peanuts, rice, and wheat. Prothioconazole is a systemic demethylation inhibitor fungicide (Group 3 fungicide) of the triazolinthione chemical class. The chemical has been submitted for joint EPA and PMRA review by Bayer CropScience. The crops proposed for joint review include barley, the dried shell and bean subgroup, the oilseed crop group, and wheat. Uses on peanuts and rice are being proposed for the U.S. only.

TABLE A.1. Prothioconazole Nomenclature.

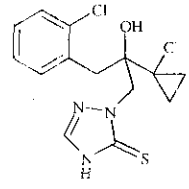
Chemical structure	
Common name	Prothioconazole (ISO accepted)
Company experimental name	JAU6476
IUPAC name	2-[(2*RS*)-2-(1-chlorocyclopropyl)-3-(2-chlorophenyl)-2-hydroxypropyl]-2*H*-1,2,4-triazole-3(4*H*)-thione
CAS name	2-[2-(1-chlorocyclopropyl)-3-(2-chlorophenyl)-2-hydroxypropyl]-1,2-dihydro-3*H*-1,2,4-triazole-3-thione
CAS registry number	178928-70-6
PC Code	113961
End-use product (EP)	Proline® 480 SC (4 lb/gal suspension concentrate), EPA File Symbol 264-IEL

TABLE A.2. Physicochemical Properties of the Technical Grade Prothioconazole.

Parameter	Value	References
Melting range	139.1 to 144.5°C	MRID 46246003/CES
pH	5.8 (1% solution)	MRID 46246003/CES
Density at 20°C	1.36 g/mL	MRID 46246003/CES
Water solubility at 20°C	pH: mg/L 4: 5 8: 300 9: 2000	MRID 46246003/CES
Solvent solubility at 20°C	Solvent: g/L Acetone: >250 Acetonitrile: 69 Dichloromethane: 88 Dimethysulfoxide: 126 Ethyl acetate: >250 n-Heptane: <0.1 1-Octanol: 58 Polyethylene glycol: >250 2-Propanol: 87 Xylene: 8	MRID 46246003/CES
Vapor pressure at 20 or 25°C	$<4 \times 10^{-7}$ Pa (calculated from determinations at 70°C)	MRID 46246003/CES
Dissociation constant, pK_a	6.9 (calculated from K_{OW})	MRID 46246003/CES
Octanol/water partition coefficient, at 20°C	pH: $Log(K_{OW})$ unbuffered water: 4.05 4: 4.16 7: 3.82 9: 2.00	MRID 46246003/CES
UV/visible absorption spectrum	Peak maxima at 257 nm. No absorption at > 300 nm.	MRID 46246003/CES

CES - Chemistry Evaluation Section of PMRA

B. EXPERIMENTAL DESIGN

B.1. Test Site and Crop Information

TABLE B.1.1. Test Site Information.

Testing Environment and location	Soil characteristics						
	Type	% Sand	% Silt	% Clay	%OM	pH	CEC (meq/100 g)
Phenyl-label sutdy							
A single planting container (1.0 m^2 surface area, soil height of 55 cm) in a greenhouse in Leverkusen, Germany; light, temperature, and humidity were controlled automatically.	Sandy loam	58.2	31.0	10.8	1.98	6.30 ($CaCl_2$) 6.50 (water)	10.00

TABLE B.1.1. Test Site Information.

Testing Environment and location	*Soil characteristics*						
	Type	% Sand	% Silt	% Clay	%OM	pH	CEC (meq/100 g)
Triazole-label study							
A single planting container (23.09 ft^2 surface area) at Bayer (Stilwell, KS)	Sandy loam	58	24	18	5.9	7.4	14.9

Phenyl-label study

Because plants were grown in a greenhouse with controlled light, temperature, and humidity, weather data are not needed to support the study. The applicant did not provide many details of the conduct of the in-life phase of the study. Before each planting, the soil was mixed to a depth of 10 cm. It appeared that the same container was used for all three tested rotations; i.e., after harvest of the crops from the first rotation, plants from the second rotation were planted in the same soil, and after harvest of the crops from the second rotation, plants from the third rotation were again planted in the same soil.

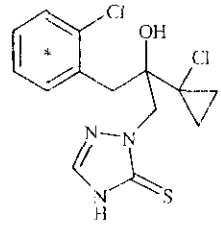
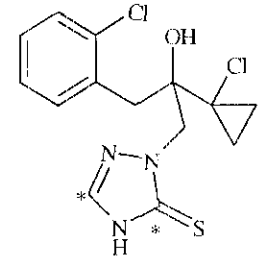
Triazole-label study

The planting container was moved inside a greenhouse prior to application of test substance and then moved back outdoors one day after application. The soil was tilled to a depth of ~12 inches prior to planting the rotational crops. The planting container was kept outdoors after planting the rotational crops, except that it was returned to the greenhouse when the outdoor temperatures were not suitable for growth of Swiss chard and turnips. The applicant stated that crops were watered, fertilized, hand weeded, and sprayed with maintenance chemicals as needed, and accumulated rainwater was drained from the planting container when necessary. A listing of the daily temperature and rainfall recordings during the course of the study was included in the submission. No unusual weather conditions were reported.

TABLE B.1.2. Crop Information.

Crop; crop group	Variety	Plantback intervals (days)	Growth stage at harvest	Harvested RAC	Harvesting procedure
Phenyl-label study					
Swiss chard; Vegetable, leafy, except *Brassica*, group 4	Lucullus	28, 146, 269	Maturity; BBCH 49	Leaves	Cut above the soil with scissors.
Turnip; Vegetable, root and tuber, group 1, and Vegetable, leaves of root and tuber, group 2	Vollenda	28, 146, 269	Maturity; BBCH 49	Tops and roots	Remove whole plants from soil, cut leaves with scissors. Remove soil particles by hand.
Spring wheat, Grain, cereal, group 15, and Grain, cereal, forage, fodder, and straw, group 16	Kadett	28, 146, 269	BBCH 34-39	Forage (day 73)	Plants were cut at the soil surface. In the case of hay, plants were dried in a fumehood for 4 days. For mature samples, grain samples were picked by hand, and the remaining chaff was mixed with the straw sample.
			Late milk, early dough stage; BBCH 83	Hay (day 111)	
			Maturity; BBCH 89	Straw and grain (day 145)	
Triazole-label study					
Swiss chard; Vegetable, leafy, except *Brassica*, group 4	Lucullus	30, 125, 366	At crop maturity (BBCH 49)	Leaves	Cut above the soil with a pair of scissors.
Turnip; Vegetable, root and tuber, group 1, and Vegetable, leaves of root and tuber, group 2	Purple Top	30, 125, 366	At crop maturity (BBCH 49)	Tops and roots	Dug whole plants from soil, cut leaves with a pair of scissors, and removed soil adhering to roots with soft brush before cutting up roots into smaller pieces for processing.
Spring wheat, Grain, cereal, group 15, and Grain, cereal, forage, fodder, and straw, group 16	Butte	30, 125, 366	Beginning of stem elongation (BBCH 30) to node 3 at least 2 cm above node 2 (BBCH 33)	Forage	Cut (approximately 2 to 3 cm) above the soil with a pair of scissors
			Early boot stage (BBCH 41) to beginning of flowering (BBCH 61)	Hay	Cut (approximately 2 to 3 cm) above the soil with a pair of scissors. Hay samples were allowed to air dry in the green house for 3 days before collection for homogenization and analysis.
			At crop maturity BBCH 89	Straw and grain	Cut (approximately 2 to 3 cm) above the soil with a pair of scissors.

B.2. Test Materials

TABLE B.2.1. Test Material Characteristics.		
Chemical structure		
Radiolabel position	[phenyl-UL-^{14}C]-Prothioconazole	[triazole-3,5-^{14}C]-Prothioconazole
Lot No.	12106/1	Vial Nos. C-885 and C-885A
Purity	>98% radiochemical purity; >99% chemical purity	100% radio-chemical purity
Specific activity	Before dilution and addition of nonlabelled prothioconazole: 3.81 MBq/mg (103 µCi/mg) After dilution and addition of nonlabelled prothioconazole: 3.31 MBq/mg (89 µCi/mg)	Before dilution and addition of nonlabelled prothioconazole: 50.8 mCi/mmole After dilution and addition of nonlabelled prothioconazole: 18.6 mCi/mmole

B.3. Study Use Pattern

TABLE B.3.1. Use Pattern Information.		
Chemical name	[phenyl-UL-^{14}C]-Prothioconazole	[triazole-^{14}C]-Prothioconazole
Application method	The test substance was dissolved in acetonitrile and mixed with nonlabelled prothioconazole, then sprayed onto bare soil.	The test substance was mixed with nonlabelled prothioconazole, JAU6476 480 SC formulation blank, acetonitrile, and water. The test material was then sprayed onto bare soil.
Application rate	578 g a.i./ha (0.52 lb a.i./A)	1: 204 g a.i./ha (0.182 lb a.i./A) 2: 217 g a.i./ha (0.194 lb a.i./A) 3: 201 g a.i./ha (0.179 lb a.i./A) 4: 193 g a.i./ha (0.172 lb a.i./A) Total: 815 g a.i./ha (0.727 lb a.i./A)
Number of applications	One	Four
Timing of applications	28, 146, and 269 days prior to planting of rotational crops.	Applications were made 14 days apart, with the last application made 30, 125, and 366 days prior to planting of rotational crops.
PHI[1] (days)	Not applicable.	Not applicable.

[1] PHI = Pre-harvest Interval.

B.4. Identification/Characterization of Residues

B.4.1. Sample Handling and Preparation

Phenyl-label study

The harvested rotational crops were cut into small pieces and homogenized in the presence of liquid nitrogen. Samples were stored frozen at approximately -20°C until analysis. All rotational crop commodities from all PBIs were subjected to extraction procedures, with the exception of wheat grain from the 146- and 269-day PBIs. Cysteine HCl was added to all extracting solvents and to extracts during concentration procedures to prevent oxidative decomposition of prothioconazole. Extracts were centrifuged to remove precipitated material including cysteine HCl. Following partitioning with dichloromethane (DCM), a small volume of ACN was added to each DCM phase prior to concentration. Subsamples of rotational crop commodities were extracted three times with ACN:water (80:20, v:v), then vacuum filtered, and the filtrates were combined and concentrated. The concentrated filtrate of 28- and 146-day PBI samples of Swiss chard, turnip tops, and wheat forage and hay were then partitioned with n-hexane, and the hexane phase was reserved for TLC analysis. The resulting concentrated aqueous phase (Aqueous 1) or concentrated ACN/water filtrate (for other samples) was partitioned with DCM or ethyl acetate (EtOAc) three times. The DCM or EtOAc phases were combined, concentrated, and reserved for TLC analysis; the remaining aqueous phase (Aqueous 2) was also reserved for TLC analysis. The remaining non-extractable residues of wheat hay (28- and 146-day PBI), grain (28-day PBI), and straw (28- and 146-day PBI) were subjected to accelerated solvent extraction (ASE) with ACN:water (65:35, v:v) at 50 and 100°C (two extractions at each temperature). The remaining non-extractable residues of wheat straw (all three PBIs) and wheat hay (269-day PBI) were subjected to acid hydrolysis using dioxane:2N HCl (9:1, v:v) at reflux for 2 hours. The hydrolysate was separated by filtration and concentrated, and the remaining non-extractable residues were lyophilized prior to combustion and LSC.

The extraction procedures for rotational crop matrices are summarized in FIGURES B.4.1 and B.4.1.1, which were copied without alteration from MRID 46246225. Please note that the TRR values reported in the extraction schemes may not be reflective of the data presented in the TABLES.

FIGURE B.4.1

Extraction procedures for 28- and 146-day PBI samples:

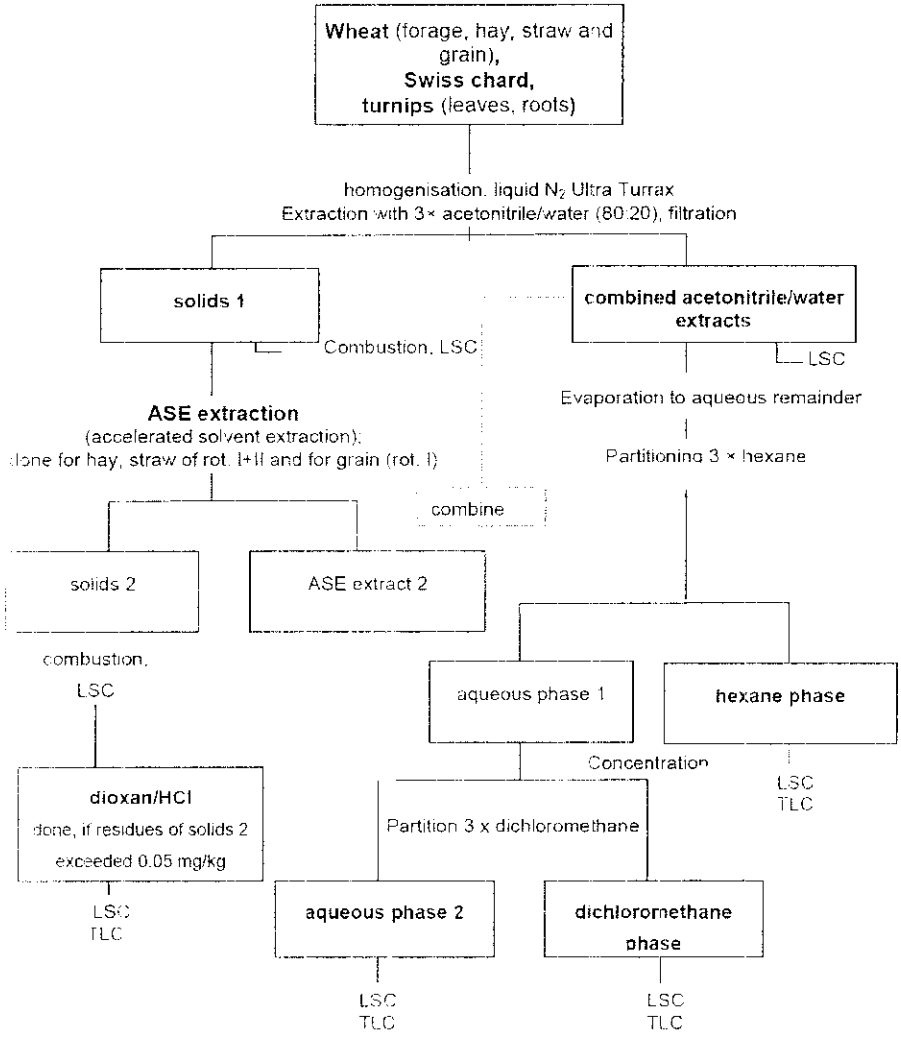
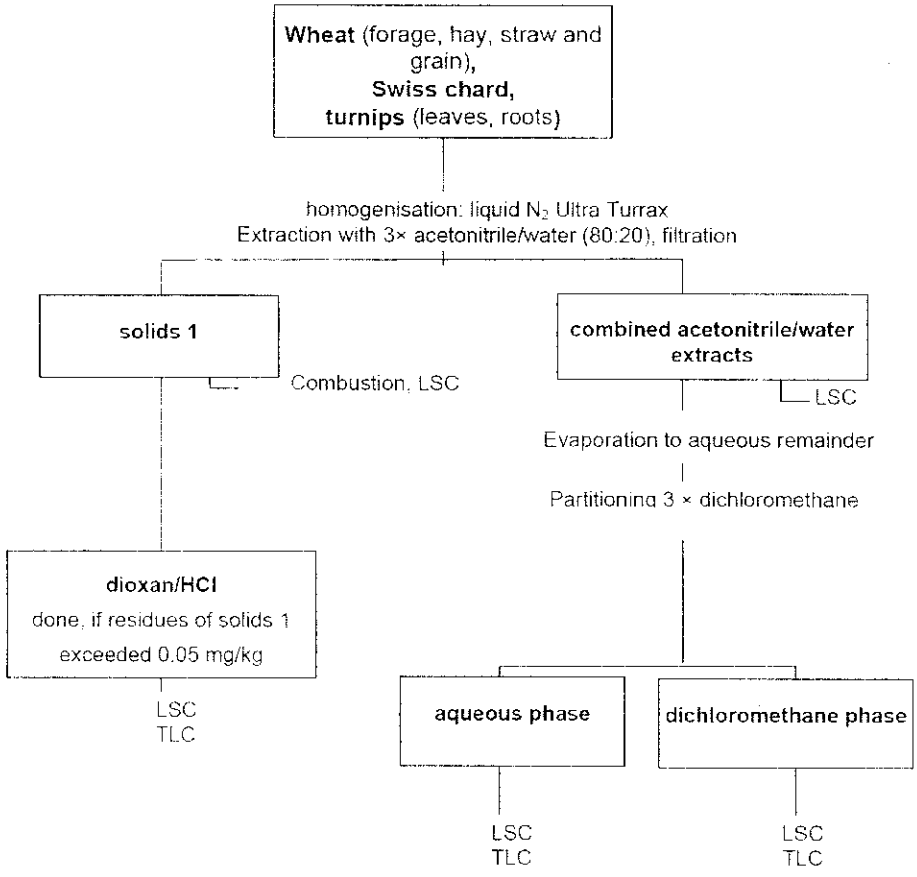


FIGURE B.4.1.1

Extraction procedures for 269-day PBI samples:



Triazole-label study

The harvested rotational Swiss chard, turnip roots and tops, and wheat forage and straw were cut into small pieces immediately after harvest. All rotational crop samples were stored frozen at -20 ± 5°C until processing for analysis. Prior to extraction, all crops were homogenized in the presence of liquid nitrogen. All rotational crop commodities from all PBIs were subjected to extraction procedures. Subsamples of rotational crop commodities were extracted three times with ACN:water (4:1, v:v; mixture contained 1 mg/mL cysteine HCl). Wheat hay, straw, and grain were then further extracted with methanol (MeOH). All extracts were combined, evaporated to dryness, and cleaned up by SPE. The dried extract was dissolved in MeOH:water (1:1, v:v) and applied to a C18 SPE cartridge; MeOH:water (4:1, v:v) was used to elute residues. The load and eluate from the SPE cartridge were combined, evaporated to dryness, and redissolved in MeOH:water (1:1,v:v) for HPLC analysis.

For rotational crops with non-extractable residues accounting for >10% of the TRRs (all rotational wheat matrices except 30-day PBI wheat forage), the non-extractable residues were subjected to ASE with ACN:water (65:35, v:v) at 50 and 100°C (two extractions at each temperature). The ASE extracts were combined, concentrated, and subjected to SPE cleanup, as described above, for HPLC analysis. For 125-day PBI wheat hay, 30-, 125-, and 366-day PBI wheat straw, and 30- and 125-day PBI wheat grain, the ASE extraction was repeated using water as the solvent at 150°C. For 125-day PBI wheat hay, 30-, 125-, and 366-day PBI wheat straw, and 30-day PBI wheat grain, the non-extractable residues remaining after ASE were subjected to acid hydrolysis using dioxane:2N HCl (4:1, v:v) at reflux overnight (approximately 16 hours). The hydrolysate was radioassayed, evaporated to dryness, and, if the hydrolysate contained sufficient radioactivity, cleaned up by SPE, as described above, for HPLC analysis. The non-extractable residues of 366-day PBI wheat grain (after ASE) were subjected to acid hydrolysis using 1N HCl at reflux overnight. The hydrolysate was evaporated to dryness and cleaned up by SPE, as described above, for HPLC analysis.

The extraction procedures for rotational crop matrices are summarized in FIGURES B.4.1.1.2 to B.4.1.3.2, which were copied without alteration from MRID 46246226. Please note that the TRR values reported in the extraction schemes may not be reflective of the data presented in the TABLES.

FIGURE B.4.1.1.2 Extraction Scheme for 30-DAT Wheat Forage

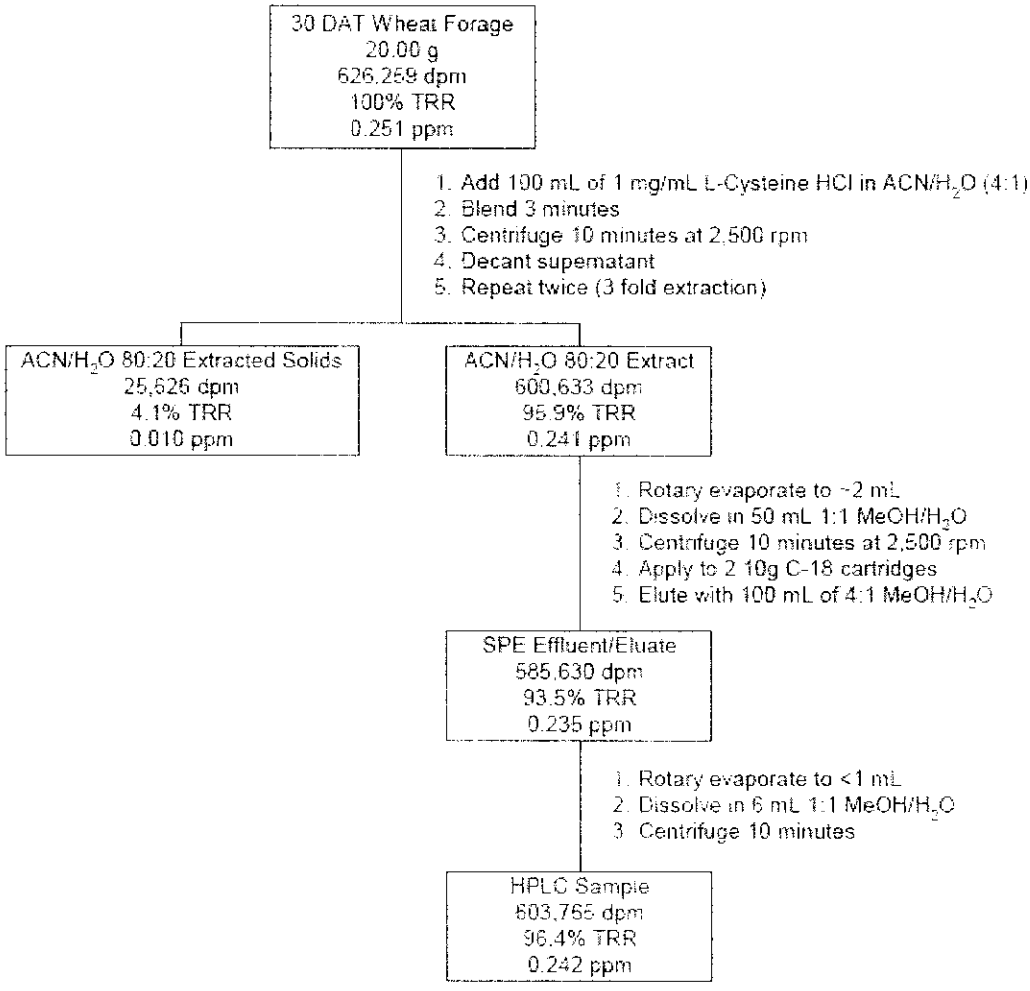


FIGURE B.4.1.1.3 Extraction Scheme for 125-DAT Wheat Forage

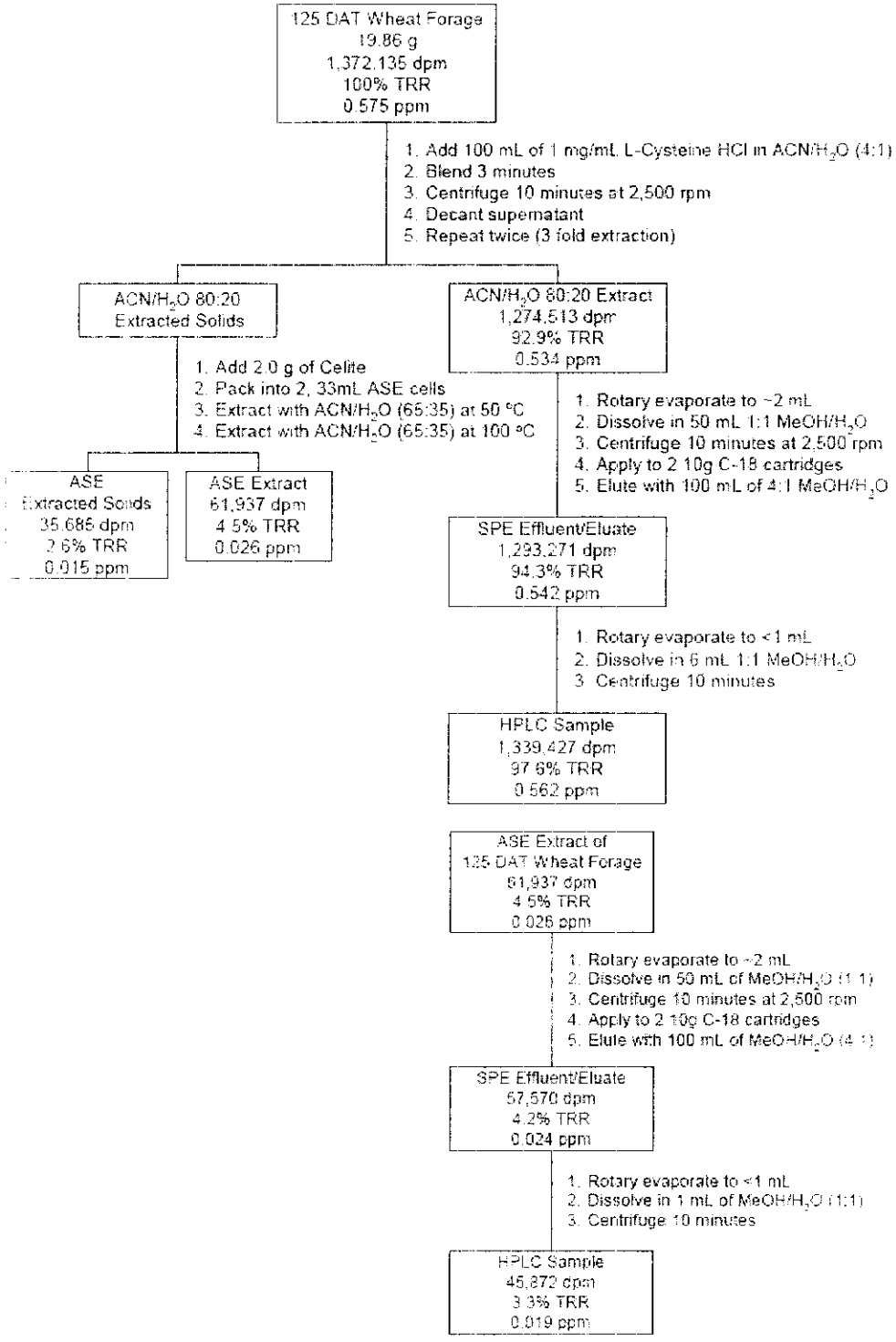


FIGURE B.4.1.1.4 Extraction Scheme for 366-DAT Wheat Forage

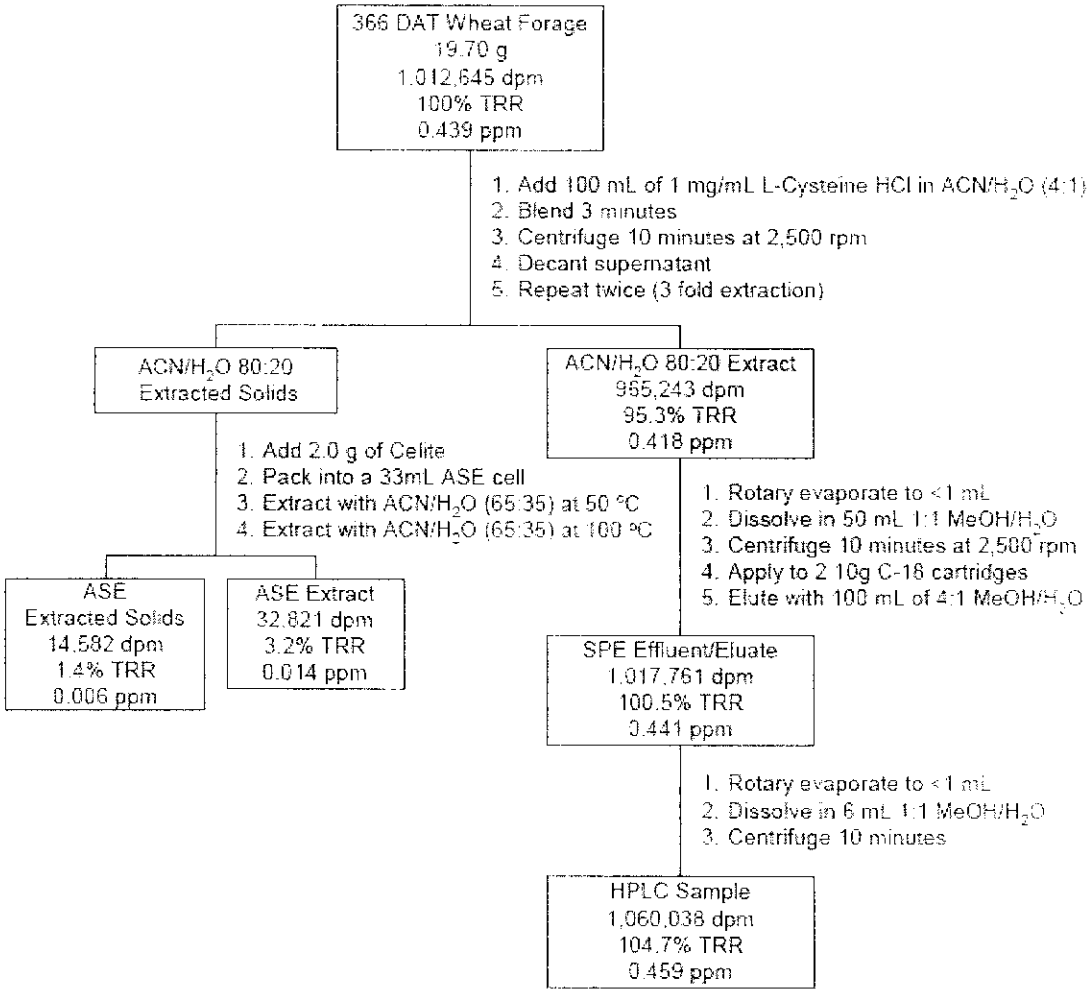


FIGURE B.4.1.1.5 Extraction Scheme for 30-DAT Wheat Hay

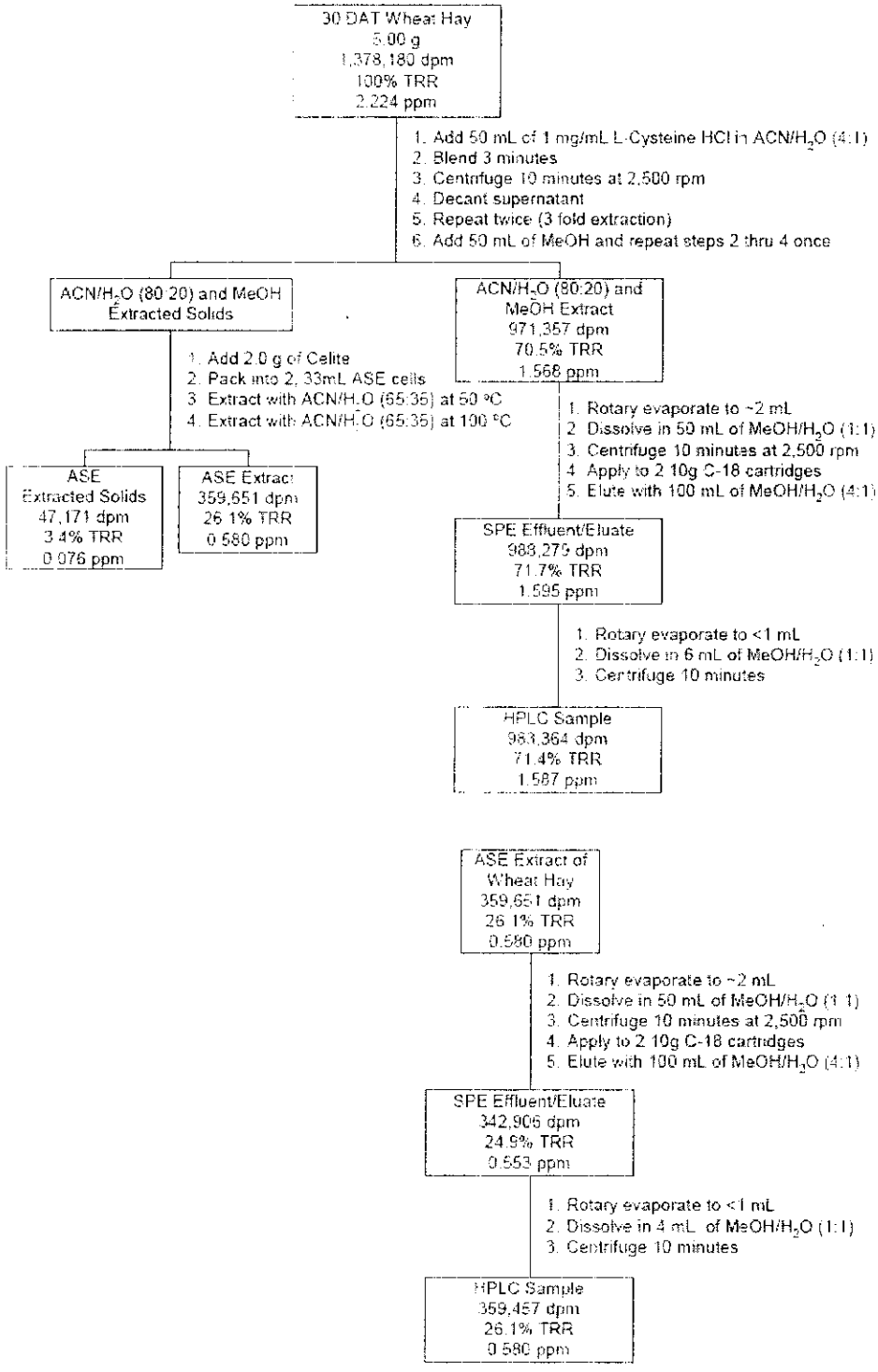


FIGURE B.4.1.1.6 Extraction Scheme for 125-DAT Wheat Hay

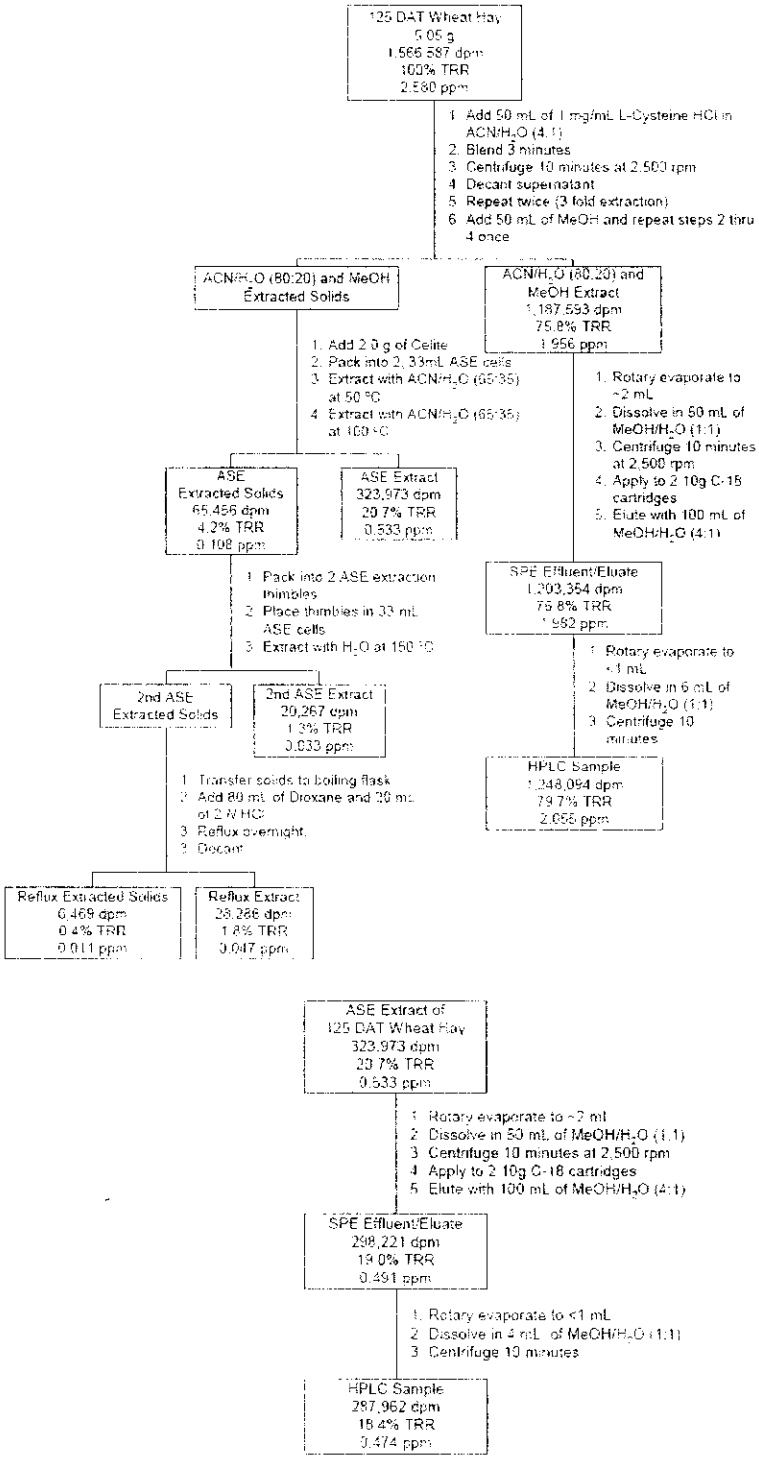


FIGURE B.4.1.1.7 Extraction Scheme for 366-DAT Wheat Hay

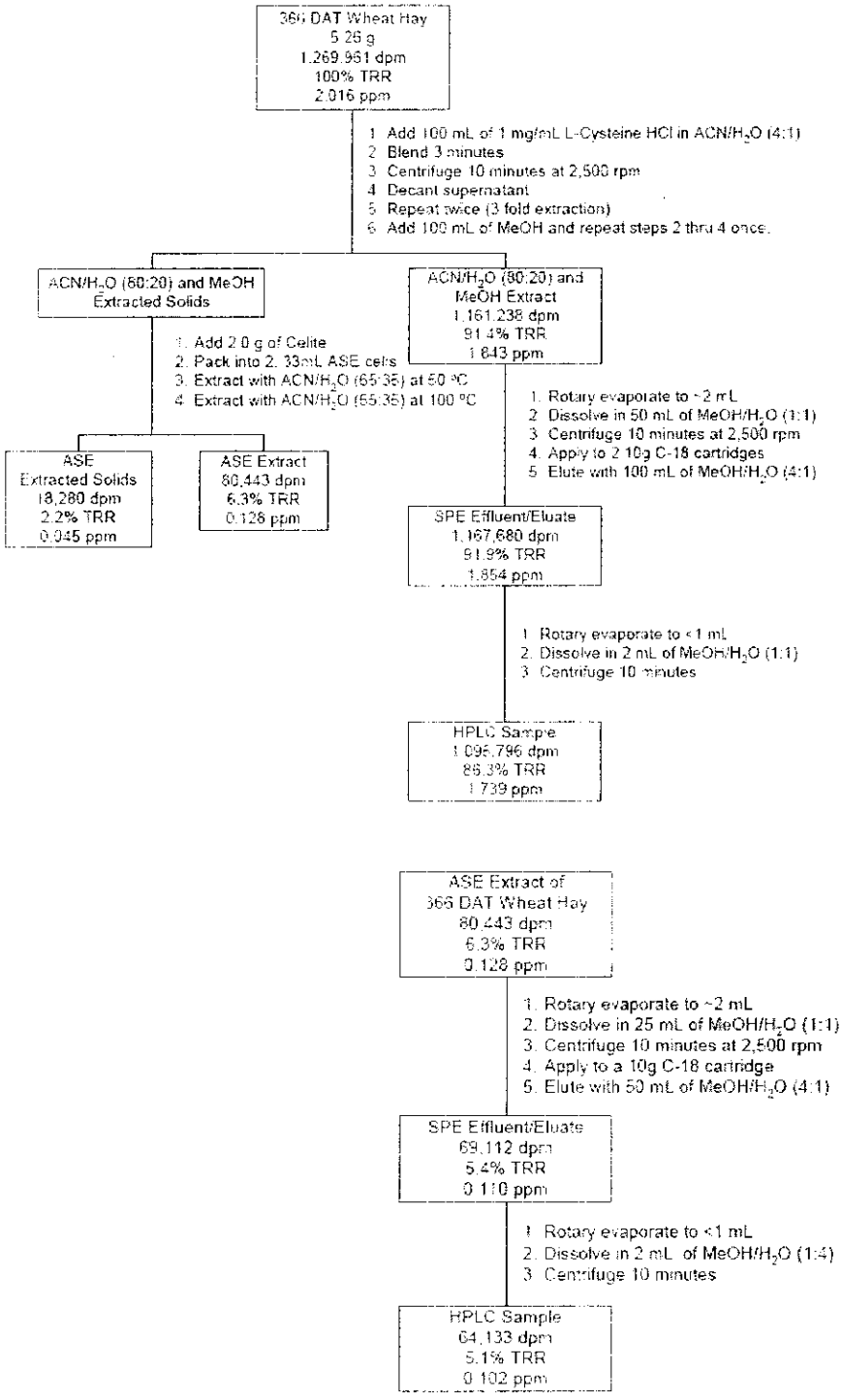


FIGURE B.4.1.1.8 Extraction Scheme for 30-DAT Wheat Straw

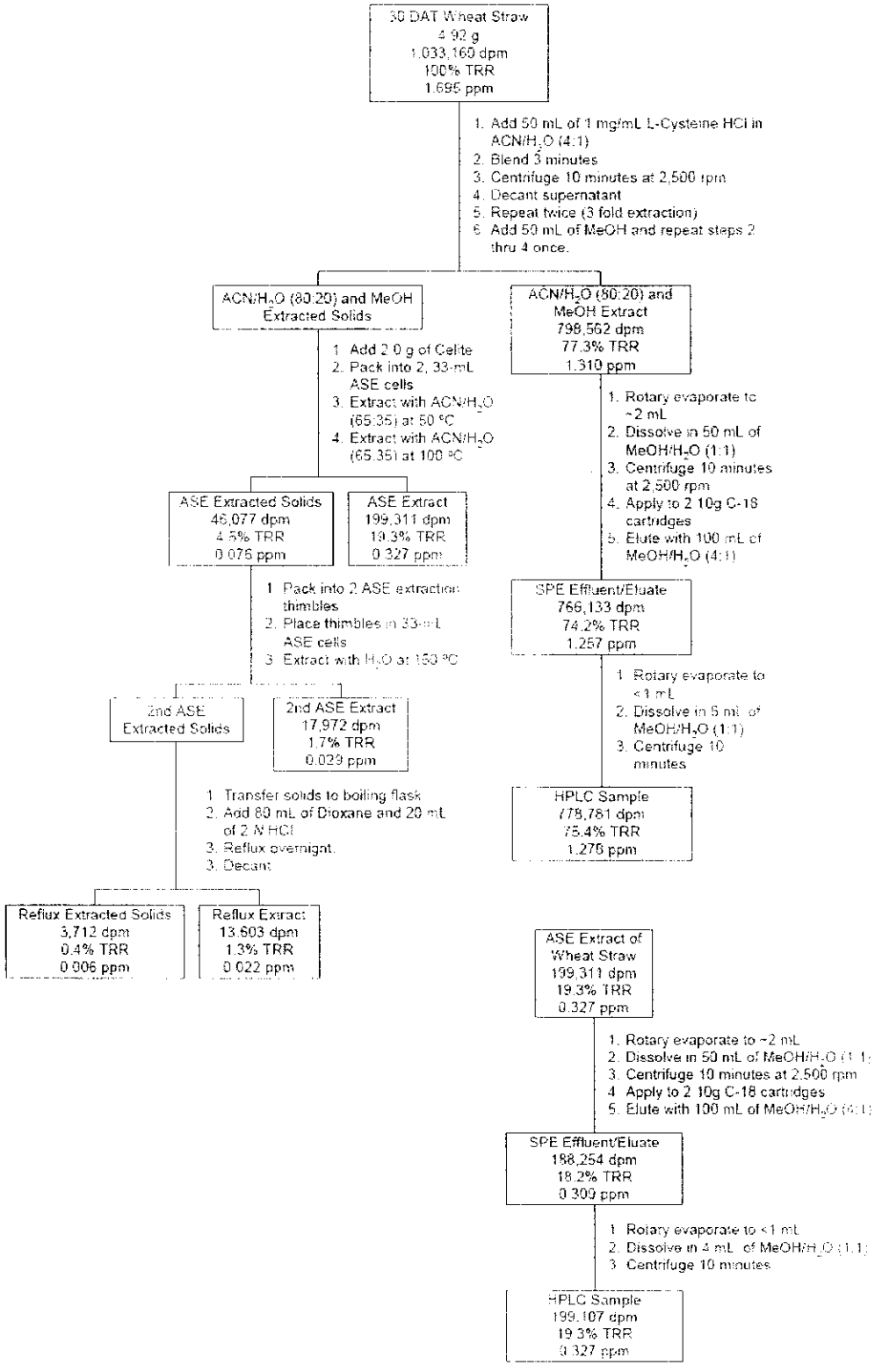


FIGURE B.4.1.1.9 Extraction Scheme for 125-DAT Wheat Straw

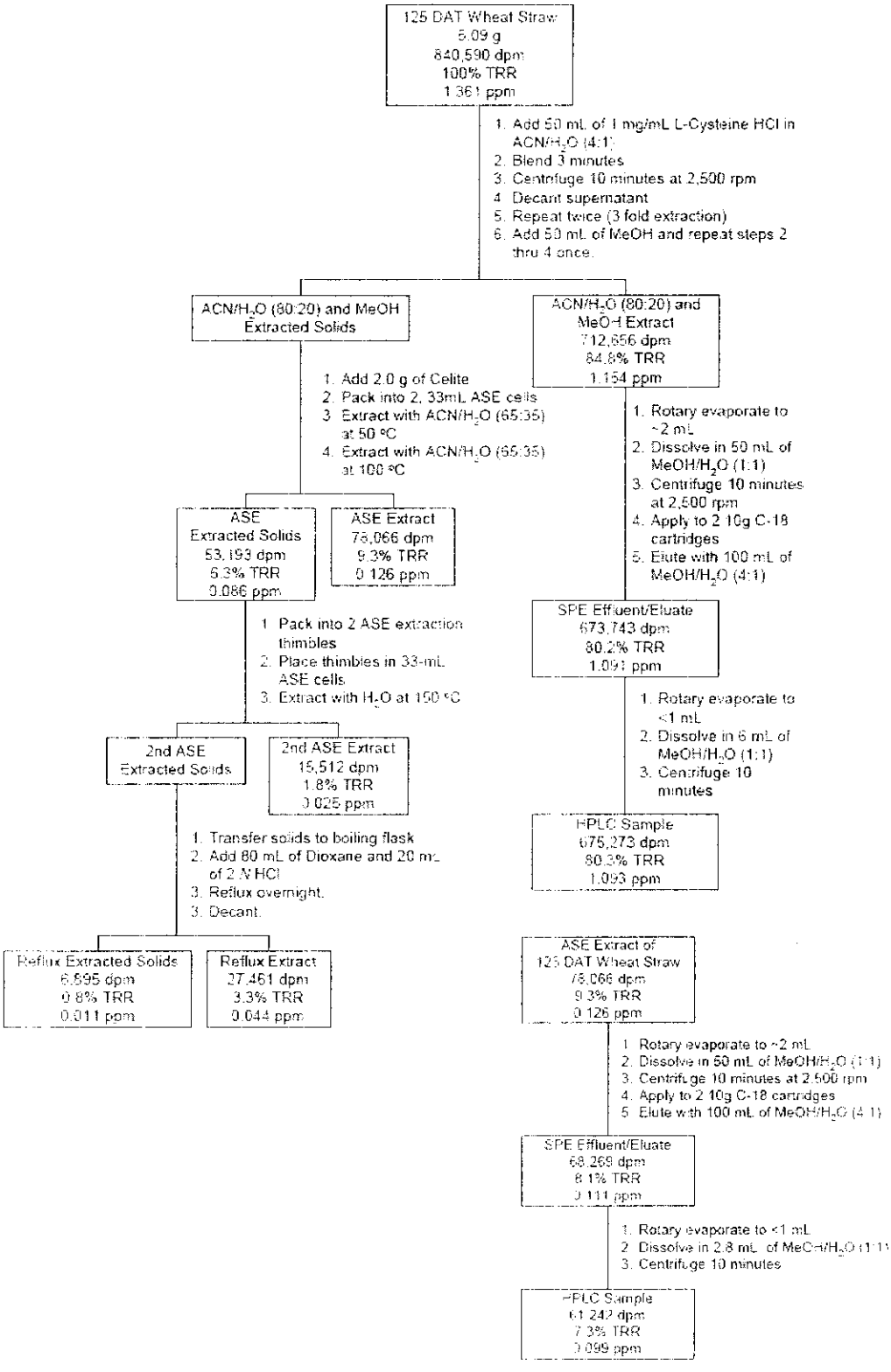


FIGURE B.4.1.2.0 Extraction Scheme for 366-DAT Wheat Straw

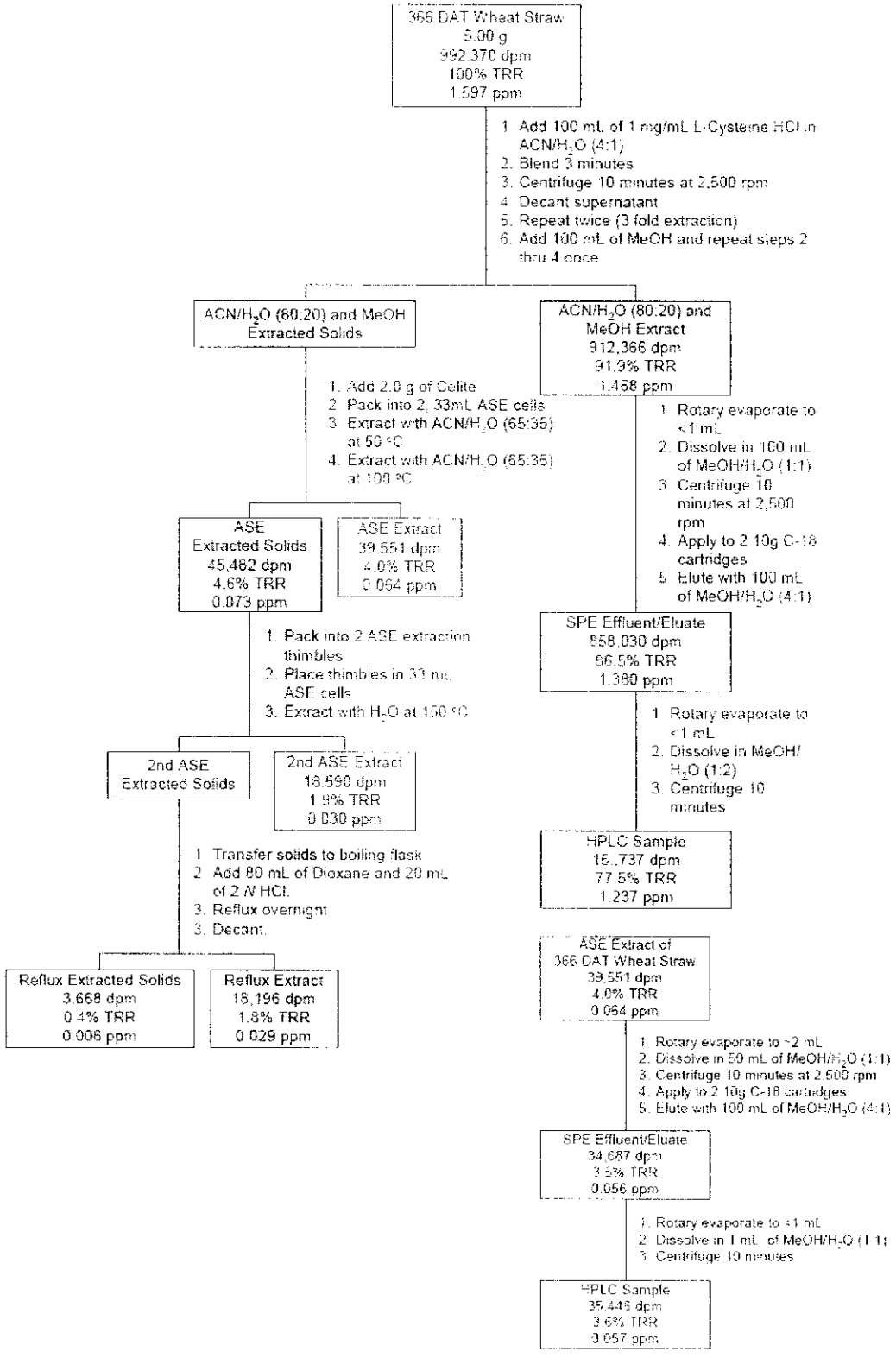


FIGURE B.4.1.2.1 Extraction Scheme for 30-DAT Wheat Grain

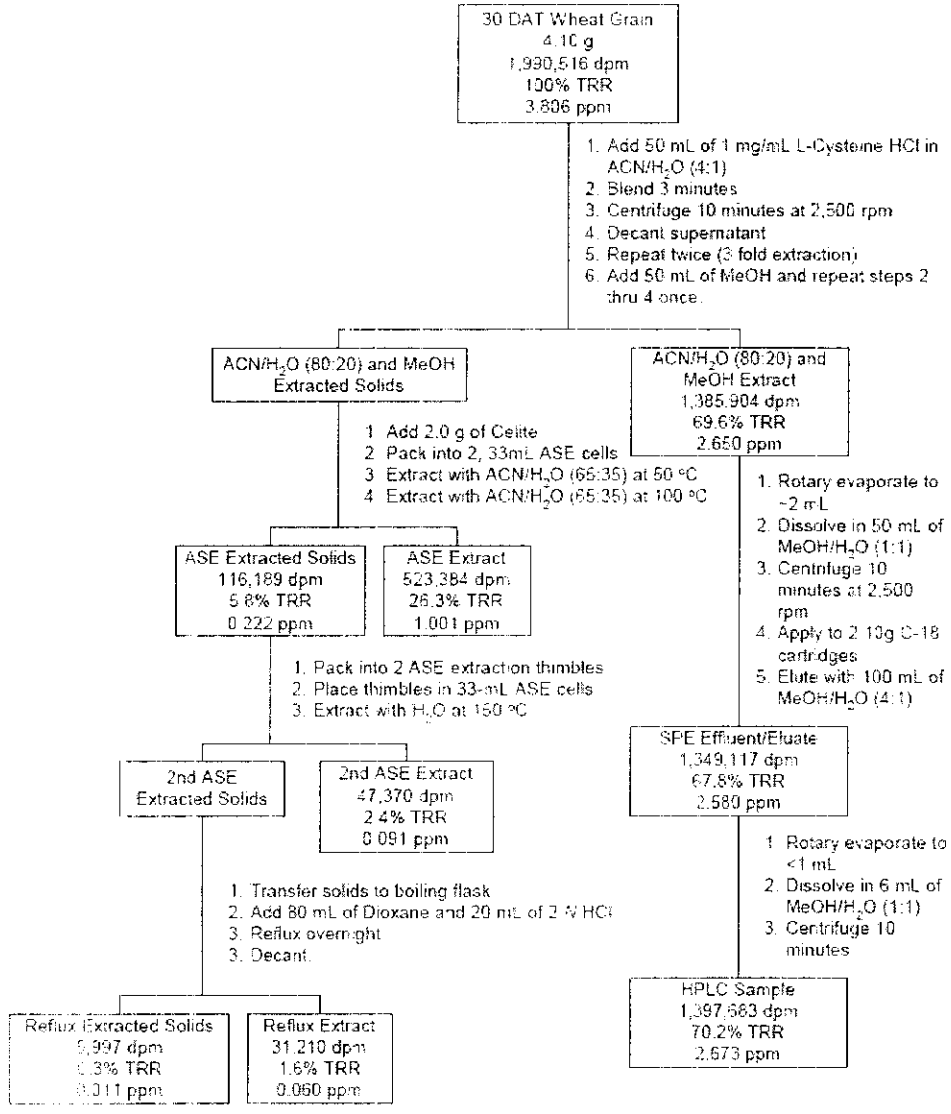


FIGURE B.4.1.2.1 Extraction Scheme for 30-DAT Wheat Grain (cont'd)

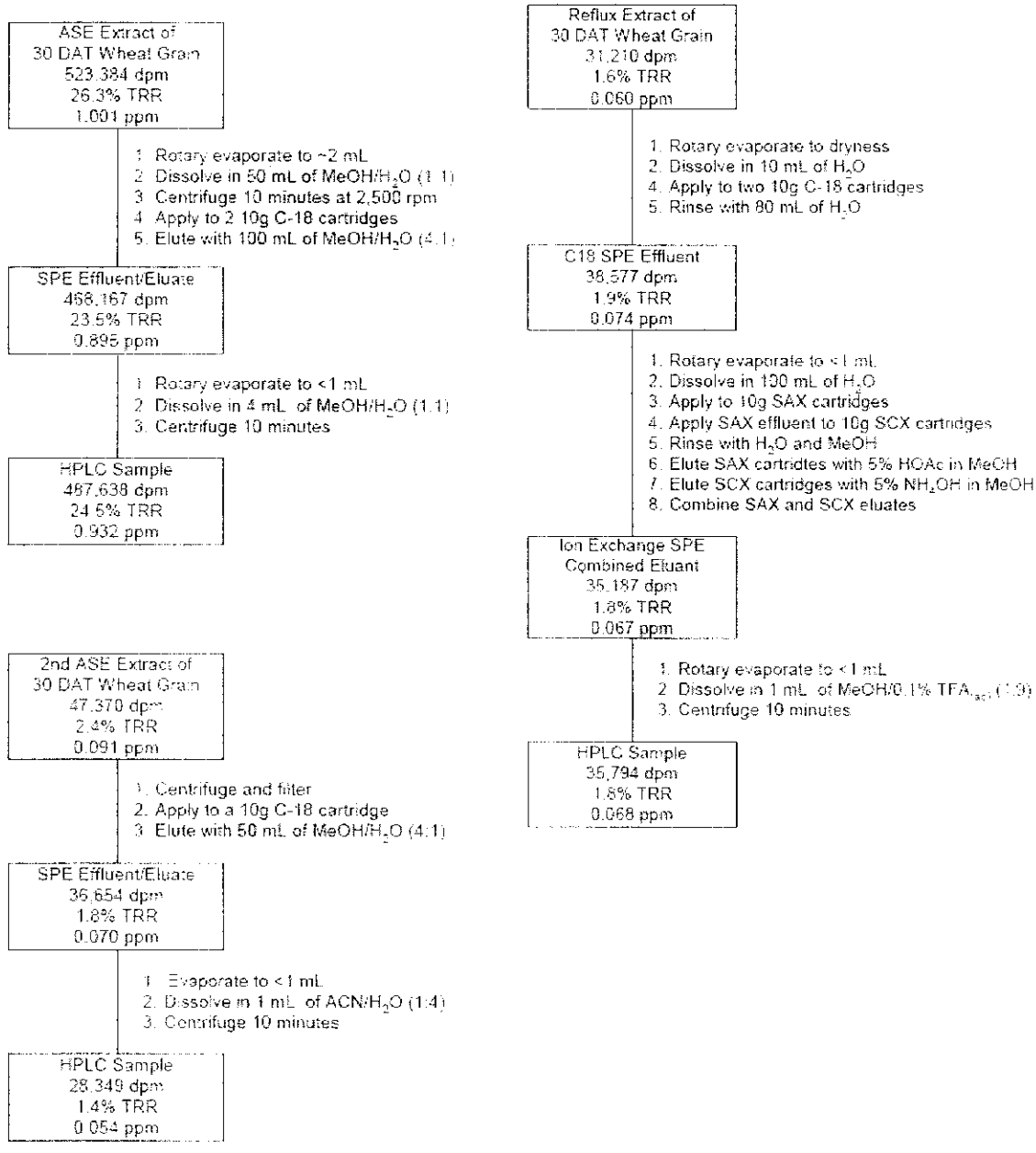


FIGURE B.4.1.2.2 Extraction Scheme for 125-DAT Wheat Grain

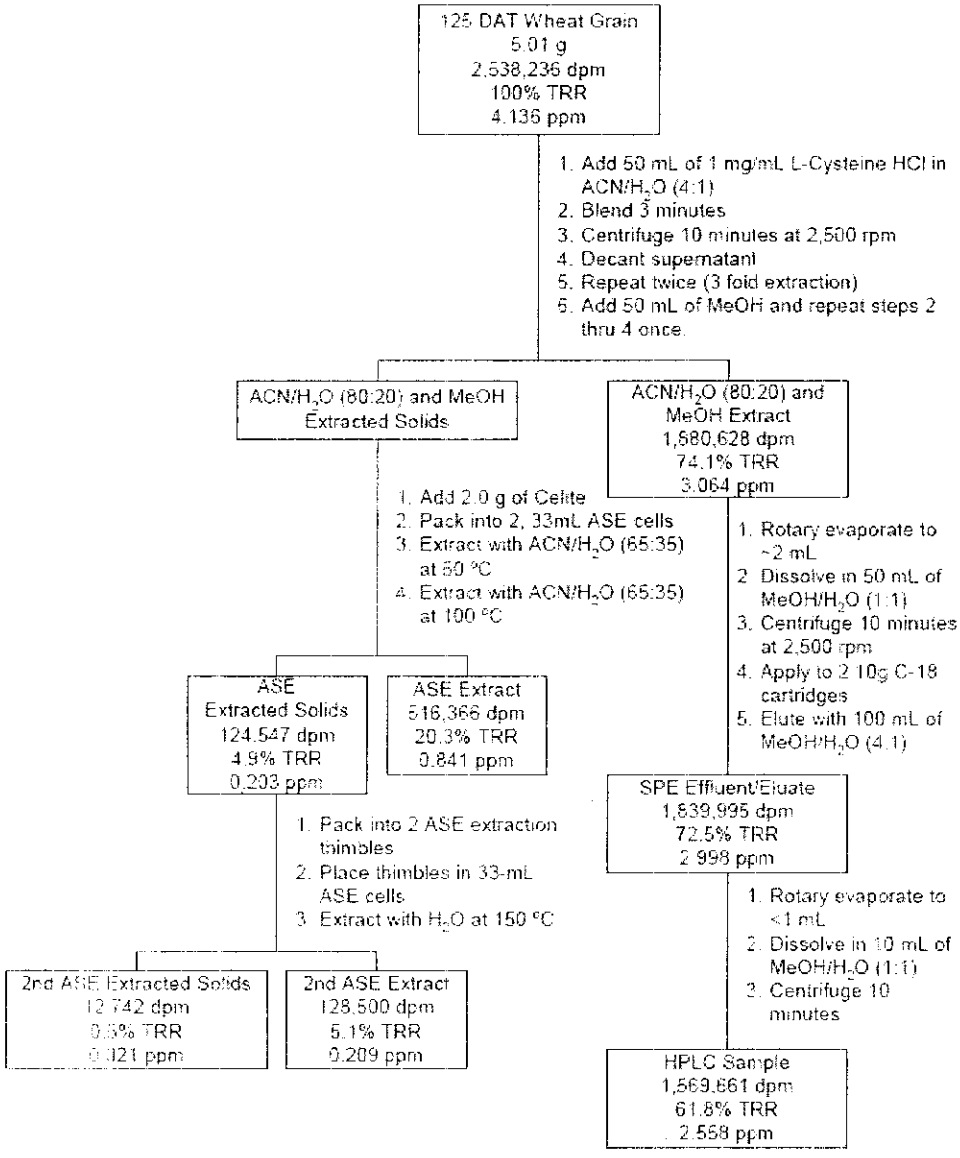


FIGURE B.4.1.2.2 Extraction Scheme for 125-DAT Wheat Grain (cont'd)

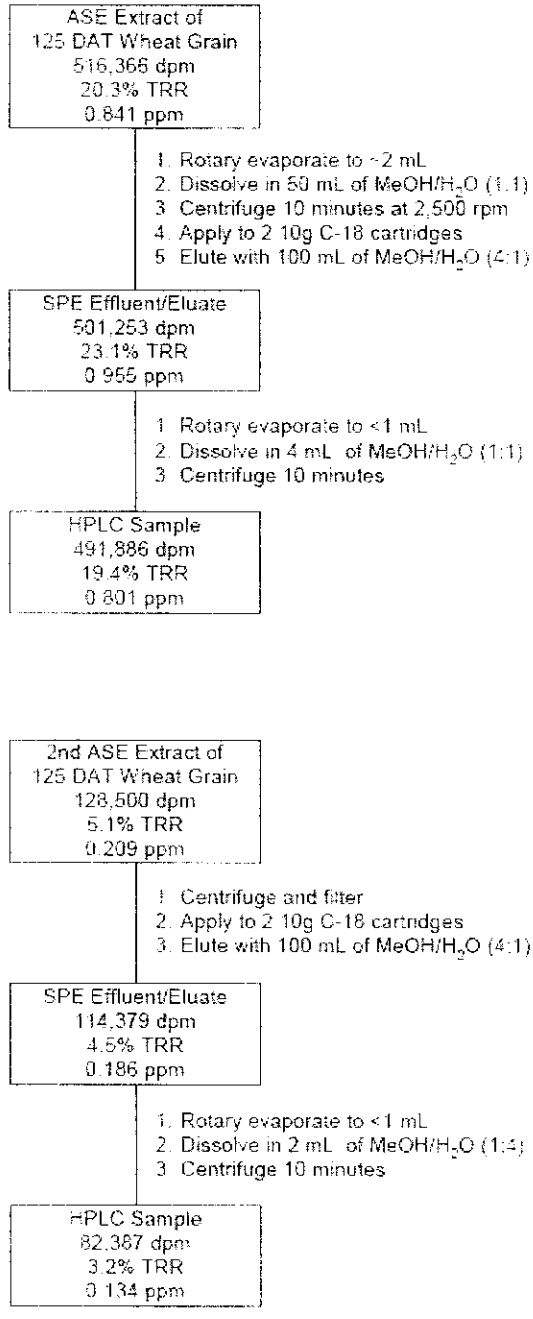


FIGURE B.4.1.2.3 Extraction Scheme for 366-DAT Wheat Grain

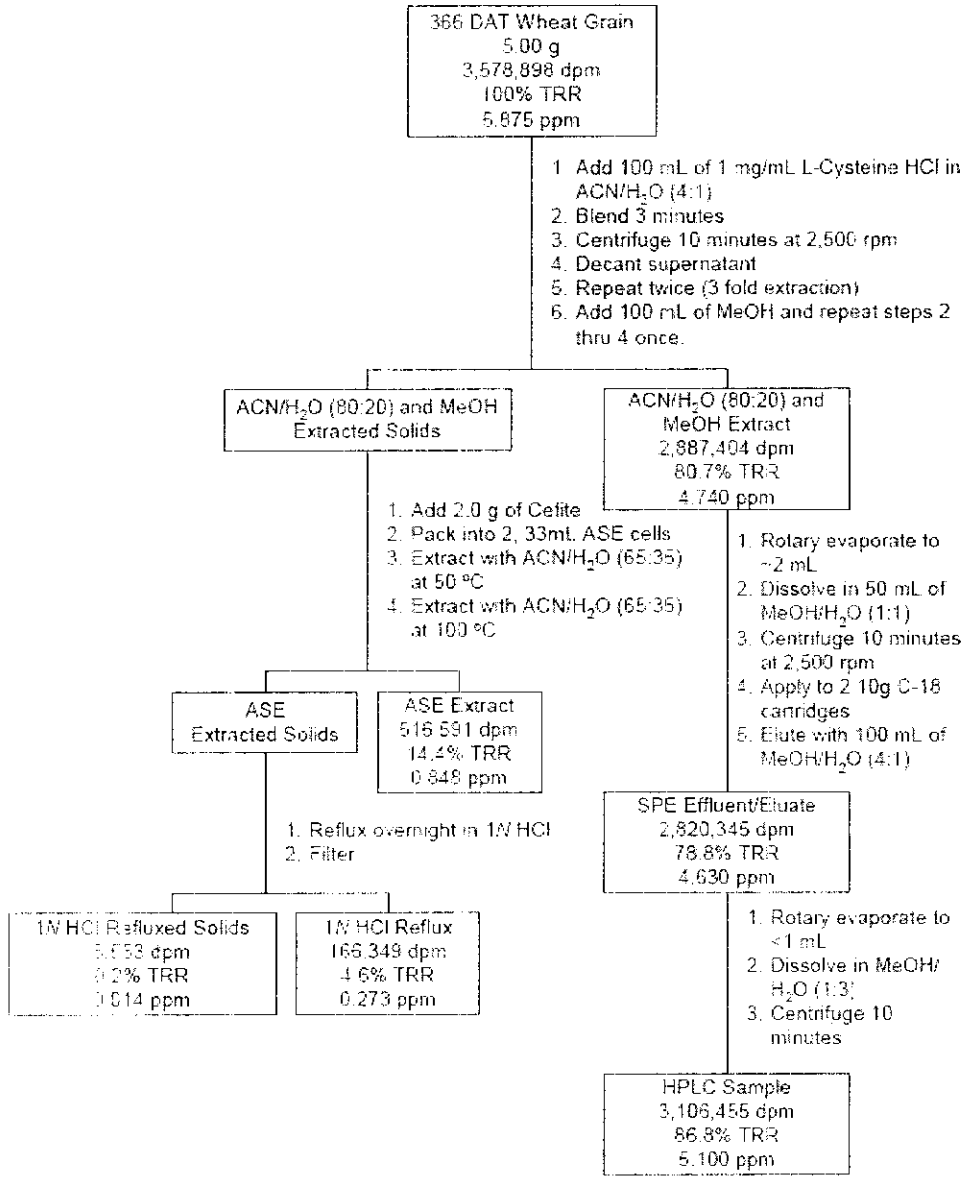


FIGURE B.4.1.2.3 Extraction Scheme for 366-DAT Wheat Grain (cont'd)

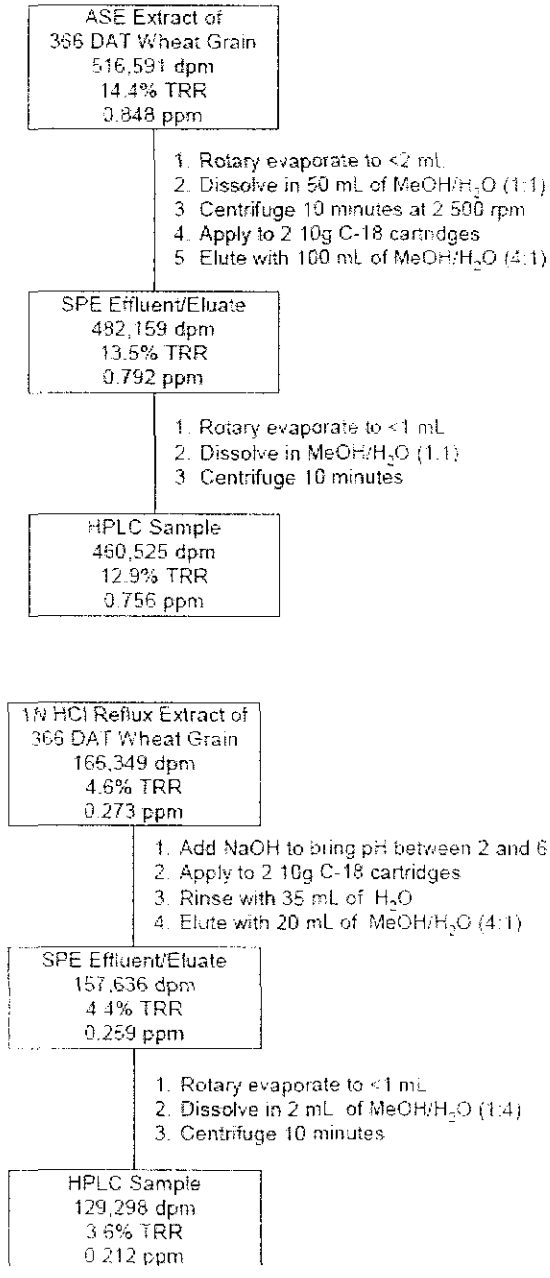


FIGURE B.4.1.2.4 Extraction Scheme for 30-DAT Swiss Chard

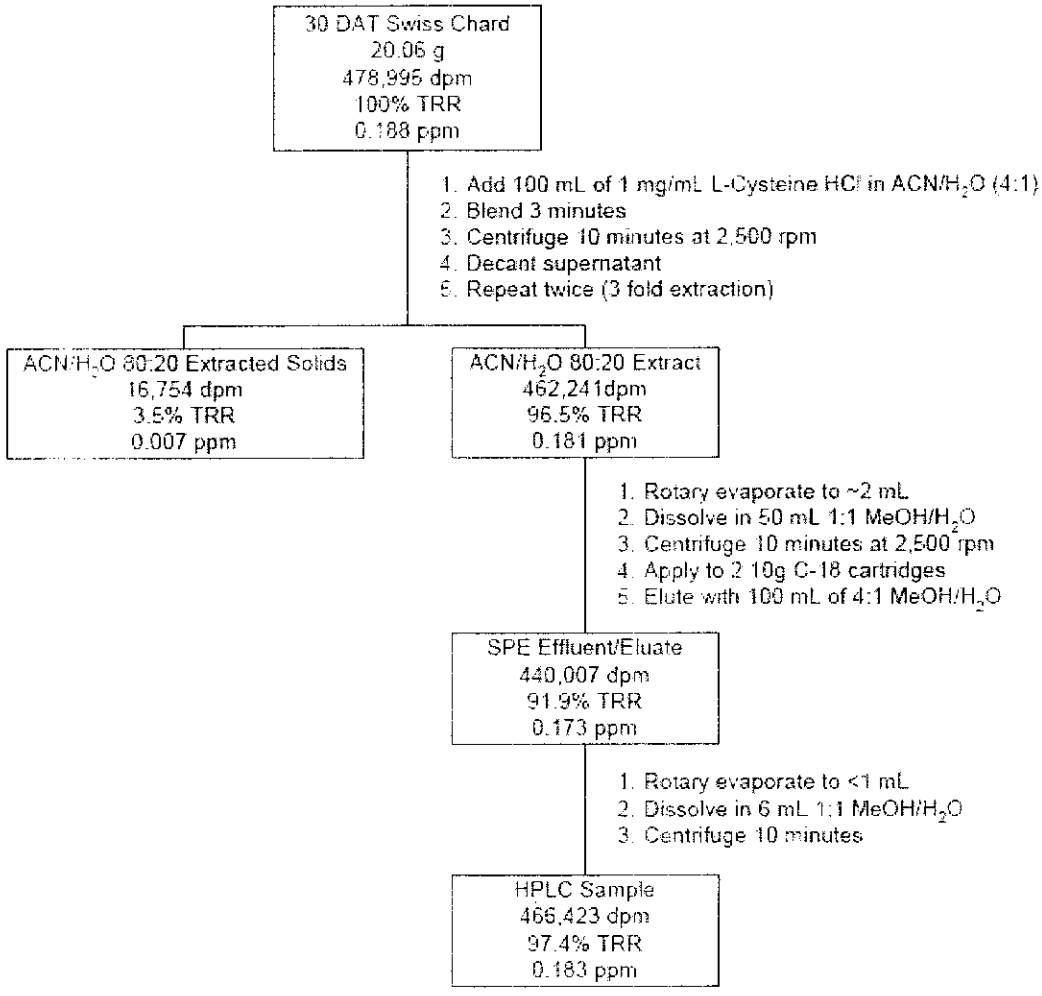


FIGURE B.4.1.2.5 Extraction Scheme for 125-DAT Swiss Chard

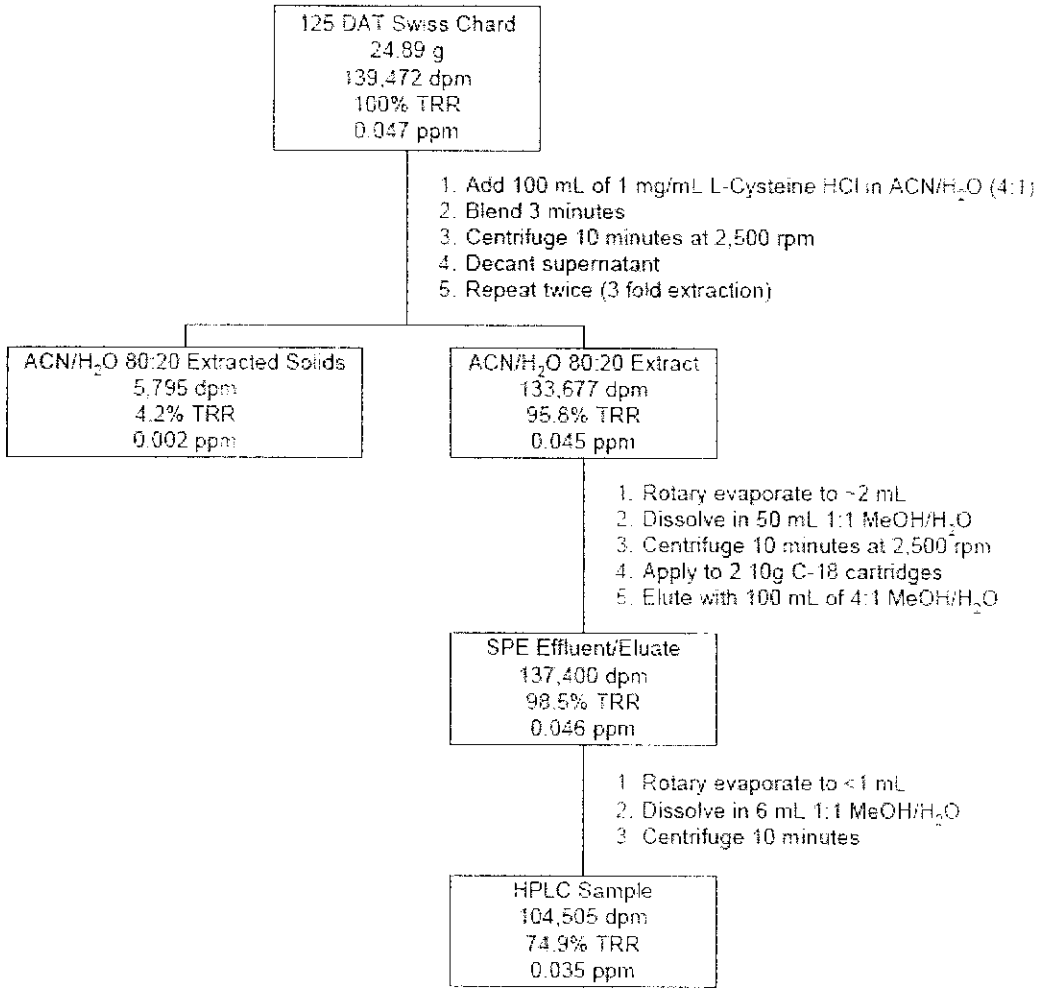


FIGURE B.4.1.2.6 Extraction Scheme for 366-DAT Swiss Chard

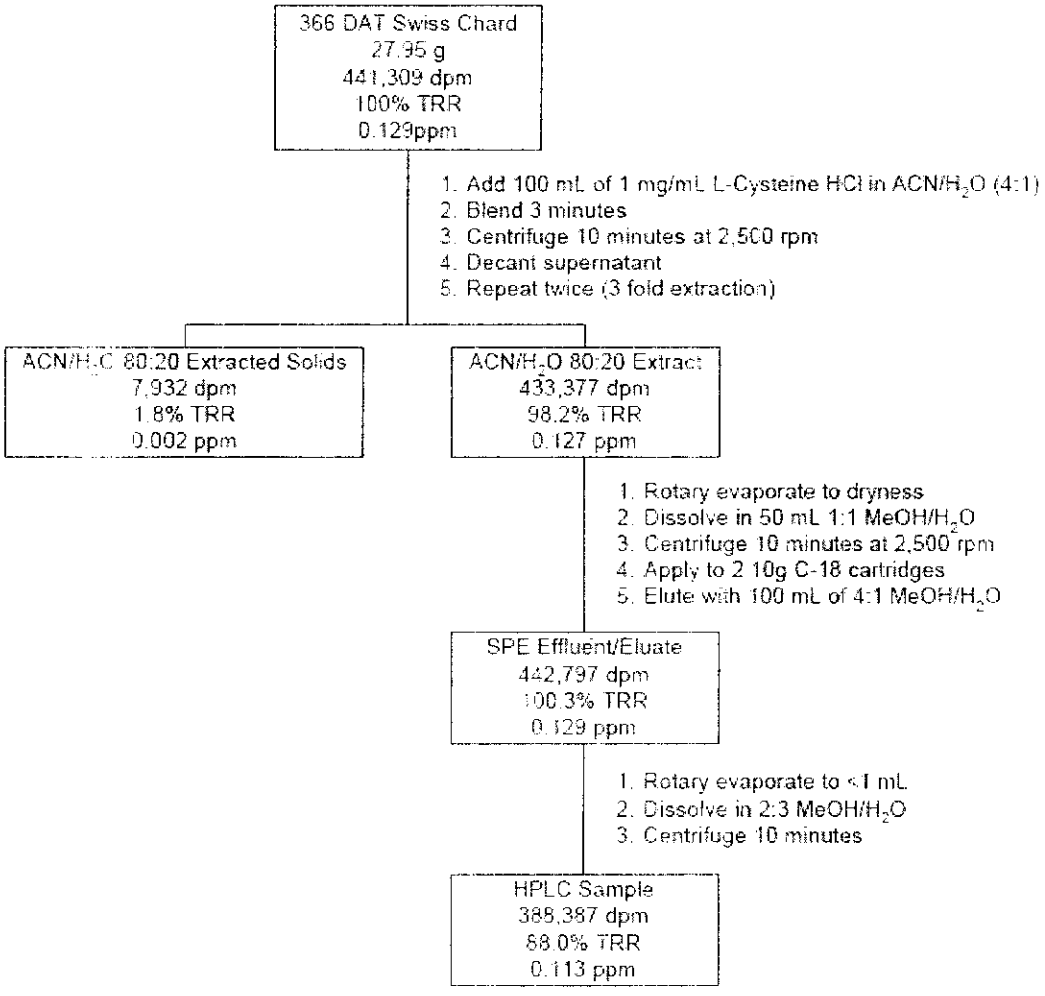


FIGURE B.4.1.2.7 Extraction Scheme for 30-DAT Turnip Tops

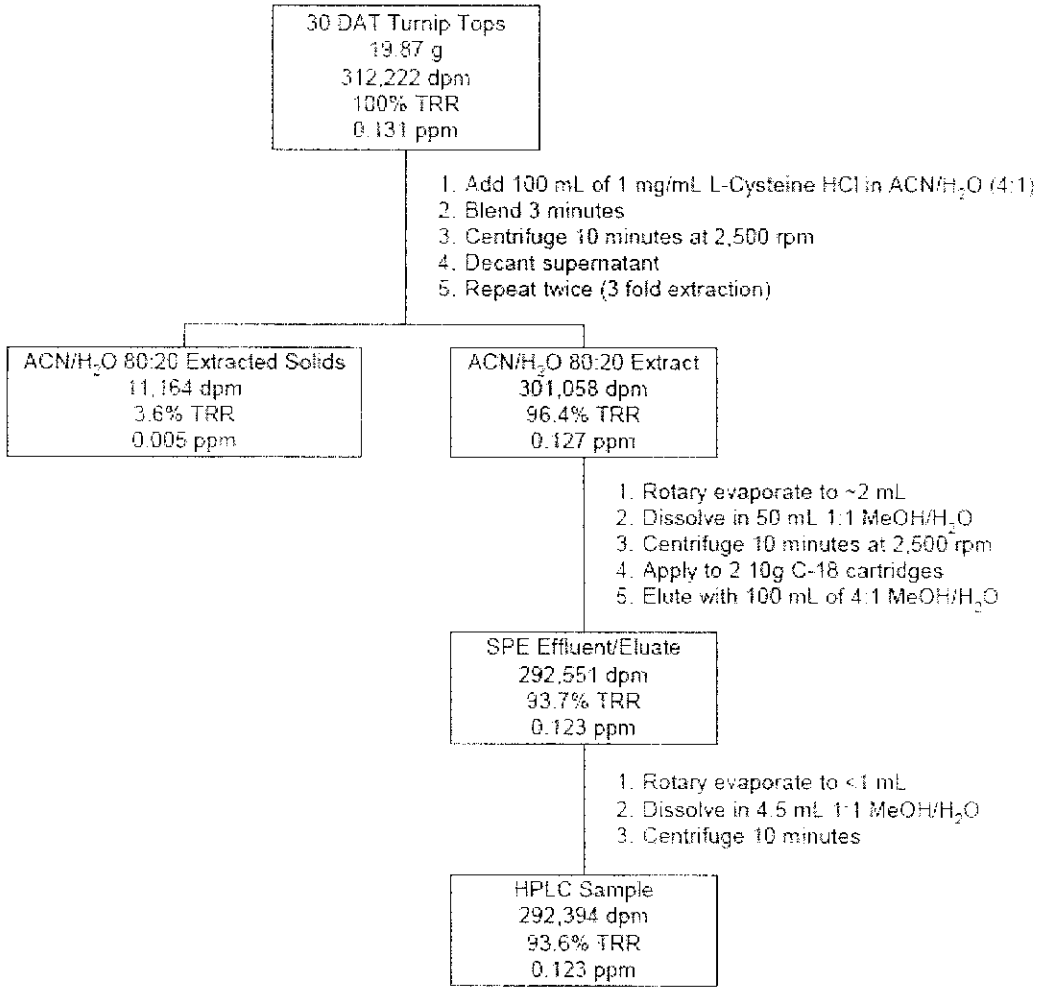


FIGURE B.4.1.2.8 Extraction Scheme for 125-DAT Turnip Tops

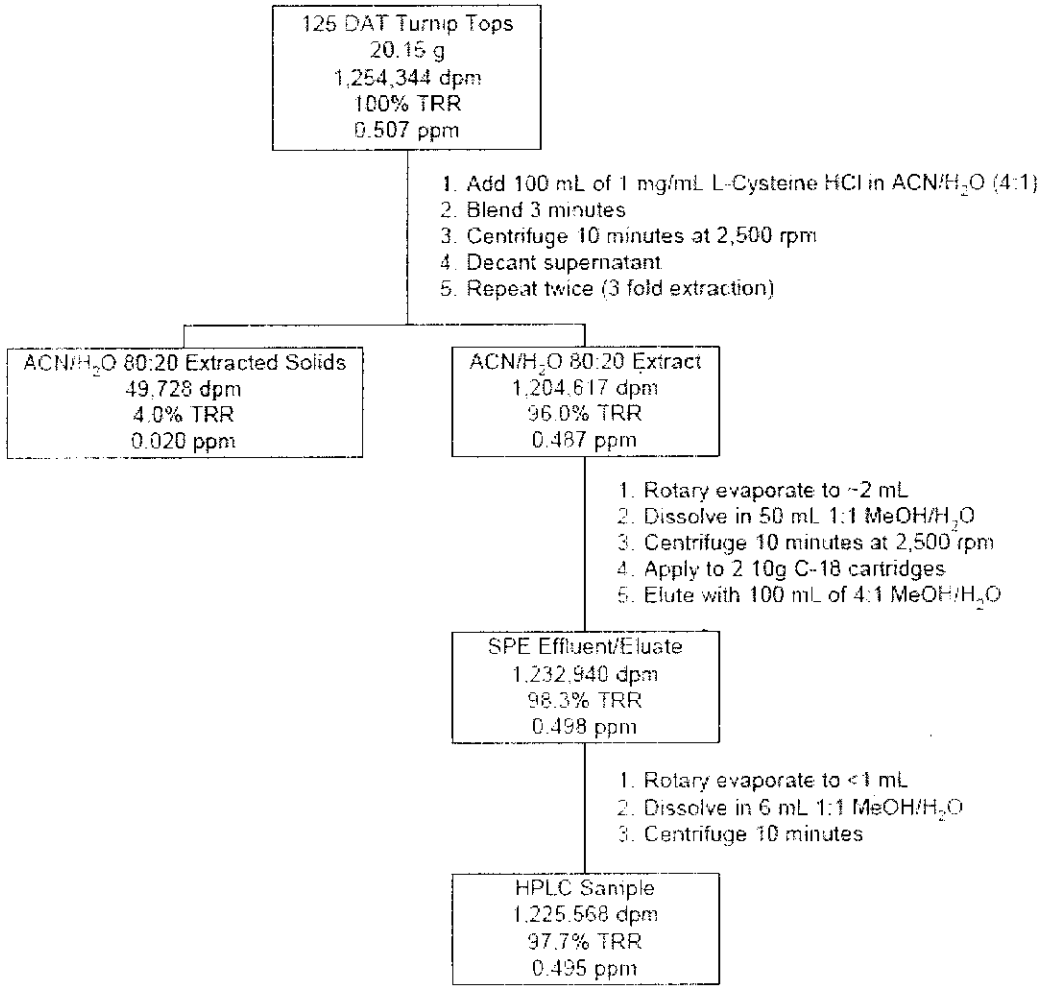


FIGURE B.4.1.2.9 Extraction Scheme for 366-DAT Turnip Tops

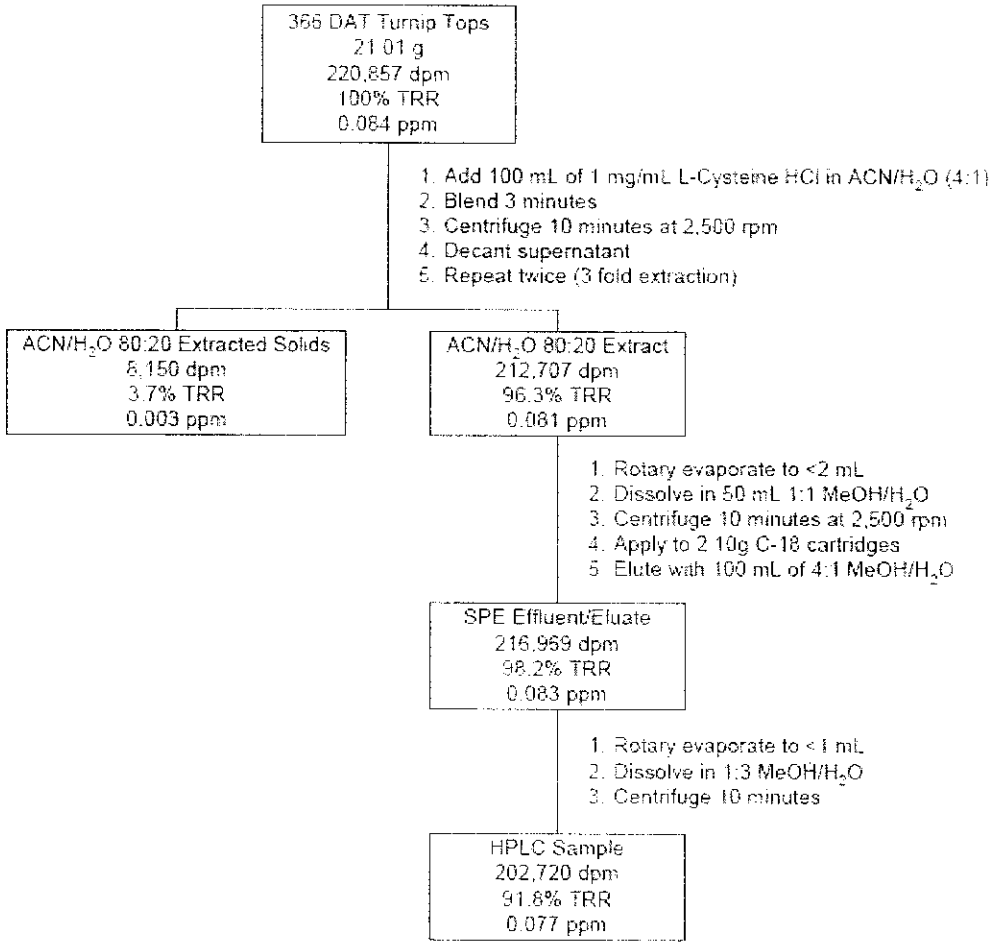


FIGURE B.4.1.3.0 Extraction Scheme for 30-DAT Turnip Roots

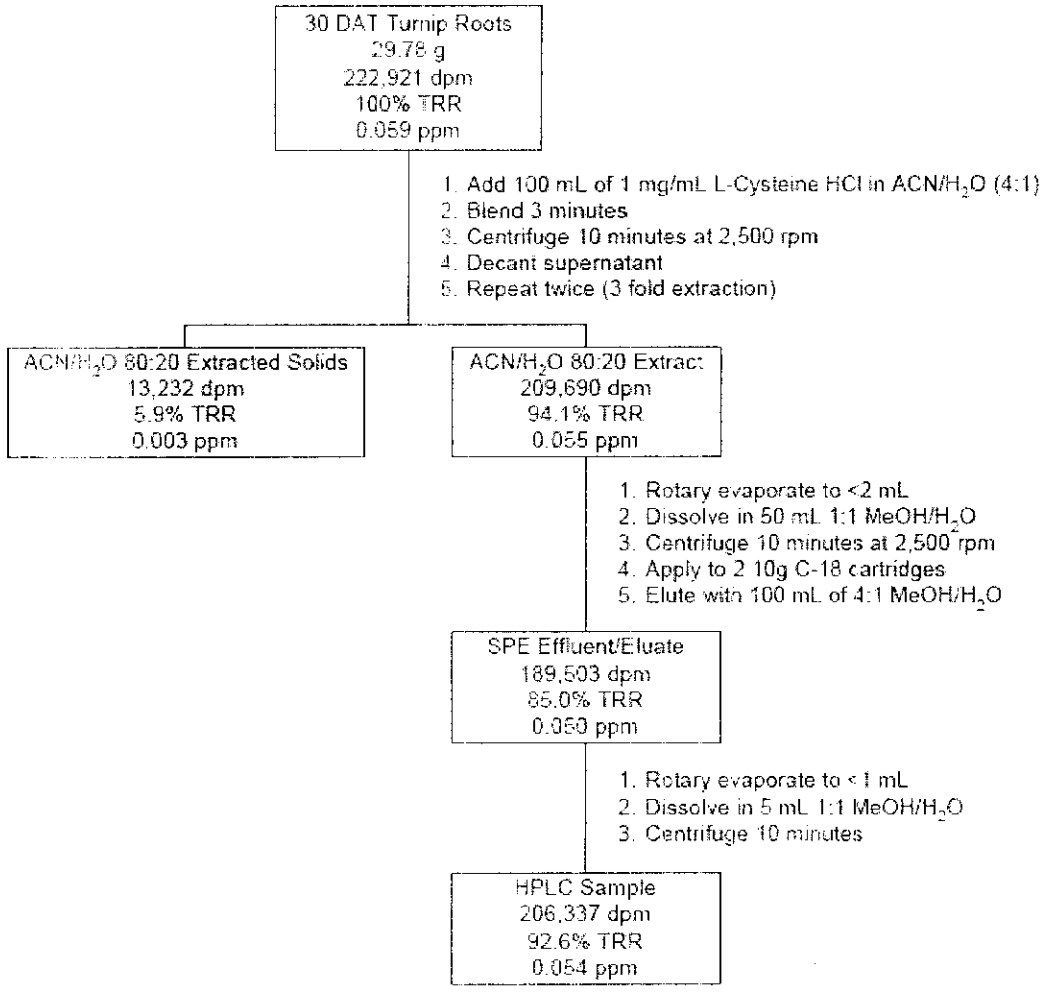


FIGURE B.4.1.3.1 Extraction Scheme for 125-DAT Turnip Roots

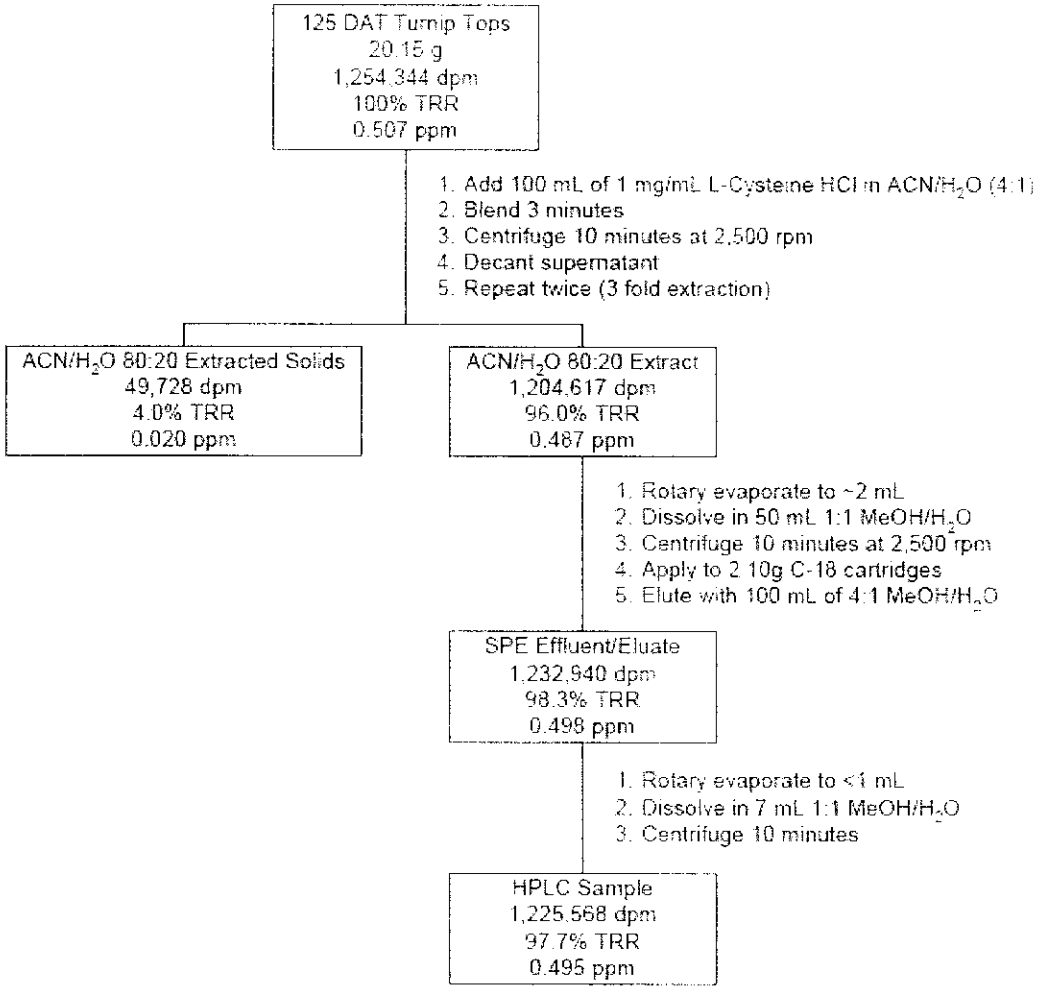
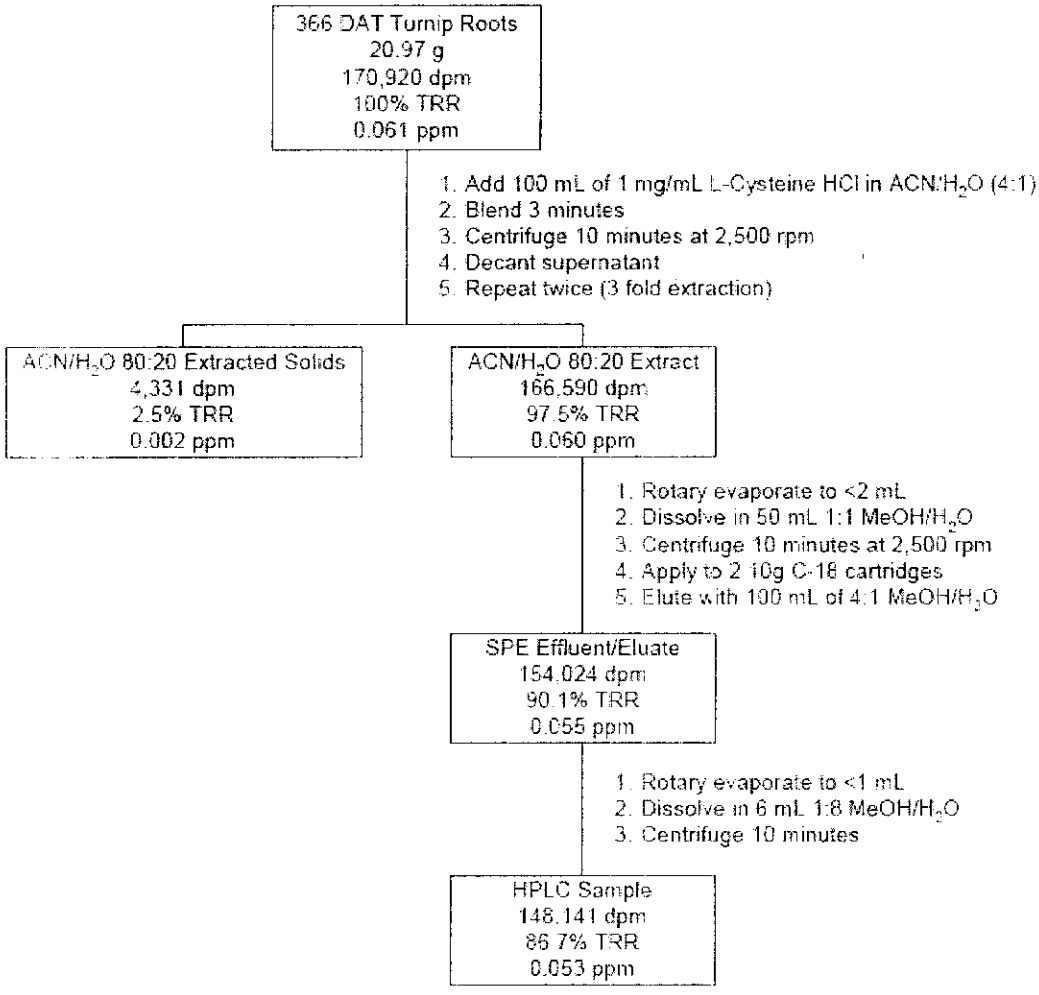


FIGURE B.4.1.3.2 Extraction Scheme for 366-DAT Turnip Roots



B.4.2. Analytical Methodology

Phenyl-label study

Total radioactive residues in rotational crop matrices were determined by summing radioactivity in extractable and non-extractable residues following extraction with ACN/water. Extracts and hydrolysates were radioassayed by LSC, and non-extractable residues were radioassayed by combustion/LSC. The limit of detection was reported as twice the background.

Extracts and hydrolysates of rotational crop matrices were subjected to reverse phase HPLC and/or reverse or normal phase TLC analysis for isolation, purification, and/or identification of metabolites. HPLC analyses were conducted on systems equipped with a UV detector and a flow-through radiodetector; a C18 column and a gradient mobile phase of water and ACN, each containing 0.1% acetic acid, were used. TLC analyses were conducted using silica gel Si60 F_{254} or C18 plates and one of the following solvent systems: DCM:methanol:25% ammonia (90:10:1, v:v:v); n-butanol:water:acetic acid (4:1:1, v:v:v); or ACN:water:methanol:acetic acid (70:25:5:1, v:v:v:v). The applicant noted that prothioconazole reference standard and cysteine HCl were spiked to each TLC origin before applying sample solutions to minimize decomposition of prothioconazole. Radioactive areas were detected and quantitatively evaluated using bioimaging, and nonradioactive reference standards were visualized under UV light. Metabolites were identified by co-chromatography and/or retention time comparisons with reference standards. Chemical names and structures for the reference standards used in this study are presented in Appendix I.

Triazole-label study

Total radioactive residues in rotational crop matrices were determined by combustion and LSC. Extracts and hydrolysates were radioassayed by LSC (or combustion/LSC for certain extracts), and non-extractable residues were radioassayed by combustion/LSC. A minimum sensitivity of 0.0033 ppm for combustion/LSC analyses was reported.

Extracts and hydrolysates of rotational crop matrices were subjected to reverse phase HPLC for isolation, purification, and/or identification of metabolites. HPLC analyses were conducted on systems equipped with a UV detector and a flow-through radiodetector. Extracts were initially analyzed using System A, consisting of a reverse-phase column and a gradient mobile phase of ACN and 0.1% TFA in water. The fraction containing triazolylalanine, THPA, and triazolylacetic acid was then injected onto HPLC System B to separate triazolylalanine from the other two compounds; System B consisted of a C8 column and a gradient mobile phase of water and MeOH each containing ion-pairing reagent pentyltriethylammonium phosphate at 0.005M. Metabolites were identified by co-chromatography and/or retention time comparisons with reference standards. Chemical names and structures for the reference standards used in this study are presented in Appendix I.

To confirm identification of triazolylalanine and to separate THPA from triazolylacetic acid, the metabolites were isolated from System B, and subjected to esterification. Triazolylalanine was purified using ion exchange chromatography, the eluate was evaporated to dryness, redissolved

in n-butanolic HCl, and heated at 110°C for 1 hour. Heptafluorobutyric anhydride was added and after standing for 10 min (or heating at 170°C for 10 min), the mixture was concentrated and purified by HPLC for LC-MS analyses. To generate the butyl ester derivatives of THPA and triazolylacetic acid, the System B fraction containing these metabolites was evaporated to dryness, redissolved in MeOH and again evaporated to dryness, and then redissolved in n-butanolic HCl and heated at 120°C for 30 minutes. The mixture was then concentrated and analyzed by HPLC using System C (C8 column and gradient mobile phase of ACN and 0.1% acetic acid in water) or System D (reverse-phase column and gradient mobile phase of ACN and 0.1% acetic acid in water); both systems allowed separation of the THPA butyl ester from the butyl ester of triazolylacetic acid.

For further identification of metabolites, LC-MS analyses were conducted. Metabolites were first purified using HPLC with a C18 column and an isocratic mobile phase of MeOH and 0.1% acetic acid in water. LC-MS analyses were conducted using a reverse-phase C8 column, a gradient mobile phase of 1% formic acid in water and MeOH, and MS detection with positive ion electrospray ionization.

C. RESULTS AND DISCUSSION

Phenyl-label study

The storage conditions for rotational crop samples are presented in TABLE C.1. The maximum number of days between harvest and HPLC analysis of the extracts was 57 days.

Total radioactive residues in rotational crops are reported in TABLE C.2.1. TRRs accumulated at ≥0.01 ppm in all rotated crops, except wheat grain, planted 28, 146, or 269 days following a single application of [phenyl-UL-^{14}C]-prothioconazole to bare soil at 0.52 lb a.i./A (582 g a.i./ha). TRRs were highest in wheat hay and straw. There was no general pattern of increasing or decreasing TRRs with increasing PBIs. At the 28-day PBI, residues were 0.114 and 0.450 ppm in wheat hay and straw, respectively, from 0.021 to 0.046 ppm in Swiss chard, turnip root and top, and wheat forage, and 0.007 ppm in wheat grain. Because of the low radioactivity levels in wheat grain, samples from the 146- and 269-day PBIs were not analyzed. At the 146-day PBI, residues were 0.135 and 0.307 ppm in wheat hay and straw, respectively, and from 0.028 to 0.062 ppm in Swiss chard, turnip root and top, and wheat forage. At the 269-day PBI, residues were 0.160 and 0.312 ppm in wheat hay and straw, respectively, and from 0.015 to 0.040 ppm in Swiss chard, turnip root and top, and wheat forage.

The extraction profiles and distribution of the radioactivity in rotational crop matrices are presented in TABLES C.2.2.1 through C.2.2.6. Extraction with ACN/water (containing cysteine HCl) released the majority of the TRRs (61-87% of the TRRs) from all rotational crop commodities except wheat grain; ACN/water released 23% of the TRRs from wheat grain. ASE with ACN/water released an additional 4 to 8% of the TRRs from wheat hay, straw, and grain, and acid hydrolysis with HCl/dioxane released approximately 9 to 21% of the TRRs from wheat hay and straw. Non-extractable residues remaining following extraction/hydrolysis accounted for <0.021 ppm in rotational crop matrices. Total accountabilites ranged from 99.1-143%. The extraction procedures extracted sufficient residues from rotational crop matrices from all PBIs.

The characterization and identification of residues in rotational crop matrices are summarized in TABLES C.2.3.1 through C.2.3.6. Total identified residues ranged from 34 to 77% of the TRRs in rotated crop commodities other than wheat grain, and were approximately 5% of the TRRs (0.003 ppm) in wheat grain. Prothioconazole was detected at very low levels (<1% of the TRRs, <0.005 ppm) in 146-day PBI Swiss chard, 28-day PBI turnip root, 146-day PBI turnip top, and 28- and 146-day PBI wheat straw; prothioconazole was not detected in any other rotational crop commodity. JAU6476-desthio was detected in all rotational crop commodities at all PBIs analyzed, and was found to be a major metabolite (present at >10% of the TRRs) in the following rotational crop commodities: 28- and 146-day PBI Swiss chard (17.5-36.8% of the TRRs, 0.010-0.014 ppm), 28-, 146-, and 269-day PBI turnip root (21.0-30.1% of the TRRs, <0.01 ppm), 28- and 269-day PBI turnip top (14.4-15.2% of the TRRs, <0.01 ppm), 28-day PBI wheat forage (13.2% of the TRRs, <0.01 ppm), and 28- and 146-day PBI wheat hay (11.4-12.4% of the TRRs, 0.014-0.016 ppm). JAU6476 sulfonic acid was found to be a major metabolite in 28-day PBI wheat hay (11.1% of the TRRs, 0.013 ppm) and 269-day PBI wheat straw (12.7% of the TRRs, 0.040 ppm); JAU6476 sulfonic acid was also detected at lower levels (≤8.3% of the TRRs;

≤0.025 ppm) in 28- and 146-day Swiss chard, 28-day PBI turnip top, 28- and 146-day PBI wheat forage and straw, and 146- and 269-day PBI wheat hay. Glucosides of JAU6476-desthio-dihydroxy-olefin (two isomers) were detected in all rotational crop commodities except 28-day PBI wheat grain, and one or both isomers were found to be major metabolites in 28-day PBI Swiss chard (13.6% of the TRRs; 0.005 ppm), 28-day PBI turnip root (10.8% of the TRRs; 0.005 ppm), turnip top (all PBIs, 10.8-14.3% of the TRRs; 0.004-0.006 ppm), 146- and 269-day PBI wheat forage (15.3-21.8% of the TRRs; 0.008-0.010 ppm), wheat hay (all PBIs, 10.3-18.3% of the TRRs; 0.012-0.029 ppm), and 269-day PBI wheat straw (10.6% of the TRRs; 0.033 ppm). Glucosides of JAU6476-hydroxy-desthio (up to three isomers) were also detected in all rotational crop commodities, except 28-day PBI wheat grain and 269-day PBI wheat forage, and at least one of the isomers accounted for significant radioactivity in 146-day PBI Swiss chard (10.1% of the TRRs; 0.006 ppm), 28-day PBI turnip root and top (10.2-13.7% of the TRRs; 0.005-0.006 ppm), and 146-day PBI wheat straw (10.3% of the TRRs; 0.031 ppm). Additional metabolites identified in rotational crops, each at <10% of the TRRs, were JAU6476-triazolinone, JAU6476-3-hydroxy-desthio, JAU6476-4-hydroxy-desthio, JAU6476-6-hydroxy-desthio, JAU6476-α-hydroxy-desthio, JAU6476-α-acetoxy-desthio, JAU6476-benzylpropyldiol and its glucoside, and JAU6476-disulfide.

The EtOAc/DCM fraction of the 28-day PBI wheat forage and grain were not intentionally fractionated but separated into two phases in a separatory funnel. Therefore, the two phases were individually analyzed by TLC. The reported TLC results represent the total of the two fractions.

The total radioactive residues found in the Swiss chard, turnip roots and leaves, and wheat forage, hay, and straw from the [phenyl-UL-^{14}C]-JAU6476 confined rotational crop study were quite low. Therefore, large quantities of the crop matrices needed to be extracted to obtain sufficient amounts of radioactivity for chromatographic analysis. Purification and concentration of extracts from large quantities of crop matrices usually lead to concurrent extraction and concentration of large quantities of plant co-extractives. For the reasons stated above, the separation and quantitation of metabolites in the phenyl-label-JAU6476 confined rotational crop study were performed with thin-layer chromatography using a Fuji Bio-Imaging Analyser. This analytical procedure allowed the separation and quantitation of residues with minimal purification and concentration of extracts.

Due to the higher levels of radioactive residues in the wheat straw, acceptable HPLC chromatograms were obtained for straw extracts. Since the HPLC chromatograms of the straw extracts from the first and third rotations were very similar, only the HPLC chromatogram from the first rotation was presented in the confined rotational crop report (as a representative chromatogram for wheat straw). Bayer's previous of analysis of samples from the JAU6476 metabolism studies, led to the discovery that polar regions in aqueous phases (or radioactivity not extractable by acetonitrile/water mixtures) were convertible to prothioconazole-desthio (or isomers of prothioconazole-hydroxy-desthio) by extracting with strong acids (ASE, under reflux) or in dioxane/HCl solutions). On the basis of these findings, it was concluded that these "highly polar metabolic group" in Swiss chard and turnip roots and tops could be converted to JAU6476-desthio upon acid hydrolysis. Therefore, these residues were

characterized as JAU6476-desthio. The primary reason to characterize these unidentified residues as JAU6476-desthio was to perform a worse-case risk assessment scenario, since it was considered the most toxicologically significant residue for JAU6476.

Triazole-label study

The storage conditions for rotational crop samples are presented in TABLE C.1.1. The maximum number of days between harvest and HPLC analysis of the extracts was 47 days. The exception was with wheat grain samples at the 30- and 125-PBI. Late attempts to extract more radioactivity with more stringent extraction methods (2nd ASE and dioxane/HCl), was pursued only to investigate if any unchanged and bound JAU6476 could be released.

Total radioactive residues in rotational crops are reported in TABLE C.2.1. TRRs accumulated at ≥0.01 ppm in all rotated crops planted 30, 125 or 366 days following four applications of [triazole-3,5-^{14}C]-prothioconazole to bare soil at a total rate of 0.727 lb a.i./A (815 g a.i./ha). TRRs were highest in wheat grain, hay, and straw. There was no general pattern of increasing or decreasing TRRs with increasing PBIs; for several crops, the highest TRRs were found at the 125-day PBI. At the 30-day PBI, residues were 1.695, 2.224, and 3.806 ppm in wheat straw, hay, and grain, respectively, and from 0.059 to 0.251 ppm in Swiss chard, turnip root and top, and wheat forage. At the 125-day PBI, residues were 1.361, 2.580, and 4.136 ppm in wheat straw, hay, and grain, respectively, and from 0.047 to 0.575 ppm in the other rotational crops. At the 366-day PBI, residues were 1.597, 2.016, and 5.875 ppm in wheat straw, hay, and grain, respectively, and from 0.061 to 0.439 ppm in the other rotational crops.

The extraction profiles and distribution of the radioactivity in rotational crop matrices are presented in TABLES C.2.2.1.1 through C.2.2.7. Extraction with ACN/water (Swiss chard and turnip root and top) or ACN/water and MeOH (wheat forage, hay, straw, and grain) released the majority of the TRRs (70-98% of the TRRs). We note that the ACN/water extraction mixture contained cysteine HCl. ASE with ACN/water at 50 and 100°C released an additional ~3 to 26% of the TRRs from all wheat matrices, and subsequent ASE with water released ~1 to 5% of the TRRs in wheat hay, straw, and grain. Acid hydrolysis with HCl/dioxane or HCl released ~1 to 5% of the TRRs in wheat hay, straw, and grain. Non-extractable residues remaining following extraction/hydrolysis accounted for ≤6% of the TRRs (≤0.076 ppm) in all rotational crop matrices. Total accountabilites ranged from 97-102%. The extraction procedures extracted sufficient residues from rotational crop matrices from all PBIs.

The characterization and identification of residues in rotational crop matrices are summarized in TABLES C.2.3.1.1 through C.2.3.7. Total identified residues ranged 71 to 99% of the TRRs (0.034-5.43 ppm) in rotated crop commodities. In general, there were no significant differences in the metabolic profiles in rotated crop commodities at different plantback intervals. Prothioconazole was not detected in any rotational crop commodity. Triazolylalanine was the major residue identified in Swiss chard, turnip root and top, and wheat forage and grain, at 44 to 56% of the TRRs (0.023-0.252 ppm) in Swiss chard and wheat forage, 58 to 68% of the TRRs (2.264-3.94 ppm) in wheat grain, and 74 to 93% of the TRRs (0.048-0.411 ppm) in turnip root and top. Triazolylalanine accounted for a major portion of the radioactivity in wheat hay and

straw, at 15 to 36% of the TRRs (0.197-0.846 ppm). Triazolylhydroxypropionic acid (THPA) was a major residue in Swiss chard and wheat forage, hay, and straw, at 18 to 39% of the TRRs (0.008-0.87 ppm). THPA was found at ≤7% of the TRRs (≤0.047 ppm) in rotated turnip root and top and wheat grain from the 30- and 125-day PBIs and was not found in these commodities from the 366-day PBI. Triazolylacetic acid accounted for significant radioactivity in wheat hay (10-22% of the TRRs; 0.222-0.578 ppm), straw (17-26% of the TRRs; 0.233-0.437 ppm), and grain (23-29% of the TRRs; 0.957-1.485 ppm); triazolylacetic acid was found at ≤6% of the TRRs (≤0.034 ppm) in Swiss chard, turnip root and top, and wheat forage. Additional metabolites identified in rotational crops, each at ≤7% of the TRRs (≤0.063 ppm), were triazolyl-ethanol (Swiss chard at all PBIs; 125-day PBI turnip root; 30- and 125-day PBI turnip top and wheat forage and straw; and wheat hay at all PBIs); triazolyl-ethanol glucoside (125-day PBI turnip root; 30- and 125-day PBI turnip top; and wheat forage, hay, and straw at all PBIs); JAU6476-desthio (30- and 125-day PBI Swiss chard and wheat hay; turnip root at all PBIs; 125-day PBI turnip top and wheat forage; and 30-day PBI wheat straw); and JAU6476-α-hydroxy-desthio (125-day PBI turnip root and wheat forage; 30- and 125-day PBI turnip top and wheat hay; and wheat straw at all PBIs). Free triazole (1*H*-1,2,4-triazole) was not identified in any rotational crop commodity.

The isolation of triazolylalanine for derivatization and identification as the butyl ester heptafluorobutyric amide derivative (BEHFBA) was performed from the 365 PBI wheat forage and grain Swiss chard, and turnip tops samples. The identity of triazolylalanine in wheat forage and grain Swiss chard, and turnip tops was confirmed by derivatizing the isolated metabolite and using HPLC to demonstrate that the derivatized compound co-chromatographed with a reference standard of triazolylalanine BEHFBA.

The metabolites THPA and triazolylacetic acid were isolated from wheat forage, hay, and straw, derivatized to their butyl esters, and analyzed by LC-MS to identify/confirm these compounds. For turnip top, the metabolites were identified based on HPLC retention time comparison of the derivatized metabolites and reference standards of the butyl esters of THPA and triazolylacetic acid. For wheat grain, the metabolites were isolated and derivatized; THPA was identified by HPLC retention time comparison and triazolylacetic acid was identified by LC-MS. For Swiss chard, the metabolites were isolated and derivatized; THPA was identified by LC-MS, and triazolylacetic acid was identified by HPLC retention time comparison. In turnip root, THPA and triazolylacetic acid were not present in sufficient quantities to allow derivatization.

The metabolites triazolyl-ethanol and triazolyl-ethanol-glucoside were isolated from wheat forage, hay, and straw and identified by LC-MS and LC-MS/MS analyses. Metabolite JAU6476-α-hydroxy-desthio was also isolated from wheat hay and identified by LC-MS and LC-MS/MS. These compounds were identified in other rotational crop commodities by HPLC retention time comparison

C.1. Storage Stability

Samples of rotated crop matrices for the phenyl-label study were stored frozen at -18°C prior to analysis and at -20±5°C for the triazole-label study.

TABLE C.1.1 Summary of Storage Conditions.

Matrix (RAC or Extract)	Plantback interval (days)	Storage Temp. (°C)	Actual Storage Duration (Days between Harvest and HPLC Analysis of Extract)	Interval of Demonstrated Storage Stability
Phenyl-label study				
Swiss chard	28 146 269	-18	45 39 19	Not required.
Turnip tops	28 146 269		31 55 19	
Turnip roots	28 146 269		31 55 18	
Wheat forage	28 146 269		31 49 23	
Wheat hay	28 146 269		14 25 57	
Wheat straw	28 146 269		16 44 30	
Wheat grain	28 146 269		26 Not collected for analysis Not collected for analysis	

TABLE C.1.1.1 Summary of Storage Conditions.					
Matrix (RAC or Extract)	Plant back interval (days)	Storage Temp. (°C)	Actual Storage Duration (Days between Harvest and HPLC Analysis of Extract)		Interval of Demonstrated Storage Stability
Triazole-label study					
Swiss chard	30	-20 ± 5	ACN/Water	2	Not required.
	125		ACN/Water	7	
	366		ACN/Water	2	
Turnip tops	30		ACN/Water	8	
	125		ACN/Water	4	
	366		ACN/Water	3	
Turnip roots	30		ACN/Water	47	
	125		ACN/Water	4	
	366		ACN/Water	6	
Wheat forage	30		ACN/Water	4	
	125		ACN/Water	2	
			ASE	9	
	366		ACN/Water	4	
			ASE	5	
Wheat hay	30		ACN/Water	10	
			ASE	11	
	125		ACN/Water	6	
			ASE	7	
	366		ACN/Water	6	
			ASE	13	
Wheat straw	30		ACN/Water	13	
			ASE	18	
	125		ACN/Water	16	
			ASE	18	
	366		ACN/Water	9	
			ASE	13	
Wheat grain	30		ACN/Water	13	
			ASE	18	
			2nd ASE	438*	
			Dioxane/HCl	434*	
	125		ACN/Water	8	
			1st ASE	10	
			2nd ASE	344*	
	366		ACN/Water	8	
			ASE	10	
			1 N HCl	15	

*Although the initial extractions performed within 13 to 18 days of harvest yielded 94% to 96% of the TRRs in the 30- and 125-day plant-back wheat grain samples, the late attempts to extract more radioactivity with more stringent extraction methods (although not really needed based on the level of extraction and identification of residues in the grain samples) was pursued only to investigate if any unchanged and bound JAU6476 could be released.

C.2. Identification, Characterization, and Distribution of Residues

TABLE C.2.1. Total Radioactive Residues (TRRs) in Rotational Swiss Chard, Turnip, and Wheat Commodities.

Matrix	Plantback interval, days	TRRs, ppm	Plantback interval, days	TRRs, ppm	Plantback interval, days	TRRs, ppm
Phenyl-label study						
Swiss chard	28	0.039	146	0.053	269	0.021
Turnip tops	28	0.046	146	0.028	269	0.036
Turnip roots	28	0.043	146	0.031	269	0.015
Wheat forage	28	0.021	146	0.062	269	0.04
Wheat hay	28	0.114	146	0.135	269	0.16
Wheat straw	28	0.45	146	0.307	269	0.312
Wheat grain	28	0.007	146	Not determined	269	Not determined
Triazole-label study						
Swiss chard	30	0.188	125	0.047	366	0.129
Turnip tops	30	0.131	125	0.507	366	0.084
Turnip roots	30	0.059	125	0.442	366	0.061
Wheat forage	30	0.251	125	0.575	366	0.439
Wheat hay	30	2.224	125	2.58	366	2.016
Wheat straw	30	1.695	125	1.361	366	1.597
Wheat grain	30	3.806	125	4.136	366	5.875

TABLE C.2.2.1. Distribution of the Parent and the Metabolites in Rotational Swiss Chard Following Application of [Phenyl-UL-^{14}C]-Prothioconazole to Bare Soil at 0.52 lb a.i./A (582 g a.i./ha). [1]

Metabolite Fraction	28-day PBI Swiss chard		146-day PBI Swiss chard		269-day PBI Swiss chard	
	TRRs = 0.039 ppm		TRRs = 0.053 ppm		TRRs = 0.021 ppm	
	%TRRs	ppm	%TRRs	ppm	%TRRs	ppm
ACN/water	86.8	0.034	84.3	0.045	61.1	0.013
n-hexane	17.8	0.007	4.5	0.002		
JAU6476-desthio	11	0.004				
JAU6476 sulfonic acid	0.4	<0.001				
JAU6476-triazolinone	1.6	0.001				
JAU6476-4-hydroxy-desthio	0.4	<0.001				
JAU6476-3-hydroxy-desthio	2.7	0.001				
JAU6476-α-hydroxy-desthio	0.2	<0.001				
JAU6476-disulfide	0.4	<0.001				
Unknowns	1.1	<0.001				
Aqueous phase 1	68.2	0.027	80.9	0.044		
DCM	27.1	0.011	32.6	0.018	11.1	0.002

TABLE C.2.2.1. Distribution of the Parent and the Metabolites in Rotational Swiss Chard Following Application of [Phenyl-UL-^{14}C]-Prothioconazole to Bare Soil at 0.52 lb a.i./A (582 g a.i./ha). [1]

Metabolite Fraction	28-day PBI Swiss chard		146-day PBI Swiss chard		269-day PBI Swiss chard	
	TRRs = 0.039 ppm		TRRs = 0.053 ppm		TRRs = 0.021 ppm	
	%TRRs	ppm	%TRRs	ppm	%TRRs	ppm
Prothioconazole	–	–	0.6	<0.001	--	--
JAU6476-desthio	25.8	0.01	17.5	0.01	6.2	0.0011
JAU6476 sulfonic acid	--	--	1.6	0.001	--	--
JAU6476-triazolinone	–	–	2.1	0.001	1	0.0002
JAU6476-4-hydroxy-desthio	--	--	0.6	<0.001	--	--
JAU6476-3-hydroxy-desthio	--	--	3.8	0.002	1.2	0.0002
JAU6476-α-hydroxy-desthio	--	--	1.5	0.001	1.2	0.0002
JAU6476-benzylpropyldiol	–	–	0.6	<0.001	0.7	0.0001
JAU6476-disulfide	1.3	0.001	1.1	0.001	0.8	0.0001
Unknowns	--	--	3.2	0.001	--	--
Aqueous phase 2	41.9	0.016	49.4	0.027	50	0.011
Glucoside of JAU6476-desthio-dihydroxy-olefin (isomer 1)	13.6	0.005	6.9	0.004	8.6	0.002
Glucoside of JAU6476-desthio-dihydroxy-olefin (isomer 2)	5.7	0.002	4.1	0.002	8.9	0.002
Glucoside of JAU6476-hydroxy-desthio (isomer 3)	9.7	0.004	10.1	0.006	5.8	0.001
Highly polar metabolic group	12.9	0.005	15.6	0.009	9.7	0.002
Unknowns[2]	--	--	12.6	0.007	17.0	0.004
Non-extractable	13.2	0.005	15.7	0.008	38.9	0.008

[1] Shading indicates that the extraction step and/or characterization analysis was not conducted for the matrix in question.
[2] Determined by partitioning and/or chromatographic behavior.

TABLE C.2.2.1.1 Distribution of the Parent and the Metabolites in Rotational Swiss Chard Following Application of [Triazole-3,5-^{14}C]-Prothioconazole to Bare Soil at 0.727 lb a.i./A (815 g a.i./ha).

Metabolite Fraction	30-day PBI Swiss chard		125-day PBI Swiss chard		366-day PBI Swiss chard	
	TRRs = 0.188 ppm		TRRs = 0.047 ppm		TRRs = 0.129 ppm	
	%TRRs	ppm	%TRRs	ppm	%TRRs	ppm
ACN/water	97	0.181	96	0.045	98	0.127
Triazolylalanine	51	0.096	49	0.023	56	0.072
THPA	32	0.06	18	0.008	30	0.038
Triazolylacetic acid	--	--			1	0.001
Triazolyl-ethanol	7	0.014	5	0.002	2	0.002
JAU6476-desthio	3	0.005	2	0.001	--	--
Unknowns[1]	5	0.008	2	0.002	--	--
Loss of radioactivity[2]	--	--	21	0.01	10	0.013
Non-extractable	3	0.007	4	0.002	2	0.002

[1]Determined by HPLC retention times.
[2]Loss of radioactivity due to transfer and preparation of sample for HPLC analysis

TABLE C.2.2.2. Distribution of the Parent and the Metabolites in Rotational Turnip Root Following Application of [Phenyl-UL-^{14}C]-Prothioconazole to Bare Soil at 0.52 lb a.i./A (582 g a.i./ha).

Metabolite Fraction	28-day PBI Turnip root		146-day PBI Turnip root		269-day PBI Turnip root	
	TRRs = 0.043 ppm		TRRs = 0.031 ppm		TRRs = 0.015 ppm	
	%TRRs	ppm	%TRRs	ppm	%TRRs	ppm
ACN/water	82.4	0.035	72.8	0.023	80	0.012
DCM	49.3	0.021	54.4	0.017	44	0.007
Prothioconazole	0.8	<0.001	--	--	--	--
JAU6476-desthio	21	0.009	30.1	0.009	28.1	0.0045
Glucoside of JAU6476-benzylpropyldiol	1.6	0.001	--	--	--	--
JAU6476-triazolinone	0.7	<0.001	1.4	<0.001	0.9	0.0001
JAU647-6-hydroxy-desthio	0.9	<0.001	2.3	0.001	3.3	0.0005
JAU6476-4-hydroxy-desthio	10.7	0.005	2.7	0.001	2.8	0.0004
JAU6476-3-hydroxy-desthio			2.4	0.001	2.6	0.0004
JAU6476-α-hydroxy-desthio	9.1	0.004	5.2	0.002	4.9	0.0008
JAU6476-benzylpropyldiol	1.7	0.001	1	<0.001	--	--
JAU6476-disulfide	--	--	1.8	0.001	0.8	0.0001
Unknowns	2.9	0.001	7.4	0.002	0.6	0.0001

TABLE C.2.2.2. Distribution of the Parent and the Metabolites in Rotational Turnip Root Following Application of [Phenyl-UL-^{14}C]-Prothioconazole to Bare Soil at 0.52 lb a.i./A (582 g a.i./ha).

Metabolite Fraction	28-day PBI Turnip root		146-day PBI Turnip root		269-day PBI Turnip root	
	TRRs = 0.043 ppm		TRRs = 0.031 ppm		TRRs = 0.015 ppm	
	%TRRs	ppm	%TRRs	ppm	%TRRs	ppm
Aqueous phase	33.1	0.014	18.4	0.006	32	0.005
Glucoside of JAU6476-desthio-dihydroxy-olefin (isomer 1+2)	10.8	0.005	4	0.001	4.9	0.001
Glucoside of JAU6476-hydroxy-desthio (isomer 1)	--	--	2.3	0.001	5.1	0.001
Glucoside of JAU6476-hydroxy-desthio (isomer 3)	13.7	0.006	2.5	0.001	4.5	0.001
Highly polar metabolic group	8.6	0.004	5.4	0.002	10.2	0.002
Unknowns [1]	-	-	4.2	0.001	7.3	0.001
Non-extractable	17.6	0.008	27.2	0.008	20	0.003

[1] Determined by partitioning and/or chromatographic behavior.

TABLE C.2.2.2.1 Distribution of the Parent and the Metabolites in Rotational Turnip Root Following Application of [Triazole-3,5-^{14}C]-Prothioconazole to Bare Soil at 0.727 lb a.i./A (815 g a.i./ha).

Metabolite Fraction	30-day PBI Turnip root		125-day PBI Turnip root		366-day PBI Turnip root	
	TRRs = 0.059 ppm		TRRs = 0.442 ppm		TRRs = 0.061 ppm	
	%TRRs	ppm	%TRRs	ppm	%TRRs	ppm
ACN/water	94	0.055	97	0.43	97	0.06
Triazolylalanine	81	0.048	93	0.411	85	0.052
THPA	5	0.003	1	0.005	--	--
Triazolylacetic acid					--	--
Triazolyl-ethanol-glucoside	--	--	<1	0.001	--	--
Triazolyl-ethanol	--	--	<1	0.002	--	--
JAU6476-4-hydroxy-desthio	--	--	<1	0.002	--	--
JAU6476-desthio	4	0.002	2	0.007	2	0.001
Unknowns [1]	2	0.001	<1	0.002	--	--
Loss of radioactivity[2]	–	--	–	–	10	0.007
Non-extractable	6	0.003	3	0.012	3	0.002

[1] Determined by chromatographic behavior.
[2]Loss of radioactivity during preparation of sample for HPLC analysis.

TABLE C.2.2.3. Distribution of the Parent and the Metabolites in Rotational Turnip Top Following Application of [Phenyl-UL-^{14}C]-Prothioconazole to Bare Soil at 0.52 lb a.i./A (582 g a.i./ha)[1].

Metabolite Fraction	28-day PBI Turnip top		146-day PBI Turnip top		269-day PBI Turnip top	
	TRRs = 0.046 ppm		TRRs = 0.028 ppm		TRRs = 0.036 ppm	
	%TRRs	ppm	%TRRs	ppm	%TRRs	ppm
ACN/water	82.9	0.038	79.8	0.022	73.9	0.027
n-hexane	12.5	0.006	4.3	0.001		
JAU6476-desthio	11.7	0.006				
JAU6476-4-hydroxy-desthio	0.3	<0.001				
JAU6476-α-hydroxy-desthio	0.5	<0.001				
Aqueous phase 1	69.7	0.032	74.5	0.021		
DCM	30.9	0.014	23.4	0.007	25.2	0.009
Prothioconazole	--	--	0.9	<0.001	--	--
JAU6476-desthio	3.5	0.002	6.3	0.002	14.4	0.005
Glucoside of JAU6476-benzylpropyldiol	2.7	0.001	1.8	0.001	--	--
JAU6476 sulfonic acid	1	<0.001	--	--	--	--
JAU6476-triazolinone	2	0.001	1	<0.001	--	--
JAU6476-6-hydroxy-desthio	1.7	0.001	1	<0.001	1.2	<0.001
JAU6476-4-hydroxy-desthio	5.2	0.002	2.3	0.001	1.8	0.001
JAU6476-3-hydroxy-desthio	3.2	0.001	1.4	<0.001		
JAU6476-α-hydroxy-desthio	6.3	0.003	5.1	0.002	2.5	0.001
JAU6476-benzylpropyldiol	0.3	<0.001	--	--	--	--
JAU6476-disulfide	--	--	0.5	<0.001	1.7	0.001
Unknowns	5	0.002	3.2	0.001	3.6	0.001
Aqueous phase 2	38.8	0.018	51.1	0.014	49.6	0.018
Glucoside of JAU6476-desthio-dihydroxy-olefin (isomer 1)	5.3	0.002	8	0.002	5.4	0.002
Glucoside of JAU6476-desthio-dihydroxy-olefin (isomer 2)	13.9	0.006	14.3	0.004	10.8	0.004
Glucoside of JAU6476-hydroxy-desthio (isomer 1)	9.4	0.004	6.3	0.002	7	0.003
Glucoside of JAU6476-hydroxy-desthio (isomer 3)	10.2	0.005	6.6	0.002	4.8	0.002
Highly polar metabolic group		--	15.9	0.004	10.5	0.004
Unknowns [2]	--	--	--	--	11.2	0.004
Non-extractable	17.1	0.008	20.2	0.006	26.1	0.009

[1] Shading indicates that the extraction step and/or characterization analysis was not conducted for the matrix in question.
[2] Determined by partitioning and/or chromatographic behavior

TABLE C.2.2.3.1 Distribution of the Parent and the Metabolites in Rotational Turnip Top Following Application of [Triazole-3,5-^{14}C]-Prothioconazole to Bare Soil at 0.727 lb a.i.A (815 g a.i./ha).

Metabolite Fraction	30-day PBI Turnip top		125-day PBI Turnip top		366-day PBI Turnip top	
	TRRs = 0.131 ppm		TRRs = 0.507 ppm		TRRs = 0.084 ppm	
	%TRRs	ppm	%TRRs	ppm	%TRRs	ppm
ACN/water	96	0.127	96	0.487	96	0.081
Triazolylalanine	76	0.1	74	0.377	92	0.077
THPA	7	0.009	7	0.035	--	--
Triazolylacetic acid			2	0.009	--	--
Triazolyl-ethanol-glucoside	3	0.004	3	0.015	--	--
Triazolyl-ethanol	3	0.004	4	0.02	--	--
JAU6476-α-hydroxy-desthio	2	0.002	1	0.007	--	--
JAU6476-desthio	--	--	1	0.005	--	--
Unknowns [1]	3	0.005	5	0.02	--	--
Loss of radioactivity[2]	2	0.003	--	--	4	0.004
Non-extractable	4	0.005	4	0.02	4	0.003

[1] Determined by chromatographic behavior.
[2]Loss of radioactivity during preparation of sample for HPLC analysis

TABLE C.2.2.4. Distribution of the Parent and the Metabolites in Rotational Wheat Forage Following Application of [Phenyl-UL-^{14}C]-Prothioconazole to Bare Soil at 0.52 lb a.i./A (582 g a.i./ha)[1].

Metabolite Fraction	28-day PBI Wheat forage		146-day PBI Wheat forage		269-day PBI Wheat forage	
	TRRs = 0.021 ppm		TRRs = 0.062 ppm		TRRs = 0.040 ppm	
	%TRRs	ppm	%TRRs	ppm	%TRRs	ppm
ACN/water	83.1	0.017	79.2	0.049	71.9	0.029
n-hexane	11.3	0.002	5.6	0.003		
JAU6476-desthio	10.3	0.002				
JAU6476-disulfide	1	<0.001				
Aqueous phase 1	73.2	0.015	73.6	0.046		
EtOAc [1]	39.4	0.008				
Fraction 1	6.7	0.002				
JAU6476-desthio	0.8	0.0002				
Glucoside of JAU6476-benzylpropyldiol	0.5	0.0002				
JAU6476 sulfonic acid	1.5	0.0004				
JAU6476-triazolinone	0.1	<0.0001				
JAU6476-3-hydroxy-desthio	0.1	<0.0001				
JAU6476-α-hydroxy-desthio	0.2	0.0001				
JAU6476-disulfide	0.2	<0.0001				

TABLE C.2.2.4. Distribution of the Parent and the Metabolites in Rotational Wheat Forage Following Application of [Phenyl-UL-^{14}C]-Prothioconazole to Bare Soil at 0.52 lb a.i./A (582 g a.i./ha)[1].

Metabolite Fraction	28-day PBI Wheat forage		146-day PBI Wheat forage		269-day PBI Wheat forage	
	TRRs = 0.021 ppm		TRRs = 0.062 ppm		TRRs = 0.040 ppm	
	%TRRs	ppm	%TRRs	ppm	%TRRs	ppm
Unknowns	3.3	0.001				
Fraction 2	32.7	0.006				
JAU6476-desthio	2.1	0.0004				
Glucoside of JAU6476-benzylpropyldiol	0.8	0.0001				
JAU6476 sulfonic acid	1.2	0.0002				
JAU6476-triazolinone	1.4	0.0003				
JAU6476-4-hydroxy-desthio	2.3	0.0004				
JAU6476-3-hydroxy-desthio	4.2	0.0008				
JAU6476-α-hydroxy-desthio	1.4	0.0003				
JAU6476-α-acetoxy-desthio	1.6	0.0003				
JAU6476-disulfide	0.8	0.0002				
Unknowns	16.9	0.0031				
DCM			16.7	0.01	11.2	0.004
JAU6476-desthio			1.5	0.0009	1.4	0.0005
Glucoside of JAU6476-benzylpropyldiol			0.3	0.0002	--	
JAU6476 sulfonic acid			0.2	0.0001	--	--
JAU6476-triazolinone			0.9	0.0006	1.9	0.0007
JAU6476-6-hydroxy-desthio			0.5	0.0003	--	--
JAU6476-4-hydroxy-desthio			1	0.0006	1	0.0004
JAU6476-3-hydroxy-desthio			1.4	0.0009		
JAU6476-α-hydroxy-desthio			1.7	0.001	2.6	0.0009
JAU6476-α-acetoxy-desthio			2.8	0.0017	2.5	0.0009
JAU6476-disulfide			0.9	0.0005	0.7	0.0003
Unknowns			5.3	0.0031	1.1	0.0004

TABLE C.2.2.4. Distribution of the Parent and the Metabolites in Rotational Wheat Forage Following Application of [Phenyl-UL-^{14}C]-Prothioconazole to Bare Soil at 0.52 lb a.i./A (582 g a.i./ha)[1].

Metabolite Fraction	28-day PBI Wheat forage		146-day PBI Wheat forage		269-day PBI Wheat forage	
	TRRs = 0.021 ppm		TRRs = 0.062 ppm		TRRs = 0.040 ppm	
	%TRRs	ppm	%TRRs	ppm	%TRRs	ppm
Aqueous phase 2	33.8	0.007	56.9	0.035	60.7	0.024
Glucoside of JAU6476-desthio-dihydroxy-olefin (isomer 1)	8.3	0.002	15.3	0.009	21.8	0.009
Glucoside of JAU6476-desthio-dihydroxy-olefin (isomer 2)	5.2	0.001	15.6	0.01	19.8	0.008
Glucoside of JAU6476-hydroxy-desthio (isomer 2)	--	--	7.4	0.005	--	--
Glucoside of JAU6476-hydroxy-desthio (isomer 3)	4	0.001	6.1	0.004	--	--
JAU6476 sulfonic acid	--	--	3.2	0.002	--	--
Unknowns	16.3	0.003	9.3	0.006	19.2	0.008
Non-extractable	16.9	0.004	20.8	0.013	28.1	0.011

[1] Shading indicates that the extraction step and/or characterization analysis was not conducted for the matrix in question.
[2] The EtOAc fraction was not further fractionated. However, two phases were observed in a separatory funnel. These phases were individually analyzed by TLC, but the results were added to the Table as one phase/fraction).

TABLE C.2.2.4.1 Distribution of the Parent and the Metabolites in Rotational Wheat Forage Following Application of [Triazole-3,5-^{14}C]-Prothioconazole to Bare Soil at 0.727 lb a.i./A (815 g a.i./ha) [1].

Metabolite Fraction	30-day PBI Wheat forage		125-day PBI Wheat forage		366-day PBI Wheat forage	
	TRRs = 0.251 ppm		TRRs = 0.575 ppm		TRRs = 0.439 ppm	
	%TRRs	ppm	%TRRs	ppm	%TRRs	ppm
ACN/water	96	0.241	93	0.534	95	0.418
Triazolylalanine	48	0.12	42	0.243	51	0.225
THPA	35	0.087	32	0.184	35	0.155
Triazolylacetic acid	3	0.008	6	0.034	1	0.006
Triazolyl-ethanol-glucoside	1	0.004	3	0.015	1	0.003
Triazolyl-ethanol	1	0.003	1	0.007	--	--
JAU6476-c-hydroxy-desthio	--	--	1	0.004	--	--
Unknowns	8	0.02	8	0.049	7	0.029
ASE extract			5	0.026	3	0.014
Triazolylalanine			2	0.009		
THPA			1	0.003		
Triazolylacetic acid						

TABLE C.2.2.4.1 Distribution of the Parent and the Metabolites in Rotational Wheat Forage Following Application of [Triazole-3,5-^{14}C]-Prothioconazole to Bare Soil at 0.727 lb a.i./A (815 g a.i./ha) [1].

Metabolite Fraction	30-day PBI Wheat forage		125-day PBI Wheat forage		366-day PBI Wheat forage	
	TRRs = 0.251 ppm		TRRs = 0.575 ppm		TRRs = 0.439 ppm	
	%TRRs	ppm	%TRRs	ppm	%TRRs	ppm
JAU6476-desthio			<1	0.001		
Unknowns [2]			1	0.007		
Loss of radioactivity[3]	-	-	1	0.007	--	--
Non-extractable	4	0.01	3	0.015	1	0.006

[1] Shading indicates that the extraction step and/or characterization analysis was not conducted for the matrix in question.
[2] Determined by chromatographic behavior.
[3] Loss of radioactivity during preparation of sample for HPLC analysis

TABLE C.2.2.5. Distribution of the Parent and the Metabolites in Rotational Wheat Hay Following Application of [Phenyl-UL-^{14}C]-Prothioconazole to Bare Soil at 0.52 lb a.i./A. (582 g a.i./ha)[1].

Metabolite Fraction	28-day PBI Wheat hay		146-day PBI Wheat hay		269-day PBI Wheat hay	
	TRRs = 0.114 ppm		TRRs = 0.135 ppm		TRRs = 0.160 ppm	
	%TRRs	ppm	%TRRs	ppm	%TRRs	ppm
ACN/water	77.4	0.088	70.3	0.095	70.6	0.113
n-hexane	4.7	0.005	2.9	0.004		
JAU6476-desthio	4.7	0.005				
Aqueous phase 1	72.6	0.083	67.9	0.092		
DCM	25.3	0.029	15.2	0.021	14.3	0.023
JAU6476-desthio	7.7	0.009	3.4	0.005	3.7	0.006
JAU6476 sulfonic acid	1.8	0.002	0.8	0.001	--	--
JAU6476-triazolinone	1.1	0.001	1.1	0.001	--	--
JAU6476-4-hydroxy-desthio	3.1	0.004	1.2	0.002	1.9	0.003
JAU6476-3-hydroxy-desthio	2.8	0.003	1	0.001	1.4	0.002
JAU6476-α-hydroxy-desthio	3	0.003	1.8	0.002	2.5	0.004
JAU6476-α-acetoxy-desthio	1.5	0.002	2	0.003	3.5	0.006
JAU6476-disulfide	2	0.002	1.2	0.002	0.6	0.001
Unknowns	2.2	0.002	2.7	0.004	0.8	0.001
Aqueous phase 2	47.4	0.054	52.7	0.071	56.2	0.09
Glucoside of JAU6476-desthio-dihydroxy-olefin (isomer 1)	7	0.008	13.3	0.018	15.2	0.024
Glucoside of JAU6476-desthio-dihydroxy-olefin (isomer 2)	10.3	0.012	15.3	0.021	18.3	0.029
Glucoside of JAU6476-hydroxy-desthio (isomer 2)	3.7	0.004	--	--	--	--
Glucoside of JAU6476-hydroxy-desthio (isomer 3)	6.1	0.007	5.4	0.007	6.3	0.01
JAU6476 sulfonic acid	9.3	0.011	5.8	0.008	--	--

TABLE C.2.2.5. Distribution of the Parent and the Metabolites in Rotational Wheat Hay Following Application of [Phenyl-UL-^{14}C]-Prothioconazole to Bare Soil at 0.52 lb a.i./A. (582 g a.i./ha)[1].

Metabolite Fraction	28-day PBI Wheat hay		146-day PBI Wheat hay		269-day PBI Wheat hay	
	TRRs = 0.114 ppm		TRRs = 0.135 ppm		TRRs = 0.160 ppm	
	%TRRs	ppm	%TRRs	ppm	%TRRs	ppm
Unknowns	11	0.012	12.9	0.017	16.4	0.027
Non-extractable	22.6	0.026	29.7	0.04	29.4	0.047
Combined ASE	4.7	0.006	8	0.011		
JAU6476-desthio			8	0.011		
Non-extractable	17.9	0.02	21.7	0.029		
Dioxane/HCl hydrolysate					16.2	0.026
JAU6476-desthio					4.6	0.007
JAU6476 sulfonic acid					2.4	0.004
Unknowns					9.2	0.015
Aqueous					7.2	0.012
Non-extractable					5.7	0.009

[1] Shading indicates that the extraction step and/or characterization analysis was not conducted for the matrix in question.

TABLE C.2.2.5.1. Distribution of the Parent and the Metabolites in Rotational Wheat Hay Following Application of [Triazole-3,5-^{14}C]-Prothioconazole to Bare Soil at 0.727 lb a.i./A (815 g a.i./ha). [1]

Metabolite Fraction	30-day PBI Wheat hay		125-day PBI Wheat hay		366-day PBI Wheat hay	
	TRRs = 2.224 ppm		TRRs = 2.580 ppm		TRRs = 2.016 ppm	
	%TRRs	ppm	%TRRs	ppm	%TRRs	ppm
ACN/water/MeOH	71	1.567	76	1.956	91	1.843
Triazolylalanine	23	0.501	24	0.622	33	0.662
THPA	29	0.651	20	0.518	27	0.549
Triazolylacetic acid	8	0.176	19	0.493	21	0.432
Triazolyl-ethanol-glucoside	2	0.045	2	0.06	1	0.03
Triazolyl-ethanol	1	0.03	1	0.029	1	0.021
JAU6476-c-hydroxy-desthio	1	0.019	1	0.023	--	--
JAU6476-desthio	1	0.02	1	0.02	--	--
Unknowns [2]	7 [3]	0.144	7 [4]	0.192	2	0.046
Loss of radioactivity [5]	-	-	-	-	5	0.104
ASE extract	26	0.58	21	0.533	6	0.128
Triazolylalanine	10	0.219	9	0.224	3	0.057
THPA	10	0.22	4	0.109	1	0.013
Triazolylacetic acid	2	0.046	3	0.085	<1	0.009
Unknowns	4 [6]	0.094	2 [7]	0.057	1	0.022
Loss of radioactivity [5]	-	-	2	0.059	1	0.026

TABLE C.2.2.5.1. Distribution of the Parent and the Metabolites in Rotational Wheat Hay Following Application of [Triazole-3,5-^{14}C]-Prothioconazole to Bare Soil at 0.727 lb a.i./A (815 g a.i./ha). [1]

Metabolite Fraction	30-day PBI Wheat hay		125-day PBI Wheat hay		366-day PBI Wheat hay	
	TRRs = 2.224 ppm		TRRs = 2.580 ppm		TRRs = 2.016 ppm	
	%TRRs	ppm	%TRRs	ppm	%TRRs	ppm
2nd ASE extract			1	0.033		
Dioxane/HCl hydrolysate			2	0.047		
Non-extractable	3	0.076	<1	0.011	2	0.045

[1] Shading indicates that the extraction step and/or characterization analysis was not conducted for the matrix in question.
[2] Determined by chromatographic behavior.
[3] A total of 6 unknowns, each ≤3% of the TRRs (≤0.074 ppm).
[4] A total of 13 unknowns, each ≤2% of the TRRs (≤0.051 ppm).
[5] Loss of radioactivity during preparation of sample for HPLC analysis
[6] A total of 6 unknowns, each ≤2% of the TRRs (≤0.048 ppm).
[7] A total of 2 unknowns, each 1% of the TRRs (0.024 and 0.033 ppm).

TABLE C.2.2.6. Distribution of the Parent and the Metabolites in Rotational Wheat Straw and Grain Following Application of [Phenyl-UL-^{14}C]-Prothioconazole to Bare Soil at 0.52 lb a.i./A (582 g a.i./ha). [1]

Metabolite Fraction	28-day PBI Wheat straw		146-day PBI Wheat straw		269-day PBI Wheat straw		28-day PBI Wheat grain	
	TRRs = 0.450 ppm		TRRs = 0.307 ppm		TRRs = 0.312 ppm		TRRs = 0.007 ppm	
	%TRRs	ppm	%TRRs	ppm	%TRRs	ppm	%TRRs	ppm
ACN/water	81.4	0.37	75.9	0.233	74.4	0.232	22.7	0.002
DCM	32.7	0.15	18	0.055	18.7	0.058	10.5 [2]	0.001
Prothioconazole	0.9	0.004	0.4	0.001	--	-	-	-
JAU6476-desthio	6.3	0.029	2.6	0.008	5.9	0.018	1.5	<0.001
Glucoside of JAU6476-benzylpropyldiol	--	-	-	-	0.9	0.003	--	
JAU6476 sulfonic acid	0.4	0.002	0.4	0.001	--	--	--	-
JAU6476-triazolinone	0.9	0.004	0.6	0.002	0.3	0.001	--	-
JAU6476-6-hydroxy-desthio	1.6	0.007	0.6	0.002	--	--	-	--
JAU6476-4-hydroxy-desthio	2.6	0.012	1.2	0.004	2.2	0.007		
JAU6476-3-hydroxy-desthio	3.7	0.017	1.4	0.004	1	0.003	1	<0.001
JAU6476-α-hydroxy-desthio	6.5	0.03	2.8	0.008	2.8	0.009	3.1	<0.001
JAU6476-benzylpropyldiol	2.2	0.01	0.5	0.002	--	--	--	--
JAU6476-α-acetoxy-desthio	2	0.009	4.4	0.013	2.8	0.009	--	--
JAU6476-disulfide	1.5	0.007	1.5	0.005	--	--	--	--
Unknowns	4.1	0.019	1.6	0.005	2.8	0.009	4.1	<0.001
Intermediate phase							7.3	<0.001

TABLE C.2.2.6. Distribution of the Parent and the Metabolites in Rotational Wheat Straw and Grain Following Application of [Phenyl-UL-^{14}C]-Prothioconazole to Bare Soil at 0.52 lb a.i./A (582 g a.i./ha). [1]

Metabolite Fraction	28-day PBI Wheat straw		146-day PBI Wheat straw		269-day PBI Wheat straw		28-day PBI Wheat grain	
	TRRs = 0.450 ppm		TRRs = 0.307 ppm		TRRs = 0.312 ppm		TRRs = 0.007 ppm	
	%TRRs	ppm	%TRRs	ppm	%TRRs	ppm	%TRRs	ppm
Aqueous phase 2	48.6	0.22	57.8	0.177	55.9	0.174	5	<0.001
Glucoside of JAU6476-desthio-dihydroxy-olefin (isomer 1)	5.2	0.023	4.9	0.015	7.3	0.023		
Glucoside of JAU6476-desthio-dihydroxy-olefin (isomer 2)	7	0.032	9.3	0.029	10.6	0.033		
Glucoside of JAU6476-hydroxy-desthio (isomer 1)	5.9	0.027	--	--	--	--		
Glucoside of JAU6476-hydroxy-desthio (isomer 2)	9.9	0.045	10.3	0.031	3.8	0.012		
Glucoside of JAU6476-hydroxy-desthio (isomer 3)	5.6	0.025	4.1	0.012	4.3	0.013		
JAU6476 sulfonic acid	--	--	7.9	0.024	12.7	0.04		
Unknowns	14.9 [3]	0.068	21.2 [4]	0.065	17.2 [5]	0.053		
Non-extractable	18.6	0.08	24.1	0.074	25.6	0.08	77.3	0.005
Combined ASE	3.8	0.02	5.8	0.018			4.6	<0.001
Non-extractable	14.7	0.06	18.3	0.056			72.7	0.005
Dioxane/HCl hydrolysate	9.5	0.04	9.2	0.028	21.3	0.066		
JAU6476-desthio	5	0.021	2.1	0.007	4.8	0.015		
JAU 6476 sulfonic acid	--	--	3.2	0.011	7.5	0.023		
Unknowns	4.4	0.019	3.9	0.013	9.1	0.028		
Aqueous	2.1	0.01	3.2	0.01	3	0.009		
Non-extractable	3.2	0.01	5.9	0.018	1.1	0.007		

[1] Shading indicates that the extraction step and/or characterization analysis was not conducted for the matrix in question.
[2] The DCM fraction of 28-day PBI wheat grain separated into two phases in a separatory funnel. Therefore, the two phases were individually analyzed by TLC. The reported TLC results represent the total of the two fractions.
[3] A total of two peaks plus origin, each representing ≤6.0% of the TRRs (≤0.027 ppm).
[4] A total of three peaks plus origin, each representing ≤7.3% of the TRRs (≤0.022 ppm).
[5] A total of two peaks plus origin, each representing ≤7.3% of the TRRs (≤0.023 ppm).

TABLE C.2.2.6. Distribution of the Parent and the Metabolites in Rotational Wheat Straw Following Application of [Triazole-3,5-^{14}C]-Prothioconazole to Bare Soil at 0.727 lb a.i./A (815 g a.i./ha).

Metabolite Fraction	30-day PBI Wheat straw		125-day PBI Wheat straw		366-day PBI Wheat straw	
	TRRs = 1.695 ppm		TRRs = 1.361 ppm		TRRs = 1.597 ppm	
	%TRRs	ppm	%TRRs	ppm	%TRRs [1]	ppm
ACN/water/MeOH	77	1.31	85	1.154	92	1.468
Triazolylalanine	14	0.244	12	0.161	24	0.378
THPA	27	0.451	28	0.382	29	0.47
Triazolylacetic acid	22	0.365	17	0.233	19	0.296
Triazolyl-ethanol-glucoside	2	0.042	5	0.063	2	0.029
Triazolyl-ethanol	1	0.023	2	0.027	--	--
JAU6476-α-hydroxy-desthio	1	0.017	2	0.026	1	0.016
JAU6476-desthio	1	0.014	--	--	--	--
Unknowns [4]	7	0.122	14 [3]	0.199	3	0.048
Loss of radioactivity[5]	2	0.032	4	0.061	14	0.231
ASE extract	19	0.327	9	0.126	4	0.064
Triazolylalanine	7	0.114	3	0.036	2	0.029
THPA	3	0.047	3	0.041	1	0.011
Triazolylacetic acid	4	0.072			<1	0.006
Unknowns [4]	6 [6]	0.094	3	0.022	1	0.011
Loss of radioactivity[5]	-	-	2	0.027	-	-
2nd ASE extract	2	0.029	2	0.025	2	0.03
Dioxane/HCl hydrolysate	1	0.022	3	0.044	2	0.029
Non-extractable	<1	0.006	1	0.011	<1	0.006

[1] The %TRRs values for individual peaks in the 366-day PBI straw have been corrected based on the data provided in the appendices.
[2] A total of 6 peaks, each ≤3% of the TRRs (≤0.055 ppm).
[3] A total of 8 peaks, each ≤3% of the TRRs (≤0.046 ppm).
[4] Determined by chromatographic behavior.
[5]Loss of radioactivity during preparation of sample for HPLC analysis
[6] A total of 6 peaks, each ≤2% of the TRRs (≤0.037 ppm).
[7] A total of 5 peaks, each ≤1% of the TRRs (≤0.017 ppm).

TABLE C.2.2.7. Distribution of the Parent and the Metabolites in Rotational Wheat Grain Following Application of [Triazole-3,5-^{14}C]-Prothioconazole to Bare Soil at 0.727 lb a.i./A (815 g a.i./ha) [1].

Metabolite Fraction	30-day PBI Wheat grain		125-day PBI Wheat grain		366-day PBI Wheat grain	
	TRRs = 3.806 ppm		TRRs = 4.136 ppm		TRRs = 5.875 ppm	
	%TRRs	ppm	%TRRs	ppm	%TRRs	ppm
ACN/water/MeOH	70	2.65	74	3.061	81	4.74
Triazolylalanine	38	1.46	39	1.606	53	3.11
THPA	1	0.047	<1	0.014	-	-
Triazolylacetic acid	26	1.002	20	0.847	24	1.436

TABLE C.2.2.7. Distribution of the Parent and the Metabolites in Rotational Wheat Grain Following Application of [Triazole-3,5-^{14}C]-Prothioconazole to Bare Soil at 0.727 lb a.i./A (815 g a.i./ha) [1].

Metabolite Fraction	30-day PBI Wheat grain		125-day PBI Wheat grain		366-day PBI Wheat grain	
	TRRs = 3.806 ppm		TRRs = 4.136 ppm		TRRs = 5.875 ppm	
	%TRRs	ppm	%TRRs	ppm	%TRRs	ppm
Unknowns [5]	4 [2]	0.163	2 [3]	0.087	3 [4]	0.194
Loss of radioactivity[6]	–	–	12	0.507	--	--
ASE extract	26	1.001	20	0.84	14	0.848
Triazolylalanine	19	0.721	16	0.655	12	0.682
THPA	--	–	<1	0.009	--	--
Triazolylacetic acid	3	0.114	3	0.11	1	0.049
Unknowns [5]	3 [7]	0.097	1	0.025	<1	0.025
Loss of radioactivity[6]	2	0.059	1	0.04		
2nd ASE extract	2	0.091	5	0.209		
Triazolylalanine	1	0.045	3	0.111		
Unknowns [5]	<1	0.009	1	0.023		
Loss of radioactivity[6]	1	0.032	2	0.075		
Dioxane/HCl hydrolysate	2	0.06				
Triazolylalanine	1	0.038				
Unknowns [5]	<1	0.016				
1 N HCl hydrolysate					5	0.273
Triazolylalanine					3	0.148
Unknowns [5]					1 [8]	0.066
Loss of radioactivity[6]	–	–	--	--	1	0.061
Non-extractable	<1	0.011	1	0.021	<1	0.014

[1] Shading indicates that the extraction step and/or characterization analysis was not conducted for the matrix in question.
[2] A total of 3 peaks, each ≤2% of the TRRs (≤0.077 ppm).
[3] A total of 3 peaks, each ≤1% of the TRRs (≤0.039 ppm).
[4] A total of 3 peaks, each ≤1% of the TRRs (≤0.078 ppm).
[5] Determined by chromatographic behavior.
[6] Loss of radioactivity during preparation of sample for HPLC analysis
[7] A total of 3 peaks, each ≤2% of the TRRs (≤0.076 ppm).
[8] A total of 3 peaks, each ≤1% of the TRRs (<0.056 ppm).

TABLE C.2.3.1. Summary of Characterization and Identification of Radioactive Residues in Rotational Swiss Chard Planted 28, 146, and 269 Days Following Application of [Phenyl-UL-^{14}C]-Prothioconazole to Bare Soil at 0.52 lb a.i./A (582 g a.i./ha).

Compound	28-day PBI Swiss chard		146-day PBI Swiss chard		269-day PBI Swiss chard	
	TRRs = 0.039 ppm		TRRs = 0.053 ppm		TRRs = 0.021 ppm	
	%TRRs	ppm	%TRRs	ppm	%TRRs	ppm
Identified						
Prothioconazole	–	–	0.6	<0.001	--	--
JAU6476-desthio	36.8	0.014	17.5	0.01	6.2	0.001
JAU6476 sulfonic acid	0.4	<0.001	1.6	0.001	--	--
JAU6476-triazolinone	1.6	0.001	2.1	0.001	1	<0.001*
JAU6476-4-hydroxy-desthio	0.4	<0.001	0.6	<0.001	--	--
JAU6476-3-hydroxy-desthio	2.7	0.001	3.8	0.002	1.2	<0.001
JAU6476-α-hydroxy-desthio	0.2	<0.001	1.5	0.001	1.2	<0.001
JAU6476-benzylpropyldiol	--	–	0.6	<0.001	0.7	<0.001
JAU6476-disulfide	1.7	0.001	1.1	0.001	0.8	<0.001
Glucoside of JAU6476-desthio-dihydroxy-olefin (isomer 1)	13.6	0.005	6.9	0.004	8.6	0.002
Glucoside of JAU6476-desthio-dihydroxy-olefin (isomer 2)	5.7	0.002	4.1	0.002	8.9	0.002
Glucoside of JAU6476-hydroxy-desthio (Isomer 3)	9.7	0.004	10.1	0.006	5.8	0.001
Characterized						
Polar metabolic group	12.9	0.005	15.6	0.009	9.7	0.002
Unknowns	1.1	<0.001	15.8	0.008	17	0.004
Fractions not further analyzed	--	--	4.5	0.002	--	--
Total identified	72.8	0.031	50.5	0.031	34.4	0.011
Total characterized	14.0	0.006	35.9	0.019	26.7	0.006
Total extractable	86.8	0.037	86.4	0.050	61.1	0.017
Unextractable (PES) [1]	13.2	0.005	15.7	0.008	38.9	0.008
Accountability [2]	108		109		119	

[1] Residues remaining after exhaustive extractions.

[2] Accountability = (Total extractable + Total unextractable)/(absolute values of the TRRs (ppm)) * 100.

* Values <0.001 ppm were included in the calculations for total identified and/or total characterized as 0.001 ppm. This resulted in high accountability values (>100%).

TABLE C.2.3.1.1 Summary of Characterization and Identification of Radioactive Residues in Rotational Swiss Chard Planted 30, 125, and 366 Days Following Application of [Triazole-3,5-^{14}C]-Prothioconazole to Bare Soil at 0.727 lb a.i./A (815 g a.i./ha).

Compound	30-day PBI Swiss chard		125-day PBI Swiss chard		366-day PBI Swiss chard	
	TRRs = 0.188 ppm		TRRs = 0.047 ppm		TRRs = 0.129 ppm	
	%TRRs	ppm	%TRRs	ppm	%TRRs	ppm
Identified						
Triazolylalanine	51	0.096	49	0.023	56	0.072
THPA	32	0.06	18	0.008	30	0.038
Triazolylacetic acid	--	--			1	0.001
Triazolyl-ethanol	7	0.014	5	0.002	2	0.002
JAU6476-desthio	3	0.005	2	0.001	--	--
Characterized						
Unknowns	5	0.008	2	0.001	--	--
Loss of Radioactivity[1]	-	-	21	0.01	10	0.013
Total identified	93	0.175	74	0.034	89	0.113
Total characterized	5	0.008	23	0.011	10	0.013
Total extractable	98	0.183	97	0.045	99	0.126
Unextractable (PES)[2]	3	0.007	4	0.002	2	0.002
Accountability[3]	101		100		99	

[1] Loss of radioactivity during preparation of sample for HPLC analysis
[2] Residues remaining after exhaustive extractions.
[3] Accountability = (Total extractable + Total unextractable)/(absolute values of the TRRs (ppm)) * 100.

TABLE C.2.3.2. Summary of Characterization and Identification of Radioactive Residues in Rotational Turnip Root Planted 28, 146, and 269 Days Following Application of [Phenyl-UL-^{14}C]-Prothioconazole to Bare Soil at 0.52 lb a.i./A (582 g a.i./ha).

Compound	28-day PBI Turnip root		146-day PBI Turnip root		269-day PBI Turnip root	
	TRRs = 0.043 ppm		TRRs = 0.031 ppm		TRRs = 0.015 ppm	
	%TRRs	ppm	%TRRs	ppm	%TRRs	ppm
Identified						
Prothioconazole	0.8	<0.001*	--	--	--	--
JAU6476-desthio	21	0.009	30.1	0.009	28.1	0.005
Glucoside of JAU6476-benzylpropyldiol	1.6	0.001	--	--	--	--
JAU6476-triazolinone	0.7	<0.001	1.4	<0.001	0.9	<0.001
JAU6476-6-hydroxy-desthio	0.9	<0.001	2.3	0.001	3.3	<0.001
JAU6476-4-hydroxy-desthio	10.7	0.005	2.7	0.001	2.8	<0.001
JAU6476-3-hydroxy-desthio			2.4	0.001	2.6	<0.001
JAU6476-α-hydroxy-desthio	9.1	0.004	5.2	0.002	4.9	<0.001
JAU6476-benzylpropyldiol	1.7	0.001	1	<0.001	--	--
JAU6476-disulfide	--	--	1.8	0.001	0.8	<0.001
Glucoside of JAU6476-desthio-dihydroxy-olefin (isomer 1+2)	10.8	0.005	4	0.001	4.9	0.001
Glucoside of JAU6476-hydroxy-desthio (isomer 1)	--	--	2.3	0.001	5.1	0.001
Glucoside of JAU6476-hydroxy-desthio (isomer 3)	13.7	0.006	2.5	0.001	4.5	0.001
Characterized						
Polar metabolic group	8.6	0.004	5.4	0.002	10.2	0.002
Unknowns[1]	2.9	0.001	11.6	0.003	7.9	0.001
Total identified	71.0	0.034	55.7	0.020	57.9	0.014
Total characterized	11.5	0.005	17.0	0.005	18.1	0.003
Total extractable	82.5	0.039	72.7	0.025	76.0	0.017
Unextractable (PES) [2]	17.6	0.008	27.2	0.008	20.0	0.003
Accountability [3]	109		106		133	

[1] Determined by partitioning and/or chromatographic behavior.
[2] Residues remaining after exhaustive extractions.
[3] Accountability = (Total extractable + Total unextractable)/(absolute values of the TRRs (ppm)) * 100.
* Values <0.001 ppm were included in the calculations for total identified and/or total characterized as 0.001 ppm. This resulted in high accountability values (>100%).

TABLE C.2.3.2.1 Summary of Characterization and Identification of Radioactive Residues in Rotational Turnip Root Planted 30, 125, and 366 Days Following Application of [Triazole-3,5-^{14}C]-Prothioconazole to Bare Soil at 0.727 lb a.i./A (815 g a.i./ha).

Compound	30-day PBI Turnip root		125-day PBI Turnip root		366-day PBI Turnip root	
	TRRs = 0.059 ppm		TRRs = 0.442 ppm		TRRs = 0.061 ppm	
	%TRRs	ppm	%TRRs	ppm	%TRRs	ppm
Identified						
Triazolylalanine	81	0.048	93	0.411	85	0.052
THPA	5	0.003	1	0.005	--	--
Triazolylacetic acid					--	--
Triazolyl-ethanol-glucoside	--	--	<1*	0.001	--	--
Triazolyl-ethanol	--	--	<1	0.002	--	--
JAU6476-α-hydroxy-desthio	--	--	<1	0.002	--	--
JAU6476-desthio	4	0.002	2	0.007	2	0.001
Characterized						
Unknowns	2	0.001	<1	0.002	--	--
Loss of Radioactivity[1]	-	-	--	--	10	0.007
Total identified	90	0.053	99	0.428	87	0.053
Total characterized	2	0.001	1	0.002	10	0.007
Total extractable	92	0.054	100	0.430	97	0.060
Unextractable (PES)[2]	6	0.003	3	0.012	3	0.002
Accountability[3]	97		100		102	

[1] Loss of radioactivity during preparation of sample for HPLC analysis
[2] Residues remaining after exhaustive extractions.
[3] Accountability = (Total extractable + Total unextractable)/(absolute values of the TRRs (ppm)) * 100.
* Values <1% of the TRRs were included in the calculations for total identified and/or total characterized as 1%.

TABLE C.2.3.3. Summary of Characterization and Identification of Radioactive Residues in Rotational Turnip Top Planted 28, 146, and 269 Days Following Application of [Phenyl-UL-^{14}C]-Prothioconazole to Bare Soil at 0.52 lb a.i./A (582 g a.i./ha).

Compound	28-day PBI Turnip top		146-day PBI Turnip top		269-day PBI Turnip top	
	TRRs = 0.046 ppm		TRRs = 0.028 ppm		TRRs = 0.036 ppm	
	%TRRs	ppm	%TRRs	ppm	%TRRs	ppm
Identified						
Prothioconazole	--	--	0.9	<0.001	--	--
JAU6476-desthio	15.2	0.008	6.3	0.002	14.4	0.005
Glucoside of JAU6476-benzylpropyldiol	2.7	0.001	1.8	0.001	--	--
JAU6476 sulfonic acid	1	<0.001*	--	--	--	--
JAU6476-triazolinone	2	0.001	1	<0.001	--	--
JAU6476-6-hydroxy-desthio	1.7	0.001	1	<0.001	1.2	<0.001
JAU6476-4-hydroxy-desthio	5.5	0.002	2.3	0.001	1.8	0.001
JAU6476-3-hydroxy-desthio	3.2	0.001	1.4	<0.001		
JAU6476-α-hydroxy-desthio	6.8	0.003	5.1	0.002	2.5	0.001
JAU6476-benzylpropyldiol	0.3	<0.001	--	--	--	--
JAU6476-disulfide	--	--	0.5	<0.001	1.7	0.001
Glucoside of JAU6476-desthio-dihydroxy-olefin (isomer 1)	5.3	0.002	8	0.002	5.4	0.002
Glucoside of JAU6476-desthio-dihydroxy-olefin (isomer 2)	13.9	0.006	14.3	0.004	10.8	0.004
Glucoside of JAU6476-hydroxy-desthio (isomer 1)	9.4	0.004	6.3	0.002	7	0.003
Glucoside of JAU6476-hydroxy-desthio (isomer 3)	10.2	0.005	6.6	0.002	4.8	0.002
Characterized						
Polar metabolic group	--	--	15.9	0.004	10.5	0.004
Unknowns [1]	5	0.002	3.2	0.001	14.8	0.005
Fractions not further analyzed	--	--	4.3	0.001	--	--
Total identified	77.2	0.036	55.5	0.021	49.6	0.020
Total characterized	5	0.002	23.4	0.006	25.3	0.009
Total extractable	82.2	0.038	78.9	0.027	74.9	0.029
Unextractable (PES) [2]	17.1	0.008	20.2	0.006	26.1	0.009
Accountability [3]	100		118		106	

[1] Determined by partitioning and/or chromatographic behavior.
[2] Residues remaining after exhaustive extractions.
[3] Accountability = (Total extractable + Total unextractable)/(absolute values of the TRRs (ppm)) * 100.
* Values <0.001 ppm were included in the calculations for total identified and/or total characterized as 0.001 ppm. This resulted in high accountability values (>100%).

TABLE C.2.3.3.1 Summary of Characterization and Identification of Radioactive Residues in Rotational Turnip Top Planted 30, 125, and 366 Days Following Application of [Triazole-3,5-^{14}C]-Prothioconazole to Bare Soil at 0.727 lb a.i./A (815 g a.i./ha).

Compound	30-day PBI Turnip top		125-day PBI Turnip top		366-day PBI Turnip top	
	TRRs = 0.131 ppm		TRRs = 0.507 ppm		TRRs = 0.084 ppm	
	%TRRs	ppm	%TRRs	ppm	%TRRs	ppm
Identified						
Triazolylalanine	76	0.1	74	0.377	92	0.077
THPA	7	0.009	7	0.035	--	--
Triazolylacetic acid			2	0.009	--	--
Triazolyl-ethanol-glucoside	3	0.004	3	0.015	--	--
Triazolyl-ethanol	3	0.004	4	0.02	--	--
JAU6476-α-hydroxy-desthio	2	0.002	1	0.007	--	--
JAU6476-desthio	--	--	1	0.005	--	--
Characterized						
Unknowns [1]	3	0.005	4	0.02	--	--
Loss of Radioactivity[2]	2	0.003	--	--	4	0.004
Total identified	91	0.119	92	0.468	92	0.077
Total characterized	5	0.008	4	0.020	4	0.004
Total extractable	97	0.127	96	0.488	96	0.081
Unextractable (PES) [3]	4	0.005	4	0.020	4	0.003
Accountability [4]	101		100		100	

[1] Determined by partitioning and/or chromatographic behavior.
[2] Loss of radioactivity during sample preparation for HPLC analysis
[3] Residues remaining after exhaustive extractions.
[4] Accountability = (Total extractable + Total unextractable)/(absolute values of the TRRs (ppm)) * 100.

TABLE C.2.3.4. Summary of Characterization and Identification of Radioactive Residues in Rotational Wheat Forage Planted 28, 146, and 269 Days Following Application of [Phenyl-UL-^{14}C]-Prothioconazole to Bare Soil at 0.52 lb a.i./A (582 g a.i./ha).

Compound	28-day PBI Wheat forage		146-day PBI Wheat forage		269-day PBI Wheat forage	
	TRRs = 0.021 ppm		TRRs = 0.062 ppm		TRRs = 0.040 ppm	
	%TRRs	ppm	%TRRs	ppm	%TRRs	ppm
Identified						
JAU6476-desthio	13.2	0.003	1.5	<0.001*	1.4	<0.001
Glucoside of JAU6476-benzylpropyldiol	1.3	<0.001	0.3	<0.001	--	--
JAU6476 sulfonic acid	2.7	<0.001	3.4	0.002	--	--
JAU6476-triazolinone	1.5	<0.001	0.9	<0.001	1.9	<0.001
JAU6476-6-hydroxy-desthio	--	--	0.5	<0.001	--	--
JAU6476-4-hydroxy-desthio	2.3	<0.001	1	<0.001	1	<0.001
JAU6476-3-hydroxy-desthio	4.3	0.001	1.4	<0.001		
JAU6476-α-hydroxy-desthio	1.6	<0.001	1.7	0.001	2.6	<0.001
JAU6476-α-acetoxy-desthio	1.6	<0.001	2.8	0.002	2.5	<0.001
JAU6476-disulfide	2	<0.001	0.9	<0.001	0.7	<0.001
Glucoside of JAU6476-desthio-dihydroxy-olefin (isomer 1)	8.3	0.002	15.3	0.009	21.8	0.009
Glucoside of JAU6476-desthio-dihydroxy-olefin (isomer 2)	5.2	0.001	15.6	0.01	19.8	0.008
Glucoside of JAU6476-hydroxy-desthio (isomer 2)	--	--	7.4	0.005	--	
Glucoside of JAU6476-hydroxy-desthio (isomer 3)	4	0.001	6.1	0.004	--	
Characterized						
Unknowns	36.5	0.007	14.9	0.009	20.3	0.008
Fractions not further analyzed	--	--	5.6	0.003	--	--
Total identified	48.0	0.015	58.8	0.040	51.7	0.023
Total characterized	36.5	0.007	20.5	0.012	20.3	0.008
Total extractable	84.5	0.022	79.3	0.052	72.0	0.031
Unextractable (PES) [1]	16.9	0.004	20.8	0.013	28.1	0.011
Accountability [2]	124		105		105	

[1] Residues remaining after exhaustive extractions.

[2] Accountability = (Total extractable + Total unextractable)/(absolute values of the TRRs (ppm)) * 100.

* Values <0.001 ppm were included in the calculations for total identified and/or total characterized as 0.001 ppm. This resulted in high accountability values (>100%).

TABLE C.2.3.4.1 Summary of Characterization and Identification of Radioactive Residues in Rotational Wheat Forage Planted 30, 125, and 366 Days Following Application of [Triazole-3,5-^{14}C]-Prothioconazole to Bare Soil at 0.727 lb a.i./A (815 g a.i./ha).

Compound	30-day PBI Wheat forage		125-day PBI Wheat forage		366-day PBI Wheat forage	
	TRRs = 0.251 ppm		TRRs = 0.575 ppm		TRRs = 0.439 ppm	
	%TRRs	ppm	%TRRs	ppm	%TRRs	ppm
Identified						
Triazolylalanine	48	0.12	44	0.252	51	0.225
THPA	35	0.087	32	0.184	35	0.155
Triazolylacetic acid	3	0.008	6	0.034	1	0.006
THPA/Triazolylacetic acid	--	--	1	0.003	--	--
Triazolyl-ethanol-glucoside	1	0.004	3	0.015	1	0.003
Triazolyl-ethanol	1	0.003	1	0.007	--	--
JAU6476-α-hydroxy-desthio	--	--	1	0.004	--	--
JAU6476-desthio	--	--	<1*	0.001	--	--
Characterized						
Unknowns	8	0.02	9	0.055	7	0.029
Fractions not further analyzed[1]	--	--	--	--	3	0.014
Loss of Radioactivity[2]	--	--	1	0.007	--	--
Total identified	88	0.222	88	0.500	88	0.389
Total characterized	8	0.020	10	0.062	10	0.043
Total extractable	96	0.242	98	0.562	98	0.432
Unextractable (PES)[3]	4	0.010	3	0.015	1	0.006
Accountability[4]	100		100		100	

[1] Radioactivity in extracts which were not analyzed
[2] Loss of radioactivity during sample preparation for HPLC analysis
[3] Residues remaining after exhaustive extractions.
[4] Accountability = (Total extractable + Total unextractable)/(absolute values of the TRRs (ppm)) * 100.
* Values <1% of the TRRs were included in the calculations for total identified and/or total characterized as 1%.

TABLE C.2.3.5. Summary of Characterization and Identification of Radioactive Residues in Rotational Wheat Hay Planted 28, 146, and 269 Days Following Application of [Phenyl-UL-^{14}C]-Prothioconazole to Bare Soil at 0.52 lb a.i./A (582 g a.i./ha).

Compound	28-day PBI Wheat hay		146-day PBI Wheat hay		269-day PBI Wheat hay	
	TRRs = 0.114 ppm		TRRs = 0.135 ppm		TRRs = 0.160 ppm	
	%TRRs	ppm	%TRRs	ppm	%TRRs	ppm
Identified						
JAU6476-desthio	12.4	0.014	11.4	0.016	3.7	0.006
JAU6476 sulfonic acid	11.1	0.013	6.6	0.009	--	--
JAU6476-triazolinone	1.1	0.001	1.1	0.001	--	--
JAU6476-4-hydroxy-desthio	3.1	0.004	1.2	0.002	1.9	0.003
JAU6476-3-hydroxy-desthio	2.8	0.003	1	0.001	1.4	0.002
JAU6476-α-hydroxy-desthio	3	0.003	1.8	0.002	2.5	0.004
JAU6476-α-acetoxy-desthio	1.5	0.002	2	0.003	3.5	0.006
JAU6476-disulfide	2	0.002	1.2	0.002	0.6	0.001
Glucoside of JAU6476-desthio-dihydroxy-olefin (isomer 1)	7	0.008	13.3	0.018	15.2	0.024
Glucoside of JAU6476-desthio-dihydroxy-olefin (isomer 2)	10.3	0.012	15.3	0.021	18.3	0.029
Glucoside of JAU6476-hydroxy-desthio (isomer 2)	3.7	0.004	--	--	--	--
Glucoside of JAU6476-hydroxy-desthio (isomer 3)	6.1	0.007	5.4	0.007	6.3	0.01
JAU6476-desthio [1]	--	--	--	--	4.6	0.007
JAU6476 sulfonic acid [1]	--	--	--	--	2.4	0.004
Characterized						
Unknowns	13.2	0.014	15.6	0.021	26.4	0.043
Fractions not further analyzed	4.7	0.006	2.9	0.004	7.2	0.012
Total identified	64.1	0.073	60.3	0.082	60.4	0.096
Total characterized	17.9	0.020	18.5	0.025	33.6	0.055
Total extractable	82.0	0.093	78.8	0.107	94.0	0.151
Unextractable (PES) [2]	17.9	0.020	21.7	0.029	5.7	0.009
Accountability [3]	99.1		101		100	

[1] Identified in acid hydrolysate.
[2] Residues remaining after exhaustive extractions.
[3] Accountability = (Total extractable + Total unextractable)/(absolute values of the TRRs (ppm)) * 100.

TABLE C.2.3.5.1 Summary of Characterization and Identification of Radioactive Residues in Rotational Wheat Hay Planted 30, 125, and 366 Days Following Application of [Triazole-3,5-^{14}C]-Prothioconazole to Bare Soil at 0.727 lb a.i./A (815 g a.i./ha).

Compound	30-day PBI Wheat hay		125-day PBI Wheat hay		366-day PBI Wheat hay	
	TRRs = 2.224 ppm		TRRs = 2.580 ppm		TRRs = 2.016 ppm	
	%TRRs	ppm	%TRRs	ppm	%TRRs	ppm
Identified						
Triazolylalanine	33	0.72	33	0.846	36	0.719
THPA	39	0.871	24	0.627	28	0.562
Triazolylacetic acid	10	0.222	22	0.578	22	0.441
Triazolyl-ethanol-glucoside	2	0.045	2	0.06	1	0.03
Triazolyl-ethanol	1	0.03	1	0.029	1	0.021
JAU6476-α-hydroxy-desthio	1	0.019	1	0.023	--	--
JAU6476-desthio	1	0.02	1	0.02	--	--
Characterized						
Unknowns	11	0.238	9	0.249	3	0.068
Fractions not further analyzed[1]	--	--	2	0.059	--	--
Loss of Radioactivity[2]	--	--	3	0.08	6	0.13
Total identified	87	1.927	85	2.183	88	1.773
Total characterized	11	0.238	14	0.388	9	0.198
Total extractable	98	2.165	99	2.571	97	1.971
Unextractable (PES)[3]	3	0.076	<1	0.011	2	0.045
Accountability[4]	101		100		100	

[1] Radioactivity in extracts which were not analyzed
[2] Loss of radioactivity during sample preparation for HPLC analysis
[3] Residues remaining after exhaustive extractions.
[4] Accountability = (*Total extractable + Total unextractable*)/(*absolute values of the TRRs (ppm)*) * 100.

TABLE C.2.3.6. Summary of Characterization and Identification of Radioactive Residues in Rotational Wheat Straw and Grain Planted 28, 146, and 269 Days Following Application of [Phenyl-UL-^{14}C]-Prothioconazole to Bare Soil at 0.52 lb a.i./A (582 g a.i./ha).

Compound	28-day PBI Wheat straw		146-day PBI Wheat straw		269-day PBI Wheat straw		28-day PBI Wheat grain	
	TRRs = 0.450 ppm		TRRs = 0.307 ppm		TRRs = 0.312 ppm		TRRs = 0.007 ppm	
	%TRRs	ppm	%TRRs	ppm	%TRRs	ppm	%TRRs	ppm
Identified								
Prothioconazole	0.9	0.004	0.4	0.001	--	--	--	--
JAU6476-desthio	6.3	0.029	2.6	0.008	5.9	0.018	1.5	<0.001*
Glucoside of JAU6476-benzylpropyldiol	–	–	–	–	0.9	0.003	--	--
JAU6476 sulfonic acid	0.4	0.002	8.3	0.025	12.7	0.04	--	--
JAU6476-triazolinone	0.9	0.004	0.6	0.002	0.3	0.001	--	--
JAU6476-6-hydroxy-desthio	1.6	0.007	0.6	0.002	--	--	--	--
JAU6476-4-hydroxy-desthio	2.6	0.012	1.2	0.004	2.2	0.007	1	<0.001
JAU6476-3-hydroxy-desthio	3.7	0.017	1.4	0.004	1	0.003		
JAU6476-α-hydroxy-desthio	6.5	0.03	2.8	0.008	2.8	0.009	3.1	<0.001
JAU6476-benzylpropyldiol	2.2	0.01	0.5	0.002	--	--	--	--
JAU6476-α-acetoxy-desthio	2	0.009	4.4	0.013	2.8	0.009	--	--
JAU6476-disulfide	1.5	0.007	1.5	0.005	--	--	--	--
Glucoside of JAU6476-desthio-dihydroxy-olefin (isomer 1)	5.2	0.023	4.9	0.015	7.3	0.023	--	--
Glucoside of JAU6476-desthio-dihydroxy-olefin (isomer 2)	7	0.032	9.3	0.029	10.6	0.033	--	--
Glucoside of JAU6476-hydroxy-desthio (isomer 1)	5.9	0.027	--	--	--	--	--	--
Glucoside of JAU6476-hydroxy-desthio (isomer 2)	9.9	0.045	10.3	0.031	3.8	0.012	--	--
Glucoside of JAU6476-hydroxy-desthio (isomer 3)	5.6	0.025	4.1	0.012	4.3	0.013	--	--
JAU6476-desthio [1]	5	0.021	2.1	0.007	4.8	0.015	--	--
JAU6476 sulfonic acid [1]	--	--	3.2	0.011	7.5	0.023	--	--
Characterized								
Unknowns[3]	23.4	0.106	26.7	0.083	29.1	0.09	4.1	<0.001
Fractions not further analyzed	5.9	0.03	9	0.028	3	0.009	16.9	0.001
Total identified	67.2	0.304	58.2	0.179	66.9	0.209	5.6	0.003
Total characterized	29.3	0.136	35.7	0.111	32.1	0.099	21.0	0.002

TABLE C.2.3.6. Summary of Characterization and Identification of Radioactive Residues in Rotational Wheat Straw and Grain Planted 28, 146, and 269 Days Following Application of [Phenyl-UL-^{14}C]-Prothioconazole to Bare Soil at 0.52 lb a.i./A (582 g a.i./ha).

Compound	28-day PBI Wheat straw		146-day PBI Wheat straw		269-day PBI Wheat straw		28-day PBI Wheat grain	
	TRRs = 0.450 ppm		TRRs = 0.307 ppm		TRRs = 0.312 ppm		TRRs = 0.007 ppm	
	%TRRs	ppm	%TRRs	ppm	%TRRs	ppm	%TRRs	ppm
Total extractable	*96.5*	*0.440*	93.9	0.290	99.0	0.308	26.6	0.005
Unextractable (PES) [2]	3.2	0.010	5.9	0.018	1.1	0.007	72.7	0.005
Accountability [4]	100		100		101		143	

[1] Identified in acid hydrolysate.
[2] Residues remaining after exhaustive extractions.
[3] Sum of single unidentified metabolites, fractions/phases, and TLC origins.
[4] Accountability = (Total extractable + Total unextractable)/(absolute values of the TRRs (ppm)) * 100.
* Values <0.001 ppm were included in the calculations for total identified and/or total characterized as 0.001 ppm.This resulted in high accountability values (>100%).

TABLE C.2.3.6.1 Summary of Characterization and Identification of Radioactive Residues in Rotational Wheat Straw Planted 30, 125, and 366 Days Following Application of [Triazole-3,5-^{14}C]-Prothioconazole to Bare Soil at 0.727 lb a.i./A (815 g a.i./ha).

Compound	30-day PBI Wheat straw		125-day PBI Wheat straw		366-day PBI Wheat straw	
	TRRs = 1.695 ppm		TRRs = 1.361 ppm		TRRs = 1.597 ppm	
	%TRRs	ppm	%TRRs	ppm	%TRRs [3]	ppm
Identified						
Triazolylalanine	21	0.358	15	0.197	26	0.407
THPA	30	0.498	28	0.382	30	0.481
Triazolylacetic acid	26	0.437	17	0.233	19	0.302
THPA/Triazolylacetic acid	--	--	3	0.041	--	--
Triazolyl-ethanol-glucoside	2	0.042	5	0.063	2	0.029
Triazolyl-ethanol	1	0.023	2	0.027	--	--
JAU6476-α-hydroxy-desthio	1	0.017	2	0.026	1	0.016
JAU6476-desthio	1	0.014	--	--	--	--
Characterized						
Unknowns	13	0.216	17	0.221	4	0.059
Fractions not further analyzed[1]	3	0.051	5	0.069	4	0.059
Loss of Radioactivity[2]	2	0.032	6	0.088	14	0.231
Total identified	82	1.389	72	0.969	78	1.235
Total characterized	18	0.299	28	0.378	22	0.349
Total extractable	100	1.688	100	1.347	100	1.584
Unextractable (PES) [3]	<1	0.006	1	0.011	<1	0.006
Accountability [4]	100		100		100	

[1] Radioactivity in extracts which were not analyzed
[2] Loss of radioactivity during sample preparation for HPLC analysis
[3] Residues remaining after exhaustive extractions.
[4] Accountability = (Total extractable + Total unextractable)/(absolute values of the TRRs (ppm)) * 100.

TABLE C.2.3.7. Summary of Characterization and Identification of Radioactive Residues in Rotational Wheat Grain Planted 30, 125, and 366 Days Following Application of [Triazole-3,5-^{14}C]-Prothioconazole to Bare Soil at 0.727 lb a.i./A (815 g a.i./ha).

Compound	30-day PBI Wheat grain		125-day PBI Wheat grain		366-day PBI Wheat grain	
	TRRs = 3.806 ppm		TRRs = 4.136 ppm		TRRs = 5.875 ppm	
	%TRRs	ppm	%TRRs	ppm	%TRRs	ppm
Identified						
Triazolylalanine	59	2.264	58	2.372	68	3.94
THPA	1	0.047	2	0.023	--	--
Triazolylacetic acid	29	1.116	23	0.957	25	1.485
Characterized						
Unknowns	9	0.285	4	0.135	5	0.285
Loss of Radioactivity[1]	3	0.091	15	0.622	1	0.061
Total identified	89	3.427	83	3.352	93	5.425
Total characterized	12	0.376	19	0.757	6	0.346
Total extractable	101	3.803	102	4.109	99	5.771
Unextractable (PES) [2]	<1	0.011	1	0.021	<1	0.014
Accountability [3]	100		100		98	

[1] Loss of radioactivity during sample preparation for HPLC analysis
[2] Residues remaining after exhaustive extractions.
[3] Accountability = (Total extractable + Total unextractable)/(absolute values of the TRRs (ppm)) * 100.

C.3. Proposed Metabolic Profile for [Phenyl-UL-^{14}C] JAU6476 and [Triazole-3,5-^{14}C] JAU6476 in Rotational Crops.

The residues found in the confined rotational crops (wheat, turnip, and Swiss chard) after soil treatment with [triazole-UL-^{14}C]-JAU6476 or [phenyl-UL-^{1}4C]-JAU6476 are shown in FIGURE C.3.1.

The major residue found in many of the rotational crops of the phenyl-label study was JAU6476-desthio. Hydroxylation was a major metabolic process. Since JAU6476 has multiple positions that could potentially undergo hydroxylation, the majority of the remaining metabolites were simply multiple isomers of monohydroxylated JAU6476-desthio and their corresponding glucosides along with JAU6476-hydroxy-diene, dihydroxy-diene, dihydroxy-olefin, and their conjugates. These compounds represented the major part of the TRRs and have both the phenyl and the triazole ring in the molecule. Cleavage of the triazole moiety occurred resulting in the formation of JAU6476-benzylpropyldiol and its glucoside representing a minor part of the TRRs.

In the triazole-label study, the major part of TRRs showed cleavage of the triazole moiety followed by rapid conjugation of the released triazole to form triazolylalanine (TA), triazolylacetic acid (TAA), and THPA. The minor residues identified in the triazole label study were JAU6476-α-hydroxy-desthio and the cleavage product, triazolyl-ethanol and its glucoside. The triazole-label study clearly elucidated the metabolic fate of the triazole portion of the JAU6476 molecule in rotational crops following soil application and was totally consistent with the results from the phenyl-label study which also adequately addressed the fate of the remainder of the molecule.

FIGURE C.3.1. Proposed Metabolic Profile of [Phenyl-UL-^{14}C] and [Triazole-3,5-^{14}C]-Prothioconazole in Rotational Swiss Chard, Turnips, and Spring Wheat.

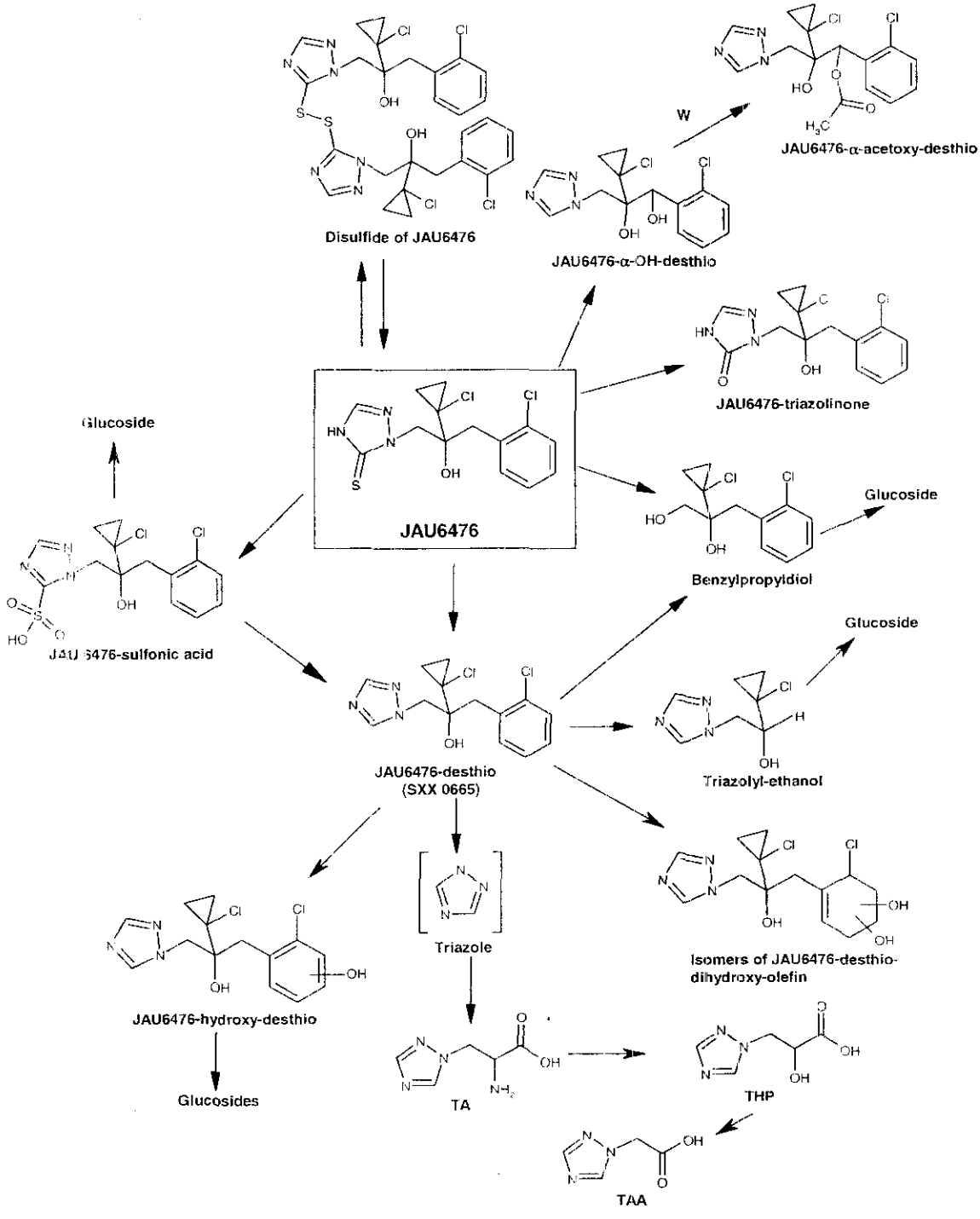


TABLE C.3.1. Identification of Compounds from the Confined Accumulation in Rotational Crop Studies.

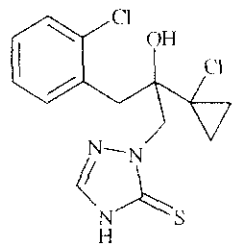
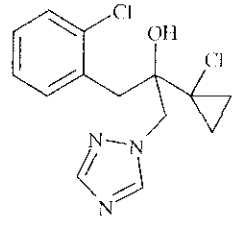
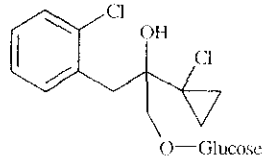
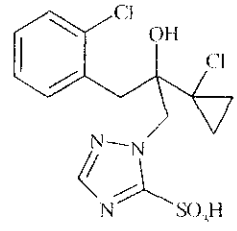
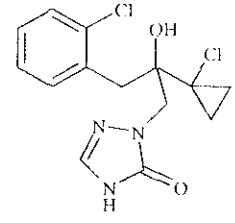
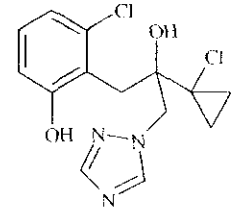
Common name/code FIGURE C.3.1 ID No.	Chemical name	Chemical structure
Prothioconazole; JAU6476	2-[2-(1-chlorocyclopropyl)-3-(2-chlorophenyl)-2-hydroxypropyl]-1,2-dihydro-3*H*-1,2,4-triazole-3-thione	
JAU6476-desthio	α-(1-chlorocyclopropyl)-α-[(2-chlorophenyl)methyl]-1*H*-1,2,4-triazole-1-ethanol	
Glucoside of JAU6476-benzylpropyldiol	2-(1-chlorocyclopropyl)-3-(2-chlorophenyl)propane-1,2-diol glucoside	
JAU6476 sulfonic acid	1-[2-(1-chlorocyclopropyl)-3-(2-chlorophenyl)-2-hydroxypropyl]-1*H*-1,2,4-triazole-5-sulfonic acid [1]	
JAU6476-triazolinone	2-[2-(1-chlorocyclopropyl)-3-(2-chlorophenyl)-2-hydroxypropyl]-1,2-dihydro-3*H*-1,2,4-triazole-3-one	
JAU6476-6-hydroxy-desthio	α-(1-chlorocyclopropyl)-α-[(2-chloro-6-hydroxyphenyl)methyl]-1*H*-1,2,4-triazole-1-ethanol	

TABLE C.3.1. Identification of Compounds from the Confined Accumulation in Rotational Crop Studies.

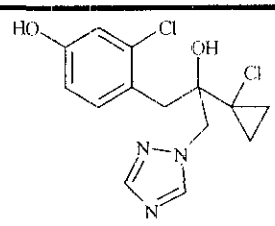
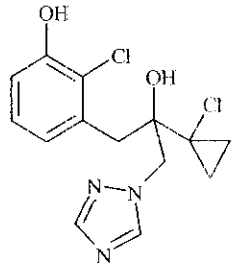
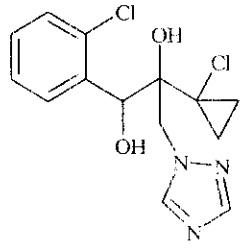
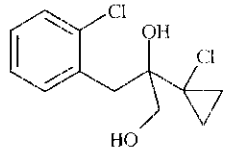
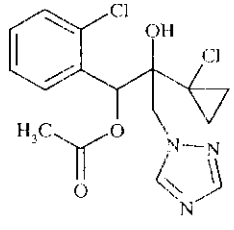
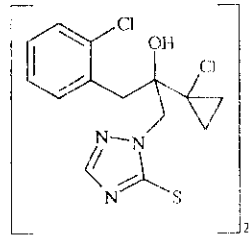
Common name/code FIGURE C.3.1 ID No.	Chemical name	Chemical structure
JAU6476-4-hydroxy-desthio	α-(1-chlorocyclopropyl)-α-[(2-chloro-4-hydroxyphenyl)methyl]-1*H*-1,2,4-triazole-1-ethanol	
JAU6476-3-hydroxy-desthio	α-(1-chlorocyclopropyl)-α-[(2-chloro-3-hydroxyphenyl)methyl]-1*H*-1,2,4-triazole-1-ethanol	
JAU6476-α-hydroxy-desthio	2-(1-chlorocyclopropyl)-1-(2-chlorophenyl)-2-hydroxy-3-(1*H*-1,2,4-triazol-1-yl)propanol	
JAU6476-benzylpropyldiol	2-(1-chlorocyclopropyl)-3-(2-chlorophenyl)propane-1,2-diol	
JAU6476-α-acetoxy-desthio	2-(1-chlorocyclopropyl)-1-(2-chlorophenyl)-2-hydroxy-3-(1*H*-1,2,4-triazol-1-yl)propyl acetate	
JAU6476-disulfide		

TABLE C.3.1. Identification of Compounds from the Confined Accumulation in Rotational Crop Studies.

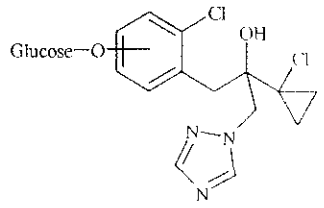
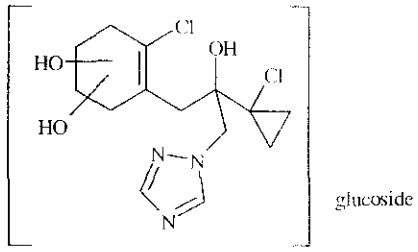
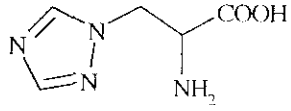
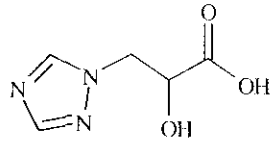
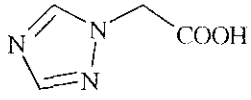
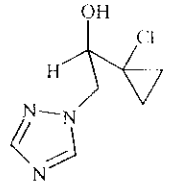
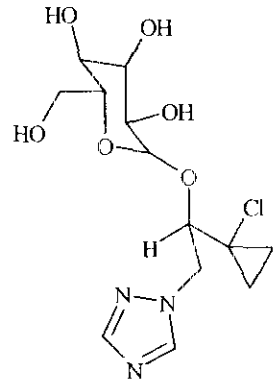
Common name/code FIGURE C.3.1 ID No.	Chemical name	Chemical structure
Glucosides of JAU6476-hydroxy-desthio		
Glucosides of JAU6476-desthio-dihydroxy-olefins		
Triazolylalanine	α-amino-1*H*-1,2,4-triazole-1-propanoic acid	
Triazolylhydroxypropionic acid; THPA	α-hydroxy-1*H*-1,2,4-triazole-1-propanoic acid	
Triazolylacetic acid	1*H*-1,2,4-triazole-1-acetic acid	
Triazolyl-ethanol	1-(1-chlorocyclopropyl)-2-(1*H*-1,2,4-triazol-1-yl)ethanol	

TABLE C.3.1. Identification of Compounds from the Confined Accumulation in Rotational Crop Studies.

Common name/code FIGURE C.3.1 ID No.	Chemical name	Chemical structure
Triazolyl-ethanol-glucoside	2-[1-(1-chlorocyclopropyl)-2-(1*H*-1,2.4-*triazol*-1-yl)ethoxy]-6-(hydroxymethyl)tetrahydro-2H-pyran-3,4.5-triol [1]	

[1] Chemical name generated using ACD chemical naming software.

D. CONCLUSION

In the phenyl-label study, prothioconazole was detected at very low levels (<1% of the TRRs) in 146-day PBI Swiss chard, 28-day PBI turnip root, 146-day PBI turnip top, and 28- and 146-day PBI wheat straw; prothioconazole was not detected in any other rotational crop commodity. JAU6476-desthio was detected in all rotational crop commodities at all PBIs analyzed, and was found to be a major metabolite (present at >10% of the TRRs) in the following rotational crop commodities: 28- and 146-day PBI Swiss chard, 28-, 146-, and 269-day PBI turnip root, 28- and 269-day PBI turnip top, 28-day PBI wheat forage, and 28- and 146-day PBI wheat hay. Additional metabolites were detected at <10% of the TRRs. In the triazole-label study, prothioconazole was not detected in any rotational crop commodity. Triazolylalanine was the major residue identified in Swiss chard, turnip root and top, and wheat forage and grain at all PBIs, at 44 to 93% of the TRRs. THPA was a major residue in Swiss chard and wheat forage, hay, and straw, at 18 to 39% of the TRRs. Triazolylacetic acid accounted for significant radioactivity in wheat hay, straw, and grain (10-29% of the TRRs). Additional metabolites identified in rotational crops, each at ≤7% of the TRRs.

The results from the two confined rotational crop studies ([phenyl-UL-^{14}C] and [triazole-3,5-^{14}C]-prothioconazole) showed that JAU6476 was completely metabolized following the soil application, subsequent uptake, and further metabolism by rotational crops. The parent compound was not detected in any rotational crop matrices at any plant-back interval. Following the initial metabolism of JAU6476 to JAU6476-desthio, two major metabolic processes were observed. One major pathway involved the hydroxylation of the phenyl ring and/or benzylic carbon to form multiple isomers of JAU6476-hydroxy-desthio, JAU6476-dihydroxy-desthio, and JAU6476-α-hydroxy-desthio followed by conjugation to form the corresponding glucosides or acetate. The other major pathway involved the cleavage of the H_2C-N bond to release the triazole moiety (and benzylpropyldiol) leading to the formation of TA and THPA and further metabolism of the triazole conjugates to TAA. The fact that no free triazole was found in any rotational crop matrix suggests an immediate or very rapid conjugation of the released triazole to form the triazole conjugates.

Minor metabolic processes involved the successive reductions of the phenyl ring to form dienes and olefins; formation of JAU6476-triazolinone; oxidation of the sulfur atom on JAU6476 to form JAU6476 sulfonic acid; and cleavage of the chlorobenzylic group to form triazolyl-ethanol and its glucoside.

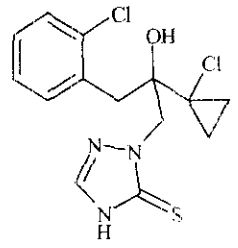
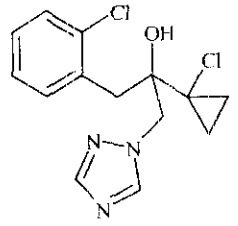
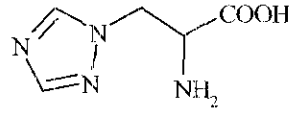
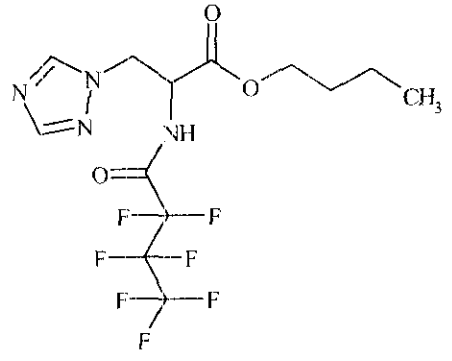
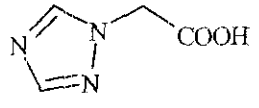
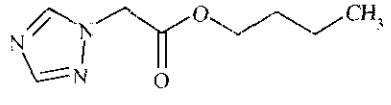
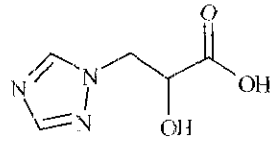
E. REFERENCES

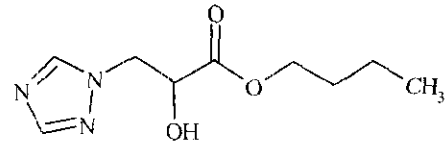
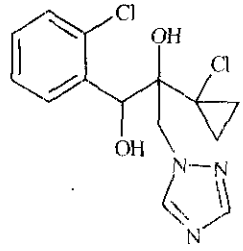
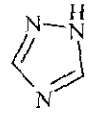
None.

F. DOCUMENT TRACKING

RDI: Louise Croteau, Suzan Mathew (PMRA) 23/01/2006; Henri Bietlot (PMRA) 24/01/2006; Stephen Funk (IO)13/03/2006; Leung Cheng (RAB3).
Petition Number: PP#4F6830
DP Barcode: D303508
PC Code: 113961

Template Version September 2003

APPENDIX I. Chemical Names and Structures of Reference Standards Used in Rotational Crop Study.		
Common name; Company code	**Chemical name**	**Chemical structure**
Prothioconazole; JAU6476	**2-[2-(1-chlorocyclopropyl)-3-(2-chlorophenyl)-2-hydroxypropyl]-1,2-dihydro-3*H*-1,2,4-triazole-3-thione**	
JAU6476-desthio	**α-(1-chlorocyclopropyl)-α-[(2-chlorophenyl)methyl]-1*H*-1,2,4-triazole-1-ethanol**	
Triazolylalanine; TA	**α-amino-1*H*-1,2,4-triazole-1-propanoic acid**	
Triazolylalanine BEHFBA	**butyl α-[(2,2,3,4,4,4,4-heptafluoro-1-oxobutyl)amino]-1*H*-1,2,4-triazole-1-propanoate**	
Triazolylacetic acid; TAA	**1*H*-1,2,4-triazole-1-acetic acid**	
TAA butyl ester	**butyl 1*H*-1,2,4-triazole-1-acetate**	
Triazolylhydroxypropionic acid; THPA	**α-hydroxy-1*H*-1,2,4-triazole-1-propanoic acid**	

APPENDIX 1. Chemical Names and Structures of Reference Standards Used in Rotational Crop Study.		
Common name; *Company code*	**Chemical name**	***Chemical structure***
THPA *butyl ester*	**butyl α-hydroxy-1*H*-1,2,4-triazole-1-propanoate**	
JAU6476-α-hydroxy-desthio	**2-(1-chlorocyclopropyl)-1-(2-chlorophenyl)-2-hydroxy-3-(1*H*-1,2,4-triazol-1-yl)propanol**	
Triazole	**1*H*-1,2,4-triazole**	

Primary Evaluators

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Date: Mar 13 2006

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Date: 23/01/06

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Date:

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Date: Jan 24/06

In the absence of signatures, this document is considered to be a draft with deliberative material for internal use only.

This DER was originally prepared under contract by Dynamac Corporation (20440 Century Boulevard, Suite 100; Germantown, MD 20874; submitted 05/05/2005). The DER has been reviewed by the Health Effects Division (HED) and Health Canada's Pest Management Regulatory Agency (PMRA). The DER has been revised to reflect current Office of Pesticide Programs (OPP) policies and Directive 98-02.

STUDY REPORT:

46246227 Lenke, V.; Lenz, C.; Murphy, J. (2004) JAU6476 480 SC - Magnitude of the Residue in Field Rotational Crops - Wheat, Mustard Greens, and Turnips. Project Number: 200535, J619RC01, J619RC02. Unpublished study prepared by Bayer Corp., Battelle and Bayer Research Farm. 1202 p.

EXECUTIVE SUMMARY:

Bayer CropScience has submitted a limited field rotational crop study on the representative crops mustard greens (leafy vegetable), turnip (root vegetable), and wheat (cereal grain). Three trial sites in GA (Region 2), IN (Region 5), and KS (Region 5), were used for each crop. At each trial site, two spray applications of the 4 lb/gal (480 g/L) suspension concentrate formulation

(flowable concentrate; FlC) were made to bare soil at ~0.36 lb a.i./A/application (~0.40 kg a.i./ha), for total application rates of ~0.72 lb a.i./A (0.81 kg a.i./ha); applications were made with a ~14-day reireatment interval. Mustard, turnip, and wheat were planted at plantback intervals (PBIs) of 1, 4, 8, and 12 months, and samples of mustard greens, turnip roots and tops, and wheat forage, hay, grain, and straw were collected at crop maturity. For winter wheat, the 1-month and 4-month PBI trials were conducted in GA (Region 2), IN (Region 5), and KS (Region 5); the 8- and 12-month PBI trials for spring wheat were conducted in ID (Region 11), ND (Region 7), and OR (Region 12).

Samples were analyzed for total prothioconazole-derived residues (prothioconazole and the metabolite prothioconazole-desthio) using LC-MS/MS method RPA JA/03/01. The validated LOQs were 0.02 ppm for each analyte in wheat grain and 0.05 ppm for each analyte in mustard greens, turnip root and top, and wheat forage, hay, and straw. Samples were analyzed for residues of 1*H*-1,2,4-triazole and the triazole conjugates triazolylalanine and triazolylacetic acid using an LC-MS/MS method (Bayer Report No. G200598). The validated LOQs for 1*H*-1,2,4-triazole were 0.01 ppm (mustard greens, turnip top, and wheat forage, hay, grain, and straw) and 0.05 ppm (turnip root). The validated LOQs for triazolylalanine were 0.01 ppm (wheat forage, hay, grain, and straw), 0.05 ppm (turnip root and top), and 0.10 ppm (mustard greens). The validated LOQs for triazolylacetic acid were 0.01 ppm (mustard greens and wheat forage, hay, and grain), 0.025 ppm (wheat straw), and 0.05 ppm (turnip root and top). The methods are adequate for data collection based on acceptable concurrent method recovery data.

At the 1-month PBI, total prothioconazole-derived residues were below the LOQ (<0.02 ppm for wheat grain and <0.05 ppm for all other commodities) in mustard greens, turnip root and top, and wheat forage, hay grain, and straw. Because residues of prothioconazole were below the LOQ in all samples from the 1-month PBI, samples from the 4-, 8-, and 12-month PBIs were not analyzed for total prothioconazole-derived residues.

At the 1-month PBI, residues of 1*H*-1,2,4-triazole were below the LOQ (<0.01 ppm) in/on all rotational crop matrices except wheat straw and grain; quantifiable residues of 0.01 ppm were detected in/on two samples each of wheat straw and grain. At the 4-month PBI, residues of 1*H*-1,2,4-triazole were below the LOQ (<0.01 ppm) in/on all rotational crop matrices. Samples from the 8- and 12-month PBIs were not analyzed for 1*H*-1,2,4-triazole residues.

At the 1-month PBI, total residues of triazole conjugates (triazolylalanine and triazolylacetic acid) were 0.102-0.313 ppm (mustard greens), 0.301-0.567 ppm (turnip root), 0.263-0.484 ppm (turnip top), 0.080-1.174 ppm (wheat forage), 0.303-2.025 ppm (wheat hay), 0.710-3.465 ppm (wheat grain), and 0.074-0.719 ppm (wheat straw). At the 4-month PBI, total triazole conjugate residues were 0.081-0.392 ppm (mustard greens), 0.066-0.201 ppm (turnip root), 0.095-0.254 ppm (turnip top), 0.164-0.333 ppm (wheat forage), 0.406-0.763 ppm (wheat hay), 0.615-1.754 ppm (wheat grain), and 0.103-0.278 ppm (wheat straw). The average triazole conjugate residues in each commodity decreased from the 1-month PBI to the 4-month PBI. Samples from the 8- and 12-month PBIs were not analyzed for triazole conjugate residues.

The maximum storage intervals from harvest to analysis for total prothioconazole-derived residues were 1263 days (41.5 months) for mustard greens, 1243 days (40.8 months) for turnip tops and roots, 1002 days (32.9 months) for wheat forage, 943 days (31.0 months) for wheat hay, 919 days (30.2 months) for wheat grain, and 911 days (29.9 months) for wheat straw. Prothioconazole-derived residues are relatively stable up to 1 year (interim report) in wheat matrices, mustard greens, turnip tops and roots. Corrections due to apparent dissipation of prothioconazole-derived residues in samples stored beyond a year are not necessary due to the low absolute (ppm) and % residue levels in plant matrices. Residues of prothioconazole-desthio are stable for up to 1 year and the degree of loss is not expected to exceed 30% after 41.5 months after freezere storage.

STUDY/WAIVER ACCEPTABILITY/DEFICIENCIES/CLARIFICATIONS:

Under the conditions and parameters used in the study, the field rotational crop residue data are tentatively classified as scientifically acceptable. The final reports of the ongoing storage stability studies with prothioconazole, prothioconazole-desthio, 1*H*-1,2,4-triazole, triazolylalanine, and triazolylacetic acid will be submitted as confirmatory data.

The acceptability of this study for regulatory purposes is addressed in the forthcoming U.S. EPA Residue Chemistry Summary Document, DP Barcode D303508, and in Canada's Regulatory Decision Document.

COMPLIANCE:

Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided. No deviations from regulatory requirements were reported which would have an impact on the validity of the study.

A. BACKGROUND INFORMATION

Prothioconazole is a broad-spectrum systemic fungicide intended for the control of many diseases in different crops such as cereals, oilseed rape or peanuts. The biochemical mode of action is the inhibition of the demethylation of lanosterol or 24-methylene-dihydrolanosterol, which are precursors of sterols in fungi. The chemical has been submitted for joint EPA and PMRA review by Bayer CropScience. The crops proposed for joint review include barley, the dried shell and bean subgroup, the oilseed crop group, and wheat. Uses on peanuts and rice are being proposed for the U.S. only.

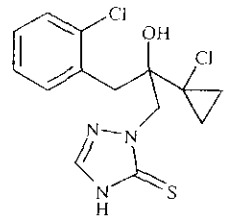
TABLE A.1. Test Compound Nomenclature.	
Chemical structure	
Common name	Prothioconazole (ISO accepted)
Company experimental name	JAU6476
IUPAC name	2-[(2*RS*)-2-(1-chlorocyclopropyl)-3-(2-chlorophenyl)-2-hydroxypropyl]-2*H*-1,2,4-triazole-3(4*H*)-thione
CAS name	2-[2-(1-chlorocyclopropyl)-3-(2-chlorophenyl)-2-hydroxypropyl]-1,2-dihydro-3*H*-1,2,4-triazole-3-thione
CAS registry number	178928-70-6
PC Code	113961
End-use product (EP)	Proline® 480 SC (4 lb/gal suspension concentrate), EPA File Symbol 264-IEL

TABLE A.2. Physicochemical Properties of the Technical Grade Test Compound.

Parameter	Value	*References*
Melting range	139.1 to 144.5°C	MRID 46246003/CES
pH	5.8 (1% solution)	MRID 46246003/CES
Density at 20°C	1.36 g/mL	MRID 46246003/CES
Water solubility at 20°C	pH: mg/L 4: 5 8: 300 9: 2000	MRID 46246003/CES
Solvent solubility at 20°C	Solvent: g/L Acetone: >250 Acetonitrile: 69 Dichloromethane: 88 Dimethysulfoxide: 126 Ethyl acetate: >250 n-Heptane: <0.1 1-Octanol: 58 Polyethylene glycol: >250 2-Propanol: 87 Xylene: 8	MRID 46246003/CES
Vapor pressure at 20 or 25°C	$<4 \times 10^{-7}$ Pa (calculated from determinations at 70°C)	MRID 46246003/CES
Dissociation constant, pK_a	6.9 (calculated from K_{OW})	MRID 46246003/CES
Octanol/water partition coefficient, at 20°C	pH: $Log(K_{OW})$ unbuffered water: 4.05 4: 4.16 7: 3.82 9: 2.00	MRID 46246003/CES
UV/visible absorption spectrum	*Peak maxima* at 257 nm. No absorption at > 300 nm.	MRID 46246003/CES

CES - Chemistry Evaluation Section of PMRA

B. EXPERIMENTAL DESIGN

B.1. Study Site Information (Copied without alteration from MRID 46246227).

Study Location (City, State)	Trial Number	Year	Soil Characteristics[a]				Meteorological Data	
			Type	% OM*	pH*	CEC*	Total Rainfall (in)[b]	Temp. Range (°F)[c]
Tifton, Georgia	J6133-00R	2000	Sandy Loam	NA	NA	NA	17.87	19 - 93
Tifton, Georgia	J6134-00R	2000	Sandy Loam	NA	NA	NA	39.23	19 - 101
Corvallis, Oregon	J6135-00R	2000	Silty Clay Loam	NA	NA	NA	19.44[d]	20 - 98
Corvallis, Oregon	J6136-00R	2000	Silty Clay Loam	NA	NA	NA	25.69[e]	20 - 98
Stilwell, Kansas	J6137-00R	2000	Silty Clay Loam	NA	NA	NA	36.02	-1 - 107
Stilwell, Kansas	J6138-00R	2000	Silty Clay Loam	NA	NA	NA	49.69	-1 - 107
Jerome, Idaho	J6139-00R	2000	Loam	NA	NA	NA	5.95[e]	5 - 106
Jerome, Idaho	J6140-00R	2000	Loam	NA	NA	NA	7.29[f]	5 - 106
Oxford, Indiana	J6141-00R	2000	Silt Loam	NA	NA	NA	25.55	-10 - 94
Oxford, Indiana	J6142-00R	2000	Silt Loam	NA	NA	NA	39.93	-10 - 94
Northwood, North Dakota	J6143-00R	2000	Loam	NA	NA	NA	20.56	-23 - 96
Northwood, North Dakota	J6144-00R	2000	Loam	NA	NA	NA	34.54	-23 - 96
Tifton, Georgia	J6145-00R	2000	Sandy Loam	NA	NA	NA	16.85[g]	19 - 100
Tifton, Georgia	J6146-00R	2000	Sandy Loam	NA	NA	NA	26.80[h]	19 - 101
Tifton, Georgia	J6147-00R	2000	Sandy Loam	NA	NA	NA	36.35	19 - 101
Tifton, Georgia	J6148-00R	2000	Sandy Loam	NA	NA	NA	20.00[h]	19 - 95
Stilwell, Kansas	J6149-00R	2000	Silty Clay Loam	NA	NA	NA	14.67	45 - 97
Stilwell, Kansas	J6150-00R	2000	Silty Clay Loam	NA	NA	NA	30.92	18 - 99
Stilwell, Kansas	J6151-00R	2000	Silty Clay Loam	NA	NA	NA	43.09	-1 - 99
Stilwell, Kansas	J6152-00R	2000	Silty Clay Loam	NA	NA	NA	58.52	-1 - 107
Oxford, Indiana	J6153-00R	2000	Silt Loam	NA	NA	NA	7.11[i]	41 - 93
Oxford, Indiana	J6154-00R	2000	Silt Loam	NA	NA	NA	18.11[j]	25 - 94
Oxford, Indiana	J6155-00R	2000	Silt Loam	NA	NA	NA	23.56	-10 - 94
Oxford, Indiana	J6156-00R	2000	Silt Loam	NA	NA	NA	35.05[j]	-10 - 94
Tifton, Georgia	J6157-00R	2000	Sandy Loam	NA	NA	NA	16.85[g]	19 - 100
Tifton, Georgia	J6158-00R	2000	Sandy Loam	NA	NA	NA	41.85[h]	19 - 101
Tifton, Georgia	J6159-00R	2000	Sandy Loam	NA	NA	NA	47.25	19 - 101
Tifton, Georgia	J6160-00R	2000	Sandy Loam	NA	NA	NA	63.63[h]	19 - 95
Stilwell, Kansas	J6161-00R	2000	Silty Clay Loam	NA	NA	NA	15.05[j]	45 - 101
Stilwell, Kansas	J6162-00R	2000	Silty Clay Loam	NA	NA	NA	32.03	18 - 99
Stilwell, Kansas	J6163-00R	2000	Silty Clay Loam	NA	NA	NA	37.96	-1 - 98
Stilwell, Kansas	J6164-00R	2000	Silty Clay Loam	NA	NA	NA	53.39	-1 - 107

* These parameters are optional except in cases where their value affects the use pattern for this chemical.

Study Location (City, State)	Trial Number	Year	Soil Characteristics[a]				Meteorological Data	
			Type	% OM*	pH*	CEC*	Total Rainfall (in)[b]	Temp. Range (°F)[c]
Oxford, Indiana	J6165-00R	2000	Silt Loam	NA	NA	NA	9.51[k]	24 - 93
Oxford, Indiana	J6166-00R	2000	Silt Loam	NA	NA	NA	24.82[j]	25 - 94
Oxford, Indiana	J6167-00R	2000	Silt Loam	NA	NA	NA	20.72	-10 - 94
Oxford, Indiana	J6168-00R	2000	Silt Loam	NA	NA	NA	20.72[j]	-10 - 94
* These parameters are optional except in cases where their value affects the use pattern for this chemical.								

[a] NA = Not applicable since these parameters do not affect the use pattern of the chemical.
[b] Study period includes the month of first application through the month of final harvest.
[c] Minimum and maximum temperatures from first application to final harvest.
[d] Irrigation added = 7.63 inches
[e] Irrigation added = 19.17 inches
[f] Irrigation added = 25.88 inches
[g] Irrigation added = 1.00 inch
[h] Irrigation added = 2.00 inches
[i] Irrigation added = 3.70 inches
[j] Irrigation added = 1.50 inches
[k] Irrigation added = 4.20 inches

The actual temperature and rainfall recordings were similar to average historical values for the residue study period. The applicant indicated that irrigation was used to supplement rainfall as needed at 17 trial sites.

TABLE B.1.2. Study Use Pattern.

Location (City, State; Year) Trial Number	EP[1]	Application					Tank Mix Adjuvants
		Method; Timing	Trtmt. No.	Rate (lb a.i./A) [kg a.i./ha]	RTI (days)[2]	Total Rate (lb a.i./A) [kg a.i./ha]	
Mustard greens trials							
Tifton, GA; 2000 J6145-00R	480 SC	Broadcast; bare soil	2	0.36, 0.36 [0.40, 0.40]	14	0.72 [0.81]	No
Tifton, GA; 2000 J6146-00R	480 SC	Broadcast; bare soil	2	0.36, 0.36 [0.40, 0.40]	15	0.72 [0.81]	No
Tifton, GA; 2000 J6147-00R	480 SC	Broadcast; bare soil	2	0.36, 0.36 [0.40, 0.40]	13	0.72 [0.81]	No
Tifton, GA; 2000 J6148-00R	480 SC	Broadcast; bare soil	2	0.36, 0.36 [0.40, 0.40]	14	0.72 [0.81]	No
Stilwell, KS; 2000 J6149-00R	480 SC	Broadcast; bare soil	2	0.36, 0.36 [0.40, 0.40]	14	0.72 [0.81]	No
Stilwell, KS; 2001 J6150-00R	480 SC	Broadcast; bare soil	2	0.36, 0.36 [0.40, 0.40]	14	0.72 [0.81]	No
Stilwell, KS; 2000 J6151-00R	480 SC	Broadcast; bare soil	2	0.36, 0.36 [0.40, 0.40]	15	0.72 [0.81]	No

TABLE B.1.2. Study Use Pattern.

Location (City, State; Year) Trial Number	EP[1]	Application					Tank Mix Adjuvants
		Method; Timing	Trtmt. No.	Rate (lb a.i./A) [kg a.i./ha]	RTI (days)[2]	Total Rate (lb a.i./A) [kg a.i./ha]	
Stilwell, KS; 2000 J6152-00R	480 SC	Broadcast; bare soil	2	0.36, 0.36 [0.40, 0.40]	14	0.72 [0.81]	No
Oxford, IN; 2000 J6153-00R	480 SC	Broadcast; bare soil	2	0.36, 0.36 [0.40, 0.40]	14	0.72 [0.81]	No
Oxford, IN; 2001 J6154-00R	480 SC	Broadcast; bare soil	2	0.37, 0.36 [0.41, 0.40]	14	0.73 [0.82]	No
Oxford, IN; 2000 J6155-00R	480 SC	Broadcast; bare soil	2	0.36, 0.36 [0.40, 0.40]	13	0.72 [0.81]	No
Oxford, IN; 2000 J6156-00R	480 SC	Broadcast; bare soil	2	0.36, 0.36 [0.40, 0.40]	13	0.72 [0.81]	No
Turnip trials							
Tifton, GA; 2000 J6157-00R	480 SC	Broadcast; bare soil	2	0.36, 0.36 [0.40, 0.40]	14	0.72 [0.81]	No
Tifton, GA; 2000 J6158-00R	480 SC	Broadcast; bare soil	2	0.36, 0.36 [0.40, 0.40]	15	0.72 [0.81]	No
Tifton, GA; 2000 J6159-00R	480 SC	Broadcast; bare soil	2	0.36, 0.36 [0.40, 0.40]	13	0.72 [0.81]	No
Tifton, GA; 2000 J6160-00R	480 SC	Broadcast; bare soil	2	0.36, 0.36 [0.40, 0.40]	14	0.72 [0.81]	No
Stilwell, KS; 2000 J6161-00R	480 SC	Broadcast; bare soil	2	0.36, 0.36 [0.40, 0.40]	14	0.72 [0.81]	No
Stilwell, KS; 2001 J6162-00R	480 SC	Broadcast; bare soil	2	0.36, 0.36 [0.40, 0.40]	14	0.72 [0.81]	No
Stilwell, KS; 2000 J6163-00R	480 SC	Broadcast; bare soil	2	0.36, 0.36 [0.40, 0.40]	15	0.72 [0.81]	No
Stilwell, KS; 2000 J6164-00R	480 SC	Broadcast; bare soil	2	0.36, 0.36 [0.40, 0.40]	14	0.72 [0.81]	No
Oxford, IN; 2000 J6165-00R	480 SC	Broadcast; bare soil	2	0.36, 0.36 [0.40, 0.40]	14	0.72 [0.81]	No
Oxford, IN; 2001 J6166-00R	480 SC	Broadcast; bare soil	2	0.36, 0.36 [0.40, 0.40]	14	0.72 [0.81]	No
Oxford, IN; 2000 J6167-00R	480 SC	Broadcast; bare soil	2	0.36, 0.36 [0.40, 0.40]	13	0.72 [0.81]	No
Oxford, IN; 2000 J6168-00R	480 SC	Broadcast; bare soil	2	0.36, 0.36 [0.40, 0.40]	13	0.72 [0.81]	No
Wheat trials							
Tifton, GA; 2000 J6133-00R	480 SC	Broadcast; bare soil	2	0.36, 0.36 [0.40, 0.40]	14	0.72 [0.81]	No
Tifton, GA; 2000 J6134-00R	480 SC	Broadcast; bare soil	2	0.36, 0.36 [0.40, 0.40]	13	0.72 [0.81]	No
Corvallis, OR; 2000 J6135-00R	480 SC	Broadcast; bare soil	2	0.37, 0.36 [0.41, 0.40]	13	0.73 [0.82]	No

TABLE B.1.2. Study Use Pattern.

Location (City, State; Year) Trial Number	EP[1]	Application					Tank Mix Adjuvants
		Method; Timing	Trtmt. No.	Rate (lb a.i./A) [kg a.i./ha]	RTI (days)[2]	Total Rate (lb a.i./A) [kg a.i./ha]	
Corvallis, OR; 2000 J6136-00R	480 SC	Broadcast; bare soil	2	0.36, 0.36 [0.40, 0.40]	0	0.72 [0.81]	No
Stilwell, KS; 2000 J6137-00R	480 SC	Broadcast; bare soil	2	0.36, 0.36 [0.40, 0.40]	14	0.72 [0.81]	No
Stilwell, KS; 2000 J6138-00R	480 SC	Broadcast; bare soil	2	0.36, 0.36 [0.40, 0.40]	14	0.72 [0.81]	No
Jerome, ID; 2000 J6139-00R	480 SC	Broadcast; bare soil	2	0.36, 0.37 [0.40, 0.41]	14	0.73 [0.82]	No
Jerome, ID; 2000 J6140-00R	480 SC	Broadcast; bare soil	2	0.36, 0.36 [0.40, 0.40]	13	0.72 [0.81]	No
Oxford, IN; 2000 J6141-00R	480 SC	Broadcast; bare soil	2	0.36, 0.36 [0.40, 0.40]	16	0.72 [0.81]	No
Oxford, IN; 2000 J6142-00R	480 SC	Broadcast; bare soil	2	0.36, 0.36 [0.40, 0.40]	17	0.72 [0.81]	No
Northwood, ND; 2000 J6143-00R	480 SC	Broadcast; bare soil	2	0.35, 0.36 [0.40, 0.40]	14	0.71 [0.80]	No
Northwood, ND; 2000 J6144-00R	480 SC	Broadcast; bare soil	2	0.36, 0.38 [0.41, 0.42]	14	0.74 [0.83]	No

[1] EP = End-use Product; 480 SC = 4 lb/gal (480 g/L) suspension concentrate (flowable concentrate; FlC) formulation
[2] RTI = Retreatment Interval

B.2. Sample Handling and Preparation

A single untreated and duplicate treated samples of rotational crop commodities (mustard greens, turnip tops and roots, and wheat forage, hay, grain, and straw) were harvested at crop maturity. The applicant did not state how wheat hay samples were dried prior to collection. All samples were frozen within 3 hours of collection and then shipped frozen to Battelle-AgriFood (Columbus, OH) for homogenization. At Battelle, the samples were homogenized with dry ice and stored frozen until shipment to Bayer Research Park (Stilwell, KS) for analysis. The percent dry matter was determined for turnip and wheat matrices at Battelle prior to shipment to Bayer. Samples were stored frozen at Bayer (<-5°C) prior to analysis.

B.3. Analytical Methodology

Samples of rotated crop commodities were analyzed for total prothioconazole-derived residues (prothioconazole and the metabolite prothioconazole-desthio) using LC-MS/MS method RPA JA/03/01. Crop matrices were 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 was spiked 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. Residue levels were quantitated from a calibration curve of mixed reference standards of prothioconazole, as the sulfonic acid, and prothioconazole-desthio. 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 LOQs were 0.02 ppm for each analyte in wheat grain and 0.05 ppm for each analyte in mustard greens, turnip root and top, and wheat forage, hay, and straw.

Samples were analyzed for residues of 1*H*-1,2,4-triazole and the triazole conjugates (triazolylalanine and triazolylacetic acid) using an LC-MS/MS method (Bayer Report No. G200598). Briefly, crop matrices were extracted with aqueous methanol, and three separate aliquots of the extract were removed for determination of each of the three analytes. Isotopically labeled internal standard was added to each aliquot. For 1*H*-1,2,4-triazole, the aliquot was mixed with dansyl chloride to form the dansyl derivative of 1*H*-1,2,4-triazole, which was partitioned into ethyl acetate and then redissolved in acetonitrile (ACN)/water for LC-MS/MS analysis. For triazolylalanine, the aliquot was cleaned up by solid-phase extraction (SPE), derivatized to the butyl ester using butanolic HCl, and then further derivatized using heptafluorobutyric anhydride (HFBA). The mixture was redissolved in ACN/water for LC-MS/MS analysis. For determination of triazolylacetic acid, the aliquot was cleaned up by SPE, derivatized to the butyl ester using butanolic HCl, and then redissolved in ACN/water for LC-MS/MS analysis. Residue levels were quantitated from a calibration curve of reference standards of dansyl-1,2,4-triazole, triazolylalanine butyl ester HFBA, and triazolylacetic acid butyl ester (generated by derivatizing reference standard solutions of triazolylacetic acid). The validated LOQs for 1*H*-1,2,4-triazole were 0.01 ppm for mustard greens, turnip top, and wheat forage, hay, grain, and straw and 0.05 ppm for turnip root. The validated LOQs for triazolylalanine were 0.01 ppm for wheat forage, hay, grain, and straw, 0.05 ppm for turnip root and top, and 0.10 ppm for mustard greens. The validated LOQs for triazolylacetic acid were 0.01 ppm for mustard greens and wheat forage, hay, and grain, 0.025 ppm for wheat straw, and 0.05 ppm for turnip root and top.

C. RESULTS AND DISCUSSION

Concurrent method recovery data are presented in TABLE C.1. Samples were analyzed for total prothioconazole-derived residues using LC-MS/MS method RPA JA/03/01. The validated LOQs were 0.02 ppm for each analyte in wheat grain and 0.05 ppm for each analyte in mustard greens, turnip root and top, and wheat forage, hay, and straw. Samples were analyzed for residues of 1*H*-1,2,4-triazole and the triazole conjugates triazolylalanine and triazolylacetic acid using an LC-MS/MS method (Bayer Report No. G200598). The validated LOQs for 1*H*-1,2,4-triazole were 0.01 ppm for mustard greens, turnip top, and wheat forage, hay, grain, and straw and 0.05 ppm for turnip root. The validated LOQs for triazolylalanine were 0.01 ppm for wheat forage, hay, grain, and straw, 0.05 ppm for turnip root and top, and 0.10 ppm for mustard greens. The validated LOQs for triazolylacetic acid were 0.01 ppm for mustard greens and wheat forage, hay, and grain, 0.025 ppm for wheat straw, and 0.05 ppm for turnip root and top. The methods are adequate for data collection based on acceptable concurrent method recovery data.

Sample storage conditions and intervals are summarized in TABLE C.2. The maximum storage intervals from harvest to analysis for total prothioconazole-derived residues were 1263 days (41.5 months) for mustard greens, 1243 days (40.8 months) for turnip tops and roots, 1002 days (32.9 months) for wheat forage, 943 days (31.0 months) for wheat hay, and 919 days (30.2 months) for wheat grain and straw. Prothioconazole-derived residues and prothioconazole-desthio residues are stable for up to 1 year based on an interim report. The need to correct the results of the current study for decline of prothioconazole-derived residues will not be necessary taking into account the absolute (ppm) and relative % residue levels in plant matrices. The degree of loss of prothioconazole-desthio residues is not expected to exceed 30% after 41.5 months.

The maximum storage intervals from harvest to analysis for 1*H*-1,2,4-triazole and triazole conjugate residues were 1257 days (41.3 months) for mustard greens, 1252 days (41.1 months) for turnip tops and roots, 1059 days (34.8 months) for wheat forage, 1008 days (33.1 months) for wheat hay, 974 days (32.0 months) for wheat straw, and 976 days (32.1 months) for wheat grain.

Residue data from the field rotational crop study are presented in TABLE C.3.1; a summary of residue data in rotational crop matrices is presented in TABLE C.4. At the 1-month PBI, total prothioconazole-derived residues were below the LOQ (<0.02 ppm for wheat grain and <0.05 ppm for all other commodities) in mustard greens, turnip root and top, and wheat forage, hay grain, and straw. Because residues of prothioconazole were below the LOQ in all samples from the 1-month PBI, samples from the 4-, 8-, and 12-month PBIs were not analyzed for total prothioconazole-derived residues.

At the 1-month PBI, residues of 1*H*-1,2,4-triazole were below the LOQ (<0.01 ppm) in/on all rotational crop matrices except wheat straw and grain; quantifiable residues of 0.01 ppm were detected in/on two samples each of wheat straw and grain. At the 4-month PBI, residues of 1*H*-1,2,4-triazole were below the LOQ (<0.01 ppm) in/on all rotational crop matrices. Samples from the 8- and 12-month PBIs were not analyzed for 1*H*-1,2,4-triazole residues.

At the 1-month PBI, total residues of triazole conjugates (triazolylalanine and triazolylacetic acid) were 0.102-0.313 ppm in/on mustard greens, 0.301-0.567 ppm in/on turnip root, 0.263-0.484 ppm in/on turnip top, 0.080-1.174 ppm in/on wheat forage, 0.303-2.025 ppm in/on wheat hay, 0.710-3.465 ppm in/on wheat grain, and 0.074-0.719 ppm in/on wheat straw. At the 4-month PBI, total triazole conjugate residues were 0.081-0.392 ppm in/on mustard greens, 0.066-0.201 ppm in/on turnip root, 0.095-0.254 ppm in/on turnip top, 0.164-0.333 ppm in/on wheat forage, 0.406-0.763 ppm in/on wheat hay, 0.615-1.754 ppm in/on wheat grain, and 0.103-0.278 ppm in/on wheat straw. The average triazole conjugate residues in each commodity decreased from the 1-month PBI to the 4-month PBI. Samples from the 8- and 12-month PBIs were not analyzed for triazole conjugate residues.

Apparent total prothioconazole-derived residues and residues of 1*H*-1,2,4-triazole and triazole conjugates in rotational crops grown in untreated soil are presented in TABLE C.3.2. Quantifiable apparent residues of 1*H*-1,2,4-triazole were observed in mustard greens, and

quantifiable apparent residues of the triazole conjugates were observed in all samples except turnip roots. The measurable control residues of the triazole conjugates may have resulted from the use of triazole fungicides from previous seasons or from naturally occurring triazole conjugates.

The percent dry matter was determined for turnip roots (9-12%) and tops (10-13%). The dry matter contents of wheat commodities were found to be 16-22% for forage, 61-77% for hay, 85-86% for grain, and 85-88% for straw.

TABLE C.1. Summary of Concurrent Recoveries of Prothioconazole Residues from Rotational Mustard Green, Turnip, and Wheat Matrices.

Matrix	Analyte	Spike level (ppm)	Sample size (n)	Recoveries (%)	Mean ± std dev
Mustard Greens	Prothioconazole	0.05	3	85, 84, 86	85.0 ± 1.0
	JAU6476-desthio	0.05	3	106, 105, 105	105 ± 0.6
	1*H*-1,2,4-triazole	0.25	6	73, 79, 77, 95, 87, 92	84 ± 8.8
	Triazolylalanine	0.25	5	68, 86, 81, 88, 86	82 ± 8.1
	Triazolylacetic acid	0.25	5	84, 84, 75, 83, 95	84 ± 7.1
Turnip Tops	Prothioconazole	0.05	3	88, 88, 87	87.7 ± 0.6
	JAU6476-desthio	0.05	3	104, 103, 102	103 ± 1.0
	1*H*-1,2,4-triazole	0.01	3	59, 81, 106	82 ± 19.2
		0.05	3	90, 87, 95	90.6 ± 3.3
	Triazolylalanine	0.05	3	98, 90, 96	94.7 ± 3.4
		0.50	3	88, 81, 92	87.0 ± 4.6
	Triazolylacetic acid	0.05	3	98, 118, 113	109.7 ± 8.5
		0.50	3	98, 88, 100	95.3 ± 5.3
		0.75	3	86, 92, 92	90 ± 2.8
Turnip Roots	Prothioconazole	0.05	3	91, 91, 95	92.3 ± 2.3
	JAU6476-desthio	0.05	3	105, 109, 105	106 ± 2.3
	1*H*-1,2,4-triazole	0.05	6	88, 102, 102, 97, 97, 93	96.5 ± 4.9
	Triazolylalanine	0.05	3	126, 92, 97	105 ± 15
		0.50	3	102, 102, 99	101 ± 1.4
		0.75	3	95, 93, 102	96.7 ± 3.9
	Triazolylacetic acid	0.05	3	96, 108, 93	99 ± 6.5
		0.50	3	104, 103, 101	102.7 ± 1.3
Wheat Forage	Prothioconazole	0.05	1	74	NA
	JAU6476-desthio	0.05	1	106	N/A
	1*H*-1,2,4-triazole	0.05	1	109	N/A
	Triazolylalanine	0.05	1	96	N/A
	Triazolylacetic acid	None	None	None	None

TABLE C.1. Summary of Concurrent Recoveries of Prothioconazole Residues from Rotational Mustard Green, Turnip, and Wheat Matrices.

Matrix	Analyte	Spike level (ppm)	Sample size (n)	Recoveries (%)	Mean ± std dev
Wheat Hay	Prothioconazole	0.10	1	79	N/A
	JAU6476-desthio	0.10	1	101	N/A
	1*H*-1,2,4-triazole	0.05	1	97	N/A
	Triazolylalanine	0.20	1	87	N/A
	Triazolylacetic acid	0.20	1	102	N/A
Wheat Straw	Prothioconazole	0.10	1	80	N/A
	JAU6476-desthio	0.10	1	101	N/A
	1*H*-1,2,4-triazole	0.05	1	93	N/A
	Triazolylalanine	0.20	1	80	N/A
	Triazolylacetic acid	0.20	1	104	N/A
Wheat Grain	Prothioconazole	0.02	1	84	N/A
	JAU6476-desthio	0.02	1	98	N/A
	1*H*-1,2,4-triazole	0.05	1	97	N/A
	Triazolylalanine	0.20	1	71	N/A
	Triazolylacetic acid	0.20	2	89, 82	N/A

N/A Not applicable

TABLE C.2. Summary of Storage Conditions.

Matrix (RAC or Extract)	Storage Temp. (°C)	Actual Storage Duration [1]	Interval of Demonstrated Storage Stability (days)
Total Prothioconazole-Derived Residues			
Mustard greens	-30.0 to -4.8	1135-1263 days (37.3-41.5 months)	Stability demonstrated for up to 12.7 months (interim report). Awaiting final report as confirmatory data.
Turnip tops		1136-1243 days (37.3-40.8 months)	
Turnip roots		1136-1243 days (37.3-40.8 months)	
Wheat forage		952-1002 days (31.3-32.9 months)	
Wheat hay		893-943 days (29.3-31.0 months)	
Wheat grain		869-919 days (28.6-30.2 months)	
Wheat straw		861-911 days (28.3-29.9 months)	

TABLE C.2. Summary of Storage Conditions.			
Matrix (RAC or Extract)	Storage Temp. (°C)	Actual Storage Duration [1]	Interval of Demonstrated Storage Stability (days)
1,2,4-Triazole and Triazole Conjugate Residues			
Mustard greens	-30.0 to -4.8	817-1257 days (26.8-41.3 months)	Not applicable at this time. Awaiting final report
Turnip tops		825-1252 days (27.1-41.1 months)	
Turnip roots		824-1251 days (27.1-41.1 months)	
Wheat forage		1001-1059 days (32.9-34.8 months)	
Wheat hay		952-1008 days (31.3-33.1 months)	
Wheat grain		929-976 days (30.5-32.1 months)	
Wheat straw		924-974 days (30.4-32.0 months)	

[1] Actual storage duration from sample collection to analysis. Sample extracts were analyzed within 4 days of extraction.

TABLE C.3.1. Prothioconazole Residues in Rotational Crops Grown in Treated Soil.									
Location (City, State; Year Trial Number)	Region	Crop Variety	Commodity	Total Rate (lb ai/A) [kg ai/ha]	Harvest DAP[1]	PBI[2] (days)	Total Prothioconazole-Derived Residues (ppm)	1*H*-1,2,4-triazole Residues (ppm)	Triazole Conjugate Residues (ppm)
Mustard Greens									
Tifton, GA; 2000 J6145-00R	2	Broadleaf Mustard	Leaves	0.72 [0.81]	86	34	<0.05	<0.01	0.104
							<0.05	<0.01	0.102
Stilwell, KS; 2000 J6149-00R	5	Curley Mustard	Leaves	0.72 [0.81]	46	34	<0.05	<0.01	0.313
							<0.05	<0.01	0.309
Oxford, IN; 2000 J6153-00R	5	Southern Giant	Leaves	0.72 [0.81]	36	31	<0.05	<0.01	0.277
							<0.05	<0.01	0.229
Tifton, GA; 2000 J6146-00R	2	Broadleaf Mustard	Leaves	0.72 [0.81]	140	119	Not analyzed (N/A)	<0.01	0.392
								<0.01	0.226
Stilwell, KS; 2000 J6150-00R	5	Curley Mustard	Leaves	0.72 [0.81]	66	119	N/A	<0.01	0.165
								<0.01	0.119
Oxford, IN; 2000 J6154-00R	5	Southern Giant	Leaves	0.73 [0.82]	44	104	N/A	<0.01	0.081
								<0.01	0.106

TABLE C.3.1. Prothioconazole Residues in Rotational Crops Grown in Treated Soil.

Location (City, State; Year Trial Number)	Region	Crop Variety	Commodity	Total Rate (lb ai/A) [kg ai/ha]	Harvest DAP[1]	PBI[2] (days)	Total Prothioconazole-Derived Residues (ppm)	*1H*-1,2,4-triazole Residues (ppm)	Triazole Conjugate Residues (ppm)
Turnip									
Tifton, GA; 2000 J6157-00R	2	Purple Top	Roots	0.72 [0.81]	86	34	<0.05	<0.05	0.384
							<0.05	<0.05	0.348
			Tops	0.72 [0.81]	86	34	<0.05	<0.01	0.263
							<0.05	<0.01	0.309
Stilwell, KS; 2000 J6161-00R	5	Purple Top	Roots	0.72 [0.81]	67	34	<0.05	<0.05	0.301
							<0.05	<0.05	0.332
			Tops	0.72 [0.81]	67	34	<0.05	<0.01	0.414
							<0.05	<0.01	0.484
Oxford, IN; 2000 J6165-00R	5	Purple Top White Glo	Roots	0.72 [0.81]	60	31	<0.05	<0.05	0.498
							<0.05	<0.05	0.567
			Tops	0.72 [0.81]	60	31	<0.05	<0.01	0.340
							<0.05	<0.01	0.322
Tifton, GA; 2000 J6158-00R	2	Purple Top	Roots	0.72 [0.81]	194	119	N/A	<0.05	0.066
								<0.05	0.096
			Tops	0.72 [0.81]	194	119	N/A	<0.01	0.095
								<0.01	0.203
Stilwell, KS; 2000 J6162-00R	5	Purple Top	Roots	0.72 [0.81]	72	119	N/A	<0.05	0.201
								<0.05	0.181
			Tops	0.72 [0.81]	72	119	N/A	<0.01	0.096
								<0.01	0.192
Oxford, IN; 2000 J6166-00R	5	Purple Top White Glo	Roots	0.72 [0.81]	69	104	N/A	<0.05	0.083
								<0.05	0.111
			Tops	0.72 [0.81]	69	104	N/A	<0.01	0.229
								<0.01	0.254
Winter Wheat									
Tifton, GA; 2000 J6133-00R	2	Coker 9663	Forage	0.72 [0.81]	105	29	<0.05	<0.01	1.053
							<0.05	<0.01	1.174
			Hay	0.72 [0.81]	156	29	<0.05	<0.01	2.025
							<0.05	<0.01	1.859
			Grain	0.72 [0.81]	188	29	<0.02	0.01	3.375
							<0.02	0.01	3.465
			Straw	0.72 [0.81]	188	29	<0.05	0.01	0.719
							<0.05	0.01	0.622

TABLE C.3.1. Prothioconazole Residues in Rotational Crops Grown in Treated Soil.

Location (City, State; Year Trial Number)	Region	Crop Variety	Commodity	Total Rate (lb ai/A) [kg ai/ha]	Harvest DAP[1]	PBI[2] (days)	Total Prothioconazole-Derived Residues (ppm)	1*H*-1,2,4-triazole Residues (ppm)	Triazole Conjugate Residues (ppm)
Stilwell, KS; 2000 J6137-00R	5	Karl 92	Forage	0.72 [0.81]	196	28	<0.05	<0.01	0.308
							<0.05	<0.01	0.299
			Hay	0.72 [0.81]	247	28	<0.05	<0.01	0.991
							<0.05	<0.01	0.854
			Grain	0.72 [0.81]	272	28	<0.02	<0.01	2.142
							<0.02	<0.01	2.306
			Straw	0.72 [0.81]	272	28	<0.05	<0.01	0.408
							<0.05	<0.01	0.324
Oxford, IN; 2000 J6141-00R	5	Becks 107	Forage	0.72 [0.81]	200	30	<0.05	<0.01	0.080
							<0.05	<0.01	0.081
			Hay	0.72 [0.81]	235	30	<0.05	<0.01	0.367
							<0.05	<0.01	0.303
			Grain	0.72 [0.81]	284	30	<0.02	<0.01	0.837
							<0.02	<0.01	0.710
			Straw	0.72 [0.81]	284	30	<0.05	<0.01	0.078
							<0.05	<0.01	0.074
Tifton, GA; 2000 J6134-00R	2	Coker 9663	Forage	0.72 [0.81]	108	119	N/A	<0.01	0.284
								<0.01	0.333
			Hay	0.72 [0.81]	159	119	N/A	<0.01	0.406
								<0.01	0.569
			Grain	0.72 [0.81]	191	119	N/A	<0.01	0.615
								<0.01	0.619
			Straw	0.72 [0.81]	191	119	N/A	<0.01	0.201
								<0.01	0.176
Stilwell, KS; 2000 J6138-00R	5	Karl 92	Forage	0.72 [0.81]	197	119	N/A	<0.01	0.326
								<0.01	0.296
			Hay	0.72 [0.81]	248	119	N/A	<0.01	0.763
								<0.01	0.670
			Grain	0.72 [0.81]	273	119	N/A	<0.01	1.724
								<0.01	1.754
			Straw	0.72 [0.81]	273	119	N/A	<0.01	0.278
								<0.01	0.262

TABLE C.3.1. Prothioconazole Residues in Rotational Crops Grown in Treated Soil.

Location (City, State; Year Trial Number)	Region	Crop Variety	Commodity	Total Rate (lb ai/A) [kg ai/ha]	Harvest DAP[1]	PBI[2] (days)	Total Prothioconazole-Derived Residues (ppm)	*1H*-1,2,4-triazole Residues (ppm)	Triazole Conjugate Residues (ppm)
Oxford, IN; 2000 J6142-00R	5	Becks 107	Forage	0.72 [0.81]	200	120	N/A	<0.01	0.164
								<0.01	0.190
			Hay	0.72 [0.81]	235	120	N/A	<0.01	0.515
								<0.01	0.453
			Grain	0.72 [0.81]	284	120	N/A	<0.01	1.010
								<0.01	1.011
			Straw	0.72 [0.81]	284	120	N/A	<0.01	0.120
								<0.01	0.103

[1] DAP = Days After Planting
[2] PBI = Plantback Interval

TABLE C.3.2. Apparent Prothioconazole Residues in Rotational Crop Samples Grown in Untreated Soil.

Matrix	Mustard greens	Turnip root	Turnip top	Wheat forage	Wheat hay	Wheat grain	Wheat straw
Total Prothioconazole-Derived Residues							
Number of samples with apparent residues <LOQ	3	3	3	3	3	3	3
Number of samples with quantifiable residues	0	0	0	0	0	0	0
Range of quantifiable residues, ppm	Not applicable						
1*H*-1,2,4-triazole							
Number of samples with apparent residues <LOQ	12	9	9	7	6	9	9
Number of samples with quantifiable residues	2	0	0	0	0	0	0
Range of quantifiable residues, ppm	0.023, 0.027	*Not applicable*					
Triazolylalanine							
Number of samples with apparent residues <LOQ	1	7	6	3	4	3	9
Number of samples with quantifiable residues	13	2	3	10	8	9	1
Range of quantifiable residues	0.013-0.113	0.038-0.039	0.029-0.062	0.011-0.179	0.014-0.066	0.048-0.212	0.013

TABLE C.3.2. Apparent Prothioconazole Residues in Rotational Crop Samples Grown in Untreated Soil.

Matrix	Mustard greens	Turnip root	Turnip top	Wheat forage	Wheat hay	Wheat grain	Wheat straw
Triazolylacetic Acid							
Number of samples with apparent residues <LOQ	10	1	0	4	3	3	6
Number of samples with quantifiable residues	1	8	9	6	8	10	9
Range of quantifiable residues	0.018	0.011-0.025	0.010-0.025	0.014-0.043	0.024-0.072	0.029-0.100	0.014-0.057

TABLE C.4. Summary of Residue Data in Rotational Crops Following Primary Treatment with Prothioconazole.

Commodity	Applic. Rate (lb a.i./A) [kg a.i./ha]	PBI (days)	Residue Levels (ppm) [1]						
			n	Min.	Max.	HAFT [2]	Median (STMdR[3])	Mean (STMR[4])	Std. Dev.
Total Prothioconazole-Derived Residues									
Mustard Greens	0.72 [0.81]	31-34	6	<0.05	<0.05	<0.05	0.025	0.025	0.00
Turnip Roots	0.72 [0.81]	31-34	6	<0.05	<0.05	<0.05	0.025	0.025	0.00
Turnip Tops	0.72 [0.81]	31-34	6	<0.05	<0.05	<0.05	0.025	0.025	0.00
Wheat Forage	0.72 [0.81]	28-30	6	<0.05	<0.05	<0.05	0.025	0.025	0.00
Wheat Hay	0.72 [0.81]	28-30	6	<0.05	<0.05	<0.05	0.025	0.025	0.00
Wheat Grain	0.72 [0.81]	28-30	6	<0.02	<0.02	<0.02	0.01	0.01	0.00
Wheat Straw	0.72 [0.81]	28-30	6	<0.05	<0.05	<0.05	0.025	0.025	0.00
***1H*-1,2,4-triazole Residues**									
Mustard Greens	0.72-0.73 [0.81-0.82]	31-34	6	<0.01	<0.01	<0.01	0.005	0.005	0.00
		104-119	6	<0.01	<0.01	<0.01	0.005	0.005	0.00
Turnip Roots	0.72 [0.81]	31-34	6	<0.05	<0.05	<0.05	0.025	0.025	0.00
		104-119	6	<0.05	<0.05	<0.05	0.025	0.025	0.00
Turnip Tops	0.72 [0.81]	31-34	6	<0.01	<0.01	<0.01	0.005	0.005	0.00
		104-119	6	<0.01	<0.01	<0.01	0.005	0.005	0.00
Wheat Forage	0.72 [0.81]	28-30	6	<0.01	<0.01	<0.01	0.005	0.005	0.00
		119-120	6	<0.01	<0.01	<0.01	0.005	0.005	0.00
Wheat Hay	0.72 [0.81]	28-30	6	<0.01	<0.01	<0.01	0.005	0.005	0.00
		119-120	6	<0.01	<0.01	<0.01	0.005	0.005	0.00
Wheat Grain	0.72 [0.81]	28-30	6	<0.01	0.01	0.01	0.005	0.007	0.002
		119-120	6	<0.01	<0.01	<0.01	0.005	0.005	0.00
Wheat Straw	0.72 [0.81]	28-30	6	<0.01	0.01	0.01	0.005	0.007	0.002
		119-120	6	<0.01	<0.01	<0.01	0.005	0.005	0.00

TABLE C.4. Summary of Residue Data in Rotational Crops Following Primary Treatment with Prothioconazole.

Commodity	Applic. Rate (lb a.i./A) [kg a.i./ha]	PBI (days)	Residue Levels (ppm) [1]						
			n	Min.	Max.	HAFT [2]	Median (STMdR[3])	Mean (STMR[4])	Std. Dev.
Triazole Conjugate Residues									
Mustard Greens	0.72-0.73 [0.81-0.82]	31-34	6	0.102	0.313	0.311	0.253	0.222	0.097
		104-119	6	0.081	0.392	0.309	0.142	0.182	0.115
Turnip Roots	0.72 [0.81]	31-34	6	0.301	0.567	0.533	0.366	0.405	0.105
		104-119	6	0.066	0.201	0.191	0.104	0.123	0.055
Turnip Tops	0.72 [0.81]	31-34	6	0.263	0.484	0.449	0.331	0.355	0.080
		104-119	6	0.095	0.254	0.242	0.198	0.178	0.068
Wheat Forage	0.72 [0.81]	28-30	6	0.080	1.174	1.114	0.304	0.499	0.488
		119-120	6	0.164	0.333	0.311	0.290	0.266	0.071
Wheat Hay	0.72 [0.81]	28-30	6	0.303	2.025	1.942	0.923	1.067	0.731
		119-120	6	0.406	0.763	0.717	0.542	0.563	0.135
Wheat Grain	0.72 [0.81]	28-30	6	0.710	3.465	3.420	2.224	2.139	1.188
		119-120	6	0.615	1.754	1.739	1.011	1.122	0.509
Wheat Straw	0.72 [0.81]	28-30	6	0.074	0.719	0.671	0.366	0.371	0.269
		119-120	6	0.103	0.278	0.270	0.189	0.190	0.072

[1] For the calculation of minimum, maximum, and HAFT values, the LOQ value (<0.01, <0.02, or <0.05 ppm) was used for residues reported as below the LOQ in TABLE C.3.1. For calculation of the median, mean and standard deviation, ½LOQ was used for residues reported as below the LOQ.
[2] HAFT = Highest Average Field Trial.
[3] STMdR = Supervised Trial Median Residue.
[4] STMR = Supervised Trial Mean Residue.

D. CONCLUSION

Total prothioconazole-derived residues were below the LOQ (<0.02 ppm for wheat grain and <0.05 ppm for all other commodities) in/on all samples of rotated mustard greens, turnip, and wheat commodities from the 1-month PBI. Residues of 1*H*-1,2,4-triazole were below the LOQ (<0.01 ppm) in/on all samples of rotated mustard greens, turnip, and wheat commodities from the 1- and 4-month PBIs, with the exception of wheat straw and grain from the 1-month PBI; residues of 1*H*-1,2,4-triazole were 0.01 ppm in/on two samples each of wheat straw and grain from the 1-month PBI. Quantifiable triazole conjugate residues were observed in/on all rotational crop matrices from the 1- and 4-month PBIs; average triazole conjugate residues in each commodity decreased from the 1-month PBI to the 4-month PBI.

E. REFERENCES

Gould T. and Timberlake B. (2005) Storage stability of JAU6476 and desthio JAU6476 in canola, wheat, mustard greens, turnip root, and tomato fruit, processed products, and solutions, Bayer CropScience Interim Report.

F. DOCUMENT TRACKING

RDI: Louise Croteau, Suzan Mathew (PMRA) 23/01/2006; Henri Bietlot (PMRA) 24/01/2006; Stephen Funk (IO) 13/03/2006; Leung Cheng (RAB3)
Petition Number: PP#4F6830
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Template Version September 2003

Primary Evaluators	Stephen Funk, Ph.D., Senior Chemist Immediate Office	Date: Mar 13 2006
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Approved by	Leung Cheng, Ph. D., Team Leader HED/RAB3	Date:
	Henri P Bietlot, Acting Section Head FREAS, HED	Date: Jan 24/06

In the absence of signatures, this document is considered to be a draft with deliberative material for internal use only.

This DER was originally prepared under contract by Dynamac Corporation (20440 Century Boulevard, Suite 100; Germantown, MD 20874; submitted 05/20/2005). The DER has been reviewed by the Health Effects Division (HED) and Health Canada's Pest Management Regulatory Agency (PMRA). The DER has been revised to reflect current Office of Pesticide Programs (OPP) policies and Directive 98-02.

STUDY REPORT:

46246221 Fischer, D.R. (2004) JAU6476 480 SC - Magnitude of the Residue in/on Dried Peas and Dried Beans (Crop Subgroup 6C - Dried, Shelled Pea and Bean - Except Soybean):Lab Project Number: J619BY02; J619DB02; RCJAX007; 200956. Unpublished study prepared by Bayer CropScience. 564 pages.

EXECUTIVE SUMMARY:

Bayer CropScience has submitted field trial data on dried peas and beans. A total of 23 field trials were conducted during the 2002 growing season in the U.S. and Canada. Thirteen trials were conducted on dried peas in Regions 5 (MN and ON; 2 trials), 11 (ID; 1 trial, OR; 3 trials, and WA; 1 trial), and 14 (AB; 2 trials, MB; 1 trial, and SK; 3 trials), and 10 trials were conducted

on dried beans in Regions 5 (IL, IN, KS, and ON; 4 trials), 7 (ND;1 trial), 7A (AB; 1 trial), 8 (TX; 1 trial), 9 (MT; 1 trial), 10 (CA; 1 trial), and 11 (WA; 1 trial). The number and locations of field trials are in accordance with OPPTS Guideline 860.1500 and Directive 98-02; Section 9.

At each test location, three broadcast foliar applications of the 4 lb/gal (480 g/L) suspension concentrate formulation (flowable concentrate; FlC) were made at ~0.180 lb a.i./A (~ 0.200 kg a.i./ha) at 9- to 15-day retreatment intervals, for a total seasonal application rate of ~0.54 lb a.i./A (~0.60 kg a.i./ha). Applications were made in ~10-33 gal/A of water using ground equipment. A non-ionic surfactant was added to the spray mixture for all applications. An additional plot at each trial was treated with three applications at a target rate of ~0.134 lb a.i./A (~ 0.150 kg a.i./ha); however, the applicant stated that the results from this application were not used because they did not support the desired product label application rate. Samples of dried shelled peas and beans were harvested 7-8 days after the last application from all test sites. It should be noted that in three of the pea field trials and five of the bean field trials, the pea and bean plants were cut and allowed to dry in the field for 2-8 days prior to collection. At two locations for dried peas and one location for dried beans, additional samples were collected to determine residue decline. Samples were harvested, at both locations, 0, 3-4, 7, 14-15, and 21-22 days after treatment for dried peas and 0, 7, 14, and 21 days after treatment for dried beans.

Samples were analyzed for total prothioconazole-derived residues (prothioconazole and the metabolite prothioconazole-desthio) using LC-MS/MS method RPA JA/03/01. The validated LOQ for total prothioconazole-derived residues was 0.05 ppm for dried peas and dried beans. Samples were analyzed for residues of 1*H*-1,2,4-triazole and the triazole conjugates triazolylalanine and triazolylacetic acid using an LC-MS/MS method (Bayer Report No. G200598). The validated LOQs were 0.01 ppm for 1*H*-1,2,4-triazole for dried peas and beans, 0.02 ppm for the triazole conjugates for dried beans, and 0.05 ppm for the triazole conjugates for dried peas. The methods are adequate for data collection based on acceptable concurrent method recovery data

The results from the pea and bean field trials show that the maximum residues of prothioconazole in/on dried peas and beans harvested 7-8 days following the last of three broadcast foliar applications at a total seasonal rate of 0.530-0.580 lb a.i./A (0.595-0.650 kg a.i./ha) were 0.684 ppm in/on dried peas and 0.288 ppm in/on dried beans for total prothioconazole-derived residues; 0.011 ppm in/on dried peas and less than the LOQ (<0.01 ppm) in/on dried beans for 1*H*-1,2,4-triazole; and 0.789 ppm in/on dried peas and 0.311 ppm in/on dried beans for the triazole conjugates.

In the residue decline trials, residues of 1*H*-1,2,4-triazole were less than the method LOQ (<0.01 ppm) at all sampling intervals in dried peas and beans. Total prothioconazole-derived residues did not increase with increasing sampling intervals in the dried bean trial and in one dried pea trial; in the other dried pea trial, residues increased slightly with increasing sampling intervals (from an average of 0.31 ppm at the 7-day PHI to an average of 0.34 ppm at the 21-day PHI). Residues of the triazole conjugates did not increase with increasing sampling intervals in dried peas, but increased slightly in dried beans with increasing sampling intervals.

The maximum storage interval of crop samples from harvest to analysis for total prothioconazole-derived residues was 542 days (17.8 months) for dried beans and peas. The degree of loss of prothioconazole-derived residues and prothioconazole-desthio residues is not expected to exceed 30% after 17.8 months in dried beans and peas.

STUDY/WAIVER ACCEPTABILITY/DEFICIENCIES/CLARIFICATIONS:

Under the conditions and parameters used in the study, the field trial residue data are classified as scientifically acceptable. The final reports of the ongoing storage stability studies with prothioconazole, prothioconazole-desthio, 1*H*-1,2,4-triazole, triazolylalanine, and triazolylacetic acid willt be submitted as confirmatory data.

The acceptability of this study for regulatory purposes is addressed in the forthcoming U.S. EPA Residue Chemistry Summary Document, DP Barcode D303508 and in Canada's Regulatory Decision Document.

COMPLIANCE:

Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided. No deviations from regulatory requirements were reported which would have an impact on the validity of the study.

A. BACKGROUND INFORMATION

Prothioconazole is a broad-spectrum systemic fungicide intended for the control of many diseases in different crops such as cereals, oilseed rape or peanuts. The biochemical mode of action in the inhibition of the demethylation of lanosterol or 24-methylene-dihydrolanosterol, which are precursors of sterols in fungi. The chemical has been submitted for joint EPA and PMRA review by Bayer CropScience. The crops proposed for joint review include barley, the dried shell and bean subgroup, the oilseed crop group, and wheat. Uses on peanuts and rice are being proposed for the U.S. only.

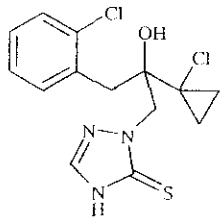
TABLE A.1. Test Compound Nomenclature.	
Chemical structure	
Common name	Prothioconazole (ISO accepted)
Company experimental name	JAU6476
IUPAC name	2-[(2*RS*)-2-(1-chlorocyclopropyl)-3-(2-chlorophenyl)-2-hydroxypropyl]-2*H*-1,2,4-triazole-3(4*H*)-thione
CAS name	2-[2-(1-chlorocyclopropyl)-3-(2-chlorophenyl)-2-hydroxypropyl]-1,2-dihydro-3*H*-1,2,4-triazole-3-thione
CAS registry number	178928-70-6
PC Code	113961
End-use product (EP)	Proline® 480 SC (4 lb/gal suspension concentrate), EPA File Symbol 264-IEL

TABLE A.2. Physicochemical Properties of the Technical Grade Test Compound.

Parameter	Value	References
Melting range	139.1 to 144.5°C	MRID 46246003/CES
pH	5.8 (1% solution)	MRID 46246003/CES
Density at 20°C	1.36 g/mL	MRID 46246003/CES
Water solubility at 20°C	pH: mg/L 4: 5 8: 300 9: 2000	MRID 46246003/CES
Solvent solubility at 20°C	Solvent: g/L Acetone: >250 Acetonitrile: 69 Dichloromethane: 88 Dimethysulfoxide: 126 Ethyl acetate: >250 n-Heptane: <0.1 1-Octanol: 58 Polyethylene glycol: >250 2-Propanol: 87 Xylene: 8	MRID 46246003/CES
Vapor pressure at 20 or 25°C	$<4 \times 10^{-7}$ Pa (calculated from determinations at 70°C)	MRID 46246003/CES
Dissociation constant, pK_a	6.9 (calculated from K_{OW})	MRID 46246003/CES
Octanol/water partition coefficient, at 20°C	pH: $Log(K_{OW})$ unbuffered water: 4.05 4: 4.16 7: 3.82 9: 2.00	MRID 46246003/CES
UV/visible absorption spectrum	Peak maxima at 257 nm. No absorption at > 300 nm.	MRID 46246003/CES

CES- Chemistry Evaluation Section of PMRA

B. EXPERIMENTAL DESIGN

B.1. Study Site Information

The tables below were copied from the data report (MRID 46246221) without alteration.

Study Location (City, State)	Trial Number	Year	Soil Characteristics[a]				Meteorological Data[b]	
			Type	% OM	pH	CEC	Total Rainfall (in)	Temp. Range (°F)
Ephrata, Washington	J6007-02H	2002	NA	NA	NA	NA	4.71[c]	45-103
Jerome, Idaho	J6008-02H	2002	NA	NA	NA	NA	3.35[c]	50-109
Madras, Oregon	J6009-02H	2002	NA	NA	NA	NA	13.45[c]	32-96
Hermiston, Oregon	J6010-02H	2002	NA	NA	NA	NA	9.14[c]	45-105
Hood River, Oregon	J6011-02D	2002	NA	NA	NA	NA	6.00[c]	43-102
Campbell, Minnesota	J6012-02H	2002	NA	NA	NA	NA	4.52	41-93
Branchton, Ontario	J6013-02H	2002	NA	NA	NA	NA	4.46	46-92
Kipp, Alberta	J6014-02H	2002	NA	NA	NA	NA	2.62	37-87
Rosthern, Saskatchewan	J6015-02H	2002	NA	NA	NA	NA	3.93	32-90
Brookdale, Manitoba	J6016-02H	2002	NA	NA	NA	NA	2.93	38-93
Wakaw, Saskatchewan	J6017-02H	2002	NA	NA	NA	NA	2.57	36-88
Rosthern, Saskatchewan	J6018-02H	2002	NA	NA	NA	NA	3.52	32-90
Edmonton, Alberta	J6019-02D	2002	NA	NA	NA	NA	3.23	30-88

[a] NA = Not Applicable, since these parameters do not affect the use pattern of the chemical.
[b] Data is for the interval from first application to last sampling.
[c] Overhead irrigation values added to rainfall values.

The actual temperature and rainfall recordings were within average historical values for the residue study period. The applicant indicated that irrigation was used to supplement as needed at seven trial sites.

TABLE B.1.2. Study Use Pattern.

Location (City, State; Year)	EP[1]	Application					Tank Mix Adjuvants
		Method; Timing	Vol. (GPA[2]) [L/ha]	Rate (lb ai/A) [kg a.i./ha]	RTI[3] (days)	Total Rate (lb a.i./A) [kg a.i./ha]	
Dried Shelled Peas							
Ephrata, WA; 2002 J6007-02H	480 SC	1: Broadcast foliar; 30% of pods have reached typical length	21 [200]	0.180 [0.201]	---	0.543 [0.607]	Non-ionic surfactant
		2: Broadcast foliar; 10% of pods ripe, seeds final color, dry and hard	22 [202]	0.180 [0.201]	12		
		3: Broadcast foliar; Fully ripe: all pods dry and brown , seeds dry and hard	21 [195]	0.183 [0.205]	12		
Jerome, ID; 2002 J6008-02H	480 SC	1: Broadcast foliar; 60% of pods have reached typical length	20 [188]	0.178 [0.199]	---	0.537 [0.601]	Non-ionic surfactant
		2: Broadcast foliar; 30% of pods ripe, seeds final color, dry and hard	21 [193]	0.180 [0.201]	14		
		3: Broadcast foliar; 80% of pods ripe, seeds final color, dry and hard	20 [191]	0.179 [0.201]	10		
Madras, OR; 2002 J6009-02H	480 SC	1: Broadcast foliar; Pods have reached typical size (green ripe)	31 [290]	0.185 [0.207]	---	0.549 [0.615]	Non-ionic surfactant
		2: Broadcast foliar; 20% of pods ripe, seeds final color, dry and hard	31 [293]	0.187 [0.210]	10		
		3: Broadcast foliar; 80% of pods ripe, seeds final color, dry and hard	30 [276]	0.177 [0.198]	11		
Hermiston, OR; 2002 J6010-02H	480 SC	1: Broadcast foliar; 30% of flowers open	28 [262]	0.179 [0.200]	---	0.533 [0.597]	Non-ionic surfactant
		2: Broadcast foliar; Beginning of ripening, seed green	28 [262]	0.179 [0.201]	14		
		3: Broadcast foliar; 50% of pods ripe, seeds final color, dry and hard	27 [255]	0.175 [0.196]	14		
Hood River, OR; 2002 J6011-02D	480 SC	1: Broadcast foliar; First pods have reached final length	21 [195]	0.182 [0.204]	---	0.544 [0.609]	Non-ionic surfactant
		2: Broadcast foliar; Pods have reached typical size (green ripe)	21 [197]	0.183 [0.205]	10		
		3: Broadcast foliar; 10% of pods ripe, seeds final color, dry and hard	23 [216]	0.179 [0.200]	10		
Campbell, MN; 2002 J6012-02H	480 SC	1: Broadcast foliar; 10% of pods have reached typical length	20 [187]	0.180 [0.202]	---	0.539 [0.604]	Non-ionic surfactant
		2: Broadcast foliar; Beginning of ripening, seed green	20 [187]	0.180 [0.201]	13		
		3: Broadcast foliar; 60% of pods ripe, seeds final color, dry and hard	20 [187]	0.179 [0.201]	13		

TABLE B.1.2. Study Use Pattern.

Location (City, State; Year)	EP[1]	Application					Tank Mix Adjuvants
		Method; Timing	Vol. (GPA[2]) [L/ha]	Rate (lb ai/A) [kg a.i./ha]	RTI[3] (days)	Total Rate (lb a.i./A) [kg a.i./ha]	
Branchton, ON; 2002 J6013-02H	480 SC	1: Broadcast foliar; First pods have reached final length	23 [215]	0.178 [0.199]	---	0.541 [0.606]	Non-ionic surfactant
		2: Broadcast foliar; 70% of pods have reached typical length	23 [215]	0.184 [0.206]	10		
		3: Broadcast foliar; 60% of pods ripe, seeds final color, dry and hard	22 [210]	0.179 [0.201]	11		
Kipp, AB; 2002 J6014-02H	480 SC	1: Broadcast foliar; Pods have reached typical size (green ripe)	11 [102]	0.184 [0.206]	---	0.548 [0.613]	Non-ionic surfactant
		2: Broadcast foliar; Pods have reached typical size (green ripe)	11 [102]	0.183 [0.205]	9		
		3: Broadcast foliar; 70% of pods ripe, seeds final color, dry and hard	11 [101]	0.181 [0.202]	12		
Rosthern, SK; 2002 J6015-02H	480 SC	1: Broadcast foliar; Full flowering, 50% of flowers open	10 [97]	0.176 [0.198]	---	0.536 [0.602]	Non-ionic surfactant
		2: Broadcast foliar; 80% of pods ripe, seeds final color, dry and hard	12 [109]	0.177 [0.199]	14		
		3: Broadcast foliar; 80% of pods ripe, seeds final color, dry and hard	11 [103]	0.183 [0.205]	10		
Brookdale, MB; 2002 J6016-02H	480 SC	1: Broadcast foliar; Full flowering; 50% of flowers open	12 [110]	0.174 [0.195]	---	0.530 [0.595]	Non-ionic surfactant
		2: Broadcast foliar; 70% of pods have reached typical length	25 [231]	0.177 [0.199]	11		
		3: Broadcast foliar; 10% of pods ripe, seeds final color, dry and hard	25 [236]	0.179 [0.201]	13		
Wakaw, SK; 2002 J6017-02H	480 SC	1: Broadcast foliar; Beginning of ripening, seed green	12 [110]	0.178 [0.200]	---	0.536 [0.601]	Non-ionic surfactant
		2: Broadcast foliar; 30% of pods ripe, seeds final color, dry and hard	12 [110]	0.180 [0.201]	13		
		3: Broadcast foliar; 80% of pods ripe, seeds final color, dry and hard	12 [111]	0.178 [0.200]	14		
Rosthern, SK; 2002 J6018-02H	480 SC	1: Broadcast foliar; Pods have reached typical size (green ripe)	12 [111]	0.181 [0.203]	---	0.539 [0.604]	Non-ionic surfactant
		2: Broadcast foliar; 30% of pods ripe, seeds final color, dry and hard	12 [110]	0.179 [0.200]	11		
		3: Broadcast foliar; Fully ripe; all pods dry and brown, seeds dry and hard	12 [110]	0.179 [0.201]	13		

TABLE B.1.2. Study Use Pattern.

Location (City, State; Year)	EP[1]	Application					Tank Mix Adjuvants
		Method; Timing	Vol. (GPA[2]) [L/ha]	Rate (lb ai/A) [kg a.i./ha]	RTI[3] (days)	Total Rate (lb a.i./A) [kg a.i./ha]	
Edmonton, AB; 2002 J6019-02D	480 SC	1: Broadcast foliar; 70% of pods have reached typical length	11 [106]	0.180 [0.201]	---	0.543 [0.608]	Non-ionic surfactant
		2: Broadcast foliar; Beginning of ripening; seed green	10 [97]	0.180 [0.202]	13		
		3: Broadcast foliar; 80% of pods ripe, seeds final color, dry and hard	11 [98]	0.183 [0.205]	12		
Dried Shelled Beans							
Lynden, ON; 2002 J6020-02H	480 SC	1: Broadcast foliar; 10% of pods ripe (beans hard) Seeds beginning to mature	31 [292]	0.175 [0.196]	---	0.580 [0.650]	Non-ionic surfactant
		2: Broadcast foliar; 40% of pods ripe (beans hard)	33 [312]	0.214 [0.244]	13		
		3: Broadcast foliar; 60% of pods ripe (beans hard)	34 [321]	0.191 [0.214]	10		
Carlyle, IL; 2002 J6021-02H	480 SC	1: Broadcast foliar; Full flowering. 50% of flowers open	19 [179]	0.184 [0.206]	---	0.547 [0.613]	Non-ionic surfactant
		2: Broadcast foliar; 70% of pods have reached typical length	10 [95]	0.182 [0.204]	14		
		3: Broadcast foliar; 20% of pods ripe (beans hard)	13 [119]	0.181 [0.203]	14		
Oxford, IN;2002 J6022-02H	480 SC	1: Broadcast foliar; 20% of pods have reached typical length	16 [150]	0.187 [0.210]	---	0.543 [0.610]	Non-ionic surfactant
		2: Broadcast foliar; 50% of pods have reached typical length	16 [146]	0.180 [0.202]	14		
		3: Broadcast foliar; 80% of pods ripe (beans hard)	15 [138]	0.176 [0.198]	13		
Stilwell, KS; 2002 J6023-02D	480 SC	1: Broadcast foliar; 20% of pods have reached typical length	21 [198]	0.188 [0.211]	---	0.555 [0.622]	Non-ionic surfactant
		2: Broadcast foliar; 20% of pods ripe (beans hard)	21 [193]	0.186 [0.208]	13		
		3: Broadcast foliar; Fully ripe; pods ripe (beans hard)	20 [191]	0.181 [0.203]	14		
Eldridge, ND; 2002 J6024-02H	480 SC	1: Broadcast foliar; 60% of pods have reached typical length	30 [283]	0.182 [0.204]	---	0.539 [0.604]	Non-ionic surfactant
		2: Broadcast foliar; 20% of pods ripe (beans hard)	29 [276]	0.177 [0.198]	13		
		3: Broadcast foliar; Fully ripe; pods ripe (beans hard)	30 [281]	0.180 [0.202]	13		

TABLE B.1.2. Study Use Pattern.

Location (City, State; Year)	EP[1]	Application					Tank Mix Adjuvants
		Method; Timing	Vol. (GPA[2]) [L/ha]	Rate (lb ai/A) [kg a.i./ha]	RTI[3] (days)	Total Rate (lb a.i./A) [kg a.i./ha]	
Taber, AB; 2002 J6025-02H	480 SC	1: Broadcast foliar; 50% of pods have reached typical length	11 [102]	0.182 [0.204]	0	0.534 [0.598]	Non-ionic surfactant
		2: Broadcast foliar; Pods, individual beans easily visible	11 [100]	0.179 [0.200]	12		
		3: Broadcast foliar; 20% of pods ripe (beans hard)	10 [98]	0.173 [0.194]	10		
Plainview, TX; 2002 J6026-02H	480 SC	1: Broadcast foliar; 30% of pods have reached typical length	15 [144]	0.179 [0.201]	---	0.536 [0.600]	Non-ionic surfactant
		2: Broadcast foliar; 10% of pods ripe (beans hard), seeds beginning to mature	16 [149]	0.182 [0.203]	11		
		3: Broadcast foliar; Fully ripe, pods ripe (beans hard)	21 [192]	0.175 [0.196]	14		
Fromberg, MT; 2002 J6027-02H	480 SC	1: Broadcast foliar; End of flowering, first pods visible	11 [102]	0.182 [0.203]	---	0.544 [0.609]	Non-ionic surfactant
		2: Broadcast foliar; 60% of pods have reached typical length	26 [239]	0.180 [0.202]	15		
		3: Broadcast foliar; 20% of pods ripe (beans hard)	23 [218]	0.182 [0.204]	14		
Fresno, CA; 2002 J6028-02H	480 SC	1: Broadcast foliar; 20% of pods have reached typical length	25 [235]	0.183 [0.205]	---	0.538 [0.603]	Non-ionic surfactant
		2: Broadcast foliar; 60% of pods have reached typical length	25 [232]	0.177 [0.199]	13		
		3: Broadcast foliar; 20% of pods ripe (beans hard)	27 [251]	0.178 [0.199]	14		
Ephrata, WA; 2002 J6029-02H	480 SC	1: Broadcast foliar; 30% of pods have reached typical length	26 [242]	0.180 [0.202]	---	0.540 [0.606]	Non-ionic surfactant
		2: Broadcast foliar; 70% of pods have reached typical length	25 [233]	0.180 [0.202]	12		
		3: Broadcast foliar; 80% of pods ripe (beans hard)	30 [282]	0.180 [0.202]	12		

[1] EP = End-use Product; 480 SC = 4 lb/gal (480 g/L) suspension concentrate (flowable concentrate; FlC) formulation
[2] GPA = Gallons per acre [or use L/ha]
[3] RTI = Retreatment Interval

TABLE B.1.3. Trial Numbers and Geographical Locations.

NAFTA Growing Region	Peas, dried shelled			Bean, dried		
	Submitted	Requested		Submitted	Requested	
		Canada	US		Canada	US
1						
1A						
2						
3						
4						
5	2	2		4	4	4
5A						
5B						
6						
7				1		1
7A				1	1	
8				1		1
9				1		1
10				1		1
11	5		5	1		1
12						
13						
14	6	6				
15						
16						
17						
18						
19						
20						
21						
Total	13	8	5	10	5	9

B.2. Sample Handling and Preparation

Samples of dried peas and beans were collected 7-8 days after the last application. In three of the pea field trials and five of the bean field trials, the pea and bean plants were cut and allowed to dry in the field for 2-8 days prior to collection. Samples were frozen within 4 hours of collection and were shipped frozen to Bayer Research Park (Stilwell, KS) for homogenization with dry ice. After homogenization was completed, the samples were stored frozen until analysis.

B.3. Analytical Methodology

Samples of dried peas and beans were analyzed for residues of total prothioconazole-derived residues (prothioconazole and the metabolite prothioconazole-desthio) using LC-MS/MS method RPA JA/03/01 with modifications. Samples were 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 was spiked 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. Residue levels were quantitated from a calibration curve of mixed reference standards of prothioconazole, as the sulfonic acid, and prothioconazole-desthio. The results for prothioconazole sulfonic acid and prothioconazole-desthio are reported in prothioconazole equivalents and then totaled to yield "total prothioconazole derived residues." The method was modified to use a different solvent for preparation of the spiking solutions and to use slightly different m/z values for the quantitation ions. The validated LOQ for total prothioconazole-derived residues was 0.05 ppm for dried peas and dried beans.

Samples were analyzed for residues of 1*H*-1,2,4-triazole and the triazole conjugates (triazolylalanine and triazolylacetic acid) using an LC-MS/MS method (Bayer Report No. G200598) with modifications. Briefly, crop matrices were extracted with aqueous methanol, and three separate aliquots of the extract were removed for determination of each of the three analytes. Isotopically labeled internal standard was added to each aliquot. For 1*H*-1,2,4-triazole, the aliquot was mixed with dansyl chloride to form the dansyl derivative of 1*H*-1,2,4-triazole, which was partitioned into ethyl acetate and then redissolved in acetonitrile (ACN)/water for LC-MS/MS analysis. For triazolylalanine, the aliquot was cleaned up by SPE, derivatized to the butyl ester using butanolic HCl, and then further derivatized using heptafluorobutyric anhydride (HFBA). The mixture was redissolved in ACN/water for LC-MS/MS analysis. For determination of triazolylacetic acid, the aliquot was cleaned up by SPE, derivatized to the butyl ester using butanolic HCl, and then redissolved in ACN/water for LC-MS/MS analysis. Residue levels were quantitated from a calibration curve of reference standards of dansyl-1,2,4-triazole, triazolylalanine butyl ester HFBA, and triazolylacetic acid butyl ester (generated by derivatizing reference standard solutions of triazolylacetic acid). The validated LOQs were 0.01 ppm for 1*H*-1,2,4-triazole for dried peas and beans, 0.02 ppm for the triazole conjugates for dried beans, and 0.05 ppm for the triazole conjugates for dried peas.

C. RESULTS AND DISCUSSION

Concurrent method recovery data are presented in TABLE C.1. Samples were analyzed for total prothioconazole-derived residues (prothioconazole and the metabolite prothioconazole-desthio) using LC-MS/MS method RPA JA/03/01. The validated LOQ for total prothioconazole-derived residues was 0.05 ppm for dried peas and dried beans. Samples were analyzed for residues of 1*H*-1,2,4-triazole and the triazole conjugates triazolylalanine and triazolylacetic acid using an LC-MS/MS method (Bayer Report No. G200598). The validated LOQs were 0.01 ppm for 1*H*-

1,2,4-triazole for dried peas and beans, 0.02 ppm for the triazole conjugates for dried beans, and 0.05 ppm for the triazole conjugates for dried peas. The methods are adequate for data collection based on acceptable concurrent method recovery data.

Apparent total prothioconazole-derived residues and residues of 1*H*-1,2,4-triazole were below the method LOQs (<0.05 and <0.01 ppm, respectively) in/on all samples of untreated dried peas and beans. Apparent residues of the triazole conjugates were below the method LOQs (<0.05 ppm for dried peas and <0.02 ppm for dried beans) in/on 20 samples of untreated dried peas and 19 samples of dried beans; quantifiable apparent residues were observed in/on 12 samples of untreated peas (residue range of 0.05-0.52 ppm) and 10 samples of untreated beans (residue range of 0.03-0.35 ppm). The measurable control residues of the triazole conjugates may have resulted from the use of triazole fungicides in previous seasons or from naturally occurring triazole conjugates.

Sample storage conditions and intervals are summarized in TABLE C.2. The maximum storage interval of crop samples from harvest to analysis for total prothioconazole-derived residues was 542 days (17.8 months) for dried beans and peas. Prothioconazole-derived residues are stable for up to 1 year based on an interim report. The need to correct the results of the current study for decline of prothioconazole-derived residues will not be necessary taking into account the absolute (ppm) and relative % residue levels in plant matrices. The degree of loss of prothioconazole-desthio residues is not expected to exceed 30% after 17.8 months.

The maximum storage interval of crop samples from harvest to analysis for 1*H*-1,2,4-triazole and triazole conjugate residues was 520 days (17.0 months) for dried beans and peas.

Residue data from the pea and bean field trials are reported in TABLE C.3. A summary of prothioconazole residue data for dried peas and beans is presented in TABLE C.4. Residues in/on peas and beans harvested 7-8 days following the last of three broadcast foliar applications were <0.05-0.684 ppm in/on dried peas and <0.05-0.288 ppm in/on dried beans for total prothioconazole-derived residues; <0.01-0.011 ppm in/on dried peas and <0.01 ppm in/on dried beans for 1*H*-1,2,4-triazole; and <0.05-0.789 ppm in/on dried peas and <0.02-0.311 ppm in/on dried beans for the triazole conjugates.

In the residue decline trials, residues of 1*H*-1,2,4-triazole were less than the method LOQ (<0.01 ppm) at all sampling intervals in dried peas and beans. Total prothioconazole-derived residues did not increase with increasing sampling intervals in the dried bean trial and in one dried pea trial; in the other dried pea trial, residues increased slightly with increasing sampling intervals (from an average of 0.31 ppm at the 7-day PHI to an average of 0.34 ppm at the 21-day PHI). Residues of the triazole conjugates did not increase with increasing sampling intervals in dried peas, but increased slightly in dried beans with increasing sampling intervals.

TABLE C.1. Summary of Concurrent Recoveries of Prothioconazole from Peas, Dried Shelled, and Beans, Dried.

Matrix	Spike level (ppm)	Sample size (n)	Recoveries (%)	Mean ± std dev
Prothioconazole				
Pea, dried shelled	0.10	2	84, 88	86 ± 2.8
Bean, dried shelled	0.10	2	87, 87	87 ± 0.0
Prothioconazole-Desthio				
Pea, dried shelled	0.10	2	93, 94	94 ± 0.7
Bean, dried shelled	0.10	2	100, 102	101 ± 1.4
1*H*-1,2,4-triazole				
Pea, dried shelled	0.05	6	70, 73, 92, 93, 102, 119	91.5 ± 18.3
	0.50	2	78, 82	80 ± 2.8
Bean, dried shelled	0.04	2	85, 97	91± 8.5
Triazolylalanine				
Pea, dried shelled	0.25	5	80, 84, 85, 86, 87	84.4 ± 2.7
	0.50	2	86, 89	87.5 ± 2.1
Bean, dried shelled	0.24	3	79, 79, 90	83 ± 6.4
Triazolylacetic acid				
Pea, dried shelled	0.25	5	70, 71, 72, 72, 82	73.4 ± 4.9
	0.50	2	74, 77	75.5 ± 2.1
Bean, dried shelled	0.08	3	70, 71, 86	76 ± 9.0

TABLE C.2. Summary of Storage Conditions.

Matrix (RAC or Extract)	Storage Temp. (°C)	Actual Storage Duration	Interval of Demonstrated Storage Stability
Total Prothioconazole-Derived Residues			
Pea, dried shelled	-22 to -24	494-542 days (16.2-17.8 months)	Stability demonstrated for up to 12.7 months (interim report). Awaiting final report as confirmatory data.
Bean, dried shelled		490-536 days (16.1-17.6 months)	
1*H*-1,2,4-triazole Residues			
Pea, dried shelled	-22 to -24	207-255 days (6.8-8.4 months)	Not applicable at this time. Awaiting final report.
Bean, dried shelled		218-518 days (7.2-17.0 months)	
Triazole Conjugate Residues			
Pea, dried shelled	-22 to -24	220-520 days (7.2-17.1 months)	Not applicable at this time. Awaiting final report.
Bean, dried shelled		222-518 days 7.3-17.0 months)	

[1] Actual storage duration from collection to analysis; pea and bean samples in seven trials were collected 2-8 days after plants were cut and left in the field. All samples were analyzed within 3 days of extraction.

TABLE C.3. Residue Data from Crop Field Trials with Prothioconazole.

Trial ID (City, State; Year)	Region	Crop; Variety	Commodity or Matrix	Total Rate (lb a.i./A) [kg a.i./ha]	PHI (d)	Total Prothioconazole-Derived Residues (ppm)	1*H*-1,2,4-triazole Residues[1] (ppm)	Triazole Conjugate Residues [1] (ppm)
Pea, Dried Shelled								
Ephrata, WA; 2002 J6007-02H	11	Dry pea; Tonic	Shelled pea seed	0.543 [0.607]	7	0.120, 0.122	<0.01, <0.01	0.168, 0.166
Jerome, ID; 2002 J6008-02H	11	Dry pea; Talbot	Shelled pea seed	0.537 [0.601]	7	0.102, 0.118	<0.01, <0.01	0.314, 0.324
Madras, OR; 2002 J6009-02H	11	Dry pea; XP8504188	Shelled pea seed	0.549 [0.615]	7	<0.05, <0.05	<0.01, <0.01	0.084, 0.086
Hermiston, OR; 2002 J6010-02H	11	Dry pea; Majorettes	Shelled pea seed	0.533 [0.597]	7	<0.05, <0.05	<0.01, <0.01	0.400, 0.450
Hood River, OR 2002 J6011-02D	11	Dry pea; Sugar Snap	Shelled pea seed	0.544 [0.609]	0	0.288, 0.318	<0.01, <0.01	0.654, 0.675
					4	0.402, 0.426	<0.01, <0.01	0.836, 0.796
					7	0.292, 0.328	<0.01, <0.01	0.762, 0.789
					14	0.279, 0.285	<0.01, <0.01	0.788, 0.797
					21	0.310, 0.365	<0.01, <0.01	0.712, 0.718
Campbell, MN 2002 J6012-02H	5	Dry pea; Carnival	Shelled pea seed	0.539 [0.604]	7	<0.05, <0.05	<0.01, <0.01	0.162, 0.163
Branchton, ON; 2002 J6013-02H	5	Dry pea; Trapper	Shelled pea seed	0.541 [0.606]	7	<0.05, <0.05	<0.01, <0.01	0.131, 0.133
Kipp, AB; 2002 J6014-02H	14	Dry pea; Eiffel	Shelled pea seed	0.548 [0.613]	7	<0.05, 0.076	<0.01, <0.01	<0.05, <0.05
Rosthern, SK; 2002 J6015-02H	14	Dry pea; Keoma	Shelled pea seed	0.536 [0.602]	7	<0.05, <0.05	<0.01, <0.01	<0.05, <0.05
Brookdale, MB; 2002 J6016-02H	14	Dry pea; DS Admiral	Shelled pea seed	0.530 [0.595]	7	<0.05, <0.05	<0.01, 0.011	0.076, 0.072
Wakaw, SK; 2002 J6017-02H	14	Dry pea; Delta	Shelled pea seed	0.536 [0.601]	7	0.519, 0.655	<0.01, <0.01	<0.05, <0.05
Rosthern, SK; 2002 J6018-02H	14	Dry pea; Delta	Shelled pea seed	0.539 [0.604]	8	0.639, 0.684	<0.01, <0.01	<0.05, <0.05
Edmonton, AB; 2002 J6019-02D	14	Dry pea; Eiffel	Shelled pea seed	0.543 [0.608]	0	0.102, 0.118	<0.01, <0.01	0.063, 0.073
					3	0.062, 0.064	<0.01, <0.01	0.075, 0.061
					7	<0.05, 0.053	<0.01, <0.01	0.060, 0.055
					15	<0.05, 0.060	<0.01, <0.01	0.056, 0.066
					22	<0.05, 0.056	<0.01, <0.01	0.064, 0.064

TABLE C.3. Residue Data from Crop Field Trials with Prothioconazole.

Trial ID (City, State; Year)	Region	Crop; Variety	Commodity or Matrix	Total Rate (lb a.i./A) [kg a.i./ha]	PHI (d)	Total Prothioconazole-Derived Residues (ppm)	*1H*-1,2,4-triazole Residues[1] (ppm)	Triazole Conjugate Residues [1] (ppm)
Beans, Dried Shelled								
Lynden, ON; 2002 J6020-02H	5	Dry bean; OAC Thunder	Shelled bean seed	0.580 [0.650]	8	<0.05, <0.05	<0.01, <0.01	0.225, 0.273
Carlyle, IL; 2002 J6021-02H	5	Dry bean; Pinto	Shelled bean seed	0.547 [0.613]	7	<0.05, <0.05	<0.01, <0.01	0.110, 0.119
Oxford, IN;2002 J6022-02H	5	Dry bean; Sanilac	Shelled bean seed	0.543 [0.610]	8	<0.05, <0.05	<0.01, <0.01	0.089, 0.097
Stilwell, KS; 2002 J6023-02D	5	Dry bean; Pinto	Shelled bean seed	0.555 [0.622]	0	0.110, 0.163	<0.01, <0.01	0.025, <0.02
					7	0.053, 0.096	<0.01, <0.01	0.064, 0.053
					14	0.051, 0.091	<0.01, <0.01	0.097, 0.084
					21	<0.05, <0.05	<0.01, <0.01	0.126, 0.128
Eldridge, ND; 2002 J6024-02H	7	Dry bean; Remington	Shelled bean seed	0.539 [0.604]	7	<0.05, <0.05	<0.01, <0.01	<0.02, <0.02
Taber, AB; 2002 J6025-02H	7A	Dry bean; Pintos Othello	Shelled bean seed	0.534 [0.598]	7	0.115, 0.137	<0.01, <0.01	<0.02, <0.02
Plainview, TX; 2002 J6026-02H	8	Dry bean; Pinto Beans	Shelled bean seed	0.536 [0.600]	7	<0.05, <0.05	<0.01, <0.01	<0.02, <0.02
Fromberg, MT; 2002 J6027-02H	9	Dry bean; Othello	Shelled bean seed	0.544 [0.609]	7	0.199, 0.288	<0.01, <0.01	<0.02, <0.02
Fresno, CA; 2002 J6028-02H	10	Dry bean; Red Kidney	Shelled bean seed	0.538 [0.603]	7	<0.05, <0.05	<0.01, <0.01	0.126, 0.311
Ephrata, WA; 2002 J6029-02H	11	Dry bean; Othello Pinto	Shelled bean seed	0.540 [0.606]	7	<0.05, <0.05	<0.01, <0.01	<0.02, 0.036

[1] Residue values are listed respectively for 1*H*-1,2,4-triazole and the triazole conjugates.

TABLE C.4. Summary of Residue Data from Crop Field Trials with Prothioconazole.									
Commodity	Total Applic. Rate (lb a.i./A) [kg a.i./ha]	PHI (days)	Residue Levels (ppm)[1]						
			n	Min.	Max.	HAFT[2]	Median (STMdR[3])	Mean (STMR[4])	Std. Dev.
Total Prothioconazole-Derived Residues									
Pea, dried shelled	0.530-0.549 [0.595-0.615]	7-8	26	<0.05	0.684	0.661	0.025	0.156	0.219
Bean, dried shelled	0.534-0.580 [0.598-0.650]	7-8	20	<0.05	0.288	0.243	0.025	0.062	0.072
1*H*-1,2,4-triazole Residues									
Pea, dried shelled	0.530-0.549 [0.595-0.615]	7-8	26	<0.01	0.011	0.01	0.005	0.005	0.001
Bean, dried shelled	0.534-0.580 [0.598-0.650]	7-8	20	<0.01	<0.01	<0.01	0.005	0.005	0.0
Triazole Conjugate Residues									
Pea, dried shelled	0.530-0.549 [0.595-0.615]	7-8	26	<0.05	0.789	0.775	0.085	0.177	0.213
Bean, dried shelled	0.534-0.580 [0.598-0.650]	7-8	20	<0.02	0.311	0.249	0.045	0.080	0.093

[1] For the determination of minimum, maximum, and HAFT values, the LOQ was used for residues reported as below the LOQ in Table C.3. For the determination of the median, mean, and standard deviation values, ½LOQ was used for residues reported as below the LOQ.
[2] HAFT = Highest Average Field Trial.
[3] STMdR = Supervised Trial Median Residue.
[4] STMR = Supervised Trial Mean Residue.

D. CONCLUSION

The study use pattern was three foliar applications of the 4 lb/gal FlC formulation for a total seasonal rate of 0.53-0.58 lb a.i./A (0.60-0.65 kg a.i./ha) with a 9- to 15-day re-treatment interval. Dried shelled pea and bean were harvested 7 to 8 days after the last application. The maximum total prothioconazole-derived residues were 0.684 ppm (dried shelled peas), and 2.88 ppm (dried shelled beans). Residues of 1*H*-1,2,4-triazole were less than the LOQ (<0.01 ppm) in/on dried shelled beans, and the maximum residue in dried shelled peas was 0.011 ppm. Maximum residues of the triazole conjugates were 0.789 ppm, and 0.311 ppm in/on dried shelled peas and beans, respectively. Acceptable methods were used for quantitation of residues in/on dried shelled peas and beans.

E. REFERENCES

Gould T. and Timberlake B. (2005) Storage stability of JAU6476 and desthio JAU6476 in canola, wheat, mustard greens, turnip root, and tomato fruit, processed products, and solutions, Bayer CropScience Interim Report.

F. DOCUMENT TRACKING

RDI: Louise Croteau, Suzan Mathew (PMRA) 23/01/2006; Henri Bietlot (PMRA) 24/01/2006; Stephen Funk (IO)13/03/2006; Leung Cheng (RAB3).
Petition Number: PP#4F6830
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Template Version September 2003

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In the absence of signatures, this document is considered to be a draft with deliberative material for internal use only.

This DER was originally prepared under contract by Dynamac Corporation (20440 Century Boulevard, Suite 100; Germantown, MD 20874; submitted 05/20/2005). The DER has been reviewed by the Health Effects Division (HED) and Health Canada's Pest Management Regulatory Agency (PMRA). The DER has been revised to reflect current Office of Pesticide Programs (OPP) policies and Directive 98-02.

STUDY REPORT:

46246215 Lemke, V., Helfrich, K. (2004) JAU6476 480 SC - *Magnitude of the Residue In/On* Canola. Project Numbers: J619CN01, RCJAY006, 200464. Unpublished study prepared by Bayer CropScience and Battelle-AgriFood. 354 p.

EXECUTIVE SUMMARY:

Bayer CropScience has submitted field trial data on canola from field trials conducted in the U.S. and Canada. A total of 22 trials were conducted in Regions 2 (GA; 1 trial), 5 (ND; 1 trial, and ON; 1 trial), 7 (ND; 1 trial, and SK; 1 trial), 11 (ID; 3 trials), and 14 (AB; 4 trials, MB; 5 trials, and SK; 5 trials) during the 2000 growing season. The number and locations of field trials were in accordance with OPPTS Guideline 860.1500 and Directive 98-02; Section 9.

At each test location, two broadcast foliar applications of the 4 lb/gal (480 g/L) suspension concentrate formulation (flowable concentrate; FlC) were made to canola at 0.17-0.19 lb a.i./A (0.19-0.21 kg a.i./ha) at an average 16-day retreatment interval (7-44 days), for a total seasonal

application rate of 0.35-0.37 lb a.i./A (0.39-0.42 kg a.i./ha). Applications were made in ~11-42 gal/A (106-395 L/ha) of water using ground equipment. An adjuvant was not added to the spray mixture for any applications. Samples of canola were harvested at 20 test sites, 36-83 days after the last application. Two locations (Ashton, ID and Branchton, ON) were designated for residue decline studies. Samples were harvested 50, 54, 59, and 64 days after treatment in the decline trial performed in ID (region 11). In the ON trial (region 5), all samples were cut inadvertently on day 41. Seed samples from this site were collected on the day of harvest, and 5, 10, and 15 days after harvest.

Samples were analyzed for total prothioconazole-derived residues (prothioconazole and the metabolite prothioconazole-desthio) using LC-MS/MS method RPA JA/03/01. The validated LOQ for total prothioconazole-derived residues was 0.02 ppm for canola seed. Samples were analyzed for residues of 1*H*-1,2,4-triazole, and the triazole conjugates triazolylalanine and triazolylacetic acid using an LC-MS/MS method (Bayer Report No. G200598). The validated LOQs were 0.02 ppm for 1*H*-1,2,4-triazole and 0.025 ppm for the triazole conjugates for canola seed. The methods were adequate for data collection based on acceptable concurrent method recovery data.

The results from the canola field trials indicated that the maximum residues of prothioconazole in/on canola seed harvested 36-83 days following the last of two broadcast foliar applications were 0.097 ppm for total prothioconazole-derived residues, <0.02 ppm for 1*H*-1,2,4-triazole, and 0.848 ppm for the triazole conjugates.

In the residue decline trial conducted in ID, total prothioconazole-derived residues and residues of 1*H*-1,2,4-triazole were less than the LOQ (<0.02 ppm each) at all sampling intervals. Residues of the triazole conjugates did not increase with increasing sampling intervals.

The maximum storage interval of canola seed samples from harvest to analysis for total prothioconazole-derived residues was 1265 days (41.6 months). Prothioconazole-derived residues and prothioconazole-desthio residues are stable up to 12.7 months (interim report) in canola matrices. The degree of loss of prothioconazole-derived residues and prothioconazole-desthio residues is not expected to exceed 30% after 41.6 months.

STUDY/WAIVER ACCEPTABILITY/DEFICIENCIES/CLARIFICATIONS:

Under the conditions and parameters used in the study, the field trial residue data are tentatively classified as scientifically acceptable. The final reports of the ongoing storage stability studies with prothioconazole, prothioconazole-desthio, 1*H*-1,2,4-triazole, triazolylalanine, and triazolylacetic acid will be submitted as confirmatory data.

The acceptability of this study for regulatory purposes is addressed in the forthcoming U.S. EPA Residue Chemistry Summary Document, DP Barcode D303508, and in Canada's Regulatory Decision Document.

COMPLIANCE:

Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided. No deviations from regulatory requirements were reported which would have an impact on the validity of the study.

A. BACKGROUND INFORMATION

Prothioconazole is a broad-spectrum systemic fungicide intended for the control of many diseases in different crops such as cereals, oilseed rape or peanuts. The biochemical mode of action is the inhibition of the demethylation of lanosterol or 24-methylene-dihydrolanosterol, which are precursors of sterols in fungi. The chemical has been submitted for joint EPA and PMRA review by Bayer CropScience. The crops proposed for joint review include barley, the dried shell and bean subgroup, the oilseed crop group, and wheat. Uses on peanuts and rice are being proposed for the U.S. only.

TABLE A.1. Test Compound Nomenclature.

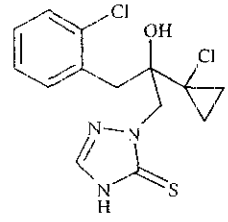
Chemical structure	
Common name	Prothioconazole (ISO accepted)
Company experimental name	JAU6476
IUPAC name	2-[(2*RS*)-2-(1-chlorocyclopropyl)-3-(2-chlorophenyl)-2-hydroxypropyl]-2*H*-1,2,4-triazole-3(4*H*)-thione
CAS name	2-[2-(1-chlorocyclopropyl)-3-(2-chlorophenyl)-2-hydroxypropyl]-1,2-dihydro-3*H*-1,2,4-triazole-3-thione
CAS registry number	178928-70-6
PC Code	113961
End-use product (EP)	Proline® 480 SC (4 lb/gal suspension concentrate), EPA File Symbol 264-IEL

TABLE A.2. Physicochemical Properties of the Technical Grade Test Compound.			
Parameter	Value		References
Melting range	139.1 to 144.5°C		MRID 46246003/CES
pH	5.8 (1% solution)		MRID 46246003/CES
Density at 20°C	1.36 g/mL		MRID 46246003/CES
Water solubility at 20°C	pH 4 8 9	mg/L 5 300 2000	MRID 46246003/CES
Solvent solubility at 20°C	Solvent Acetone Acetonitrile Dichloromethane Dimethysulfoxide Ethyl acetate n-Heptane 1-Octanol Polyethylene glycol 2-Propanol Xylene	g/L >250 69 88 126 >250 <0.1 58 >250 87 8	MRID 46246003/CES
Vapor pressure at 20 or 25°C	$<4 \times 10^{-7}$ Pa (calculated from determinations at 70°C)		MRID 46246003/CES
Dissociation constant, pK_a	6.9 (calculated from K_{OW})		MRID 46246003/CES
Octanol/water partition coefficient, at 20°C	pH unbuffered water 4 7 9	$Log(K_{OW})$ 4.05 4.16 3.82 2.00	MRID 46246003/CES
UV/visible absorption spectrum	Peak maxima at 257 nm. No absorption at > 300 nm.		MRID 46246003/CES

CES - Chemistry Evaluation Section of PMRA

B. EXPERIMENTAL DESIGN

B.1. Study Site Information

TABLE B.1.1. Trial Site Conditions

The table below was copied from the data report (MRID 46246215) without alteration.

			Soil Characteristics[a]				Meteorological data[b]	
Study location (City, State)	Trial Number	Year	Type	% OM*	pH*	CEC*	Total rainfall (in)	Temp. range (°F)
Bethany, Manitoba	J6014-00H	2000	NA	NA	NA	NA	7.5	37-93
Dundern, Saskatchewan	J6015-00H	2000	NA	NA	NA	NA	4.2	35-93
Brookdale, Manitoba	J6016-00H	2000	NA	NA	NA	NA	9.6	36-92
Wakaw, Saskatchewan	J6017-00H	2000	NA	NA	NA	NA	2.2	30-90
Hepburn, Saskatchewan	J6018-00H	2000	NA	NA	NA	NA	6.7	31-93
Rosthern, Saskatchewan	J6019-00H	2000	NA	NA	NA	NA	6.7	34-91
Blaine Lake, Saskatchewan	J6020-00H	2000	NA	NA	NA	NA	4.5	25-91
Lacombe, Alberta	J6021-00H	2000	NA	NA	NA	NA	6.3	32-83
Kemnay, Manitoba	J6022-00H	2000	NA	NA	NA	NA	9.2	36-92
Carberry, Manitoba	J6023-00HA	2000	NA	NA	NA	NA	5.2	12-92
Kipp, Alberta	J6024-00H	2000	NA	NA	NA	NA	3.7	34-99
Leduc, Alberta	J6025-00H	2000	NA	NA	NA	NA	5.2	30-84
Gwynne, Alberta	J6026-00H	2000	NA	NA	NA	NA	9.9	25-84
Glenboro, Manitoba	J6027-00H	2000	NA	NA	NA	NA	9.0	36-92
Cnula, Georgia	J6125-00H	2000	NA	NA	NA	NA	14.7	33-93
Northwood, North Dakota	J6126-00H	2000	NA	NA	NA	NA	9.5	47-92
New Rockford, North Dakota	J6127-00H	2000	NA	NA	NA	NA	8.9	45-96
Jerome, Idaho	J6128-00D	2000	NA	NA	NA	NA	0.1	37-104
Ashton, Idaho	J6129-00H	2000	NA	NA	NA	NA	2.3	27-98
Ashton, Idaho	J6130-00HA	2000	NA	NA	NA	NA	0.02	39-94
Branchton, Ontario	J6131-00D	2000	NA	NA	NA	NA	13.3	33-87
Melfort, Saskatchewan	J6132-00H	2000	NA	NA	NA	NA	3.6	31-91

* These parameters are optional except in cases where their value affects the use pattern for this chemical

[a] NA = Not applicable since these parameters do not affect the use pattern of the chemical.
[b] The data is for the interval from the first application to the last sampling unless noted otherwise
[c] The data is for the interval from the month of the first application through the month of the last sampling.

The actual temperature and rainfall recordings were within average historical values for the residue study period. Irrigation was used to supplement as needed at five trial sites (one each in AB and GA, and three in ID).

TABLE B.1.2. Study Use Pattern.

Location (City, State; Year)	EP[1]	Application				
		Method; Timing	Vol. (GPA[2]) [L/ha]	Rate (lb a.i./A) [kg a.i./ha]	RTI[3] (days)	Total Rate (lb a.i./A) [kg a.i./ha]
Bethany, MB; 2000 J6014-00H	480 SC	1: Broadcast foliar; Flower buds free, level with the youngest leaves	12 [108]	0.17 [0.198]	---	0.364 [0.407]
		2: Broadcast foliar; Full flowering, 50% of flowers on main raceme open	12 [114]	0.18 [0.209]	11	
Dundern, SK; 2000 J6015-00H	480 SC	1: Broadcast foliar; Flower buds raised above the youngest leaves	12 [111]	0.181 [0.202]	---	0.359 [0.401]
		2: Broadcast foliar; Full flowering, 50% of flowers on main raceme open	12 [109]	0.178 [0.199]	10	
Brookdale, MB; 2000 J6016-00H	480 SC	1: Broadcast foliar; Flower buds visible from above	12 [111]	0.181 [0.202]	---	0.361 [0.404]
		2: Broadcast foliar; Full flowering, 50% of flowers on main raceme open	12 [111]	0.181 [0.202]	9	
Wakaw, SK; 2000 J6017-00H	480 SC	1: Broadcast foliar; Flower buds raised above the youngest leaves	12 [110]	0.183 [0.204]	---	0.361 [0.404]
		2: Broadcast foliar; Full flowering, 50% of flowers on main raceme open	12 [109]	0.179 [0.200]	10	
Hepburn, SK; 2000 J6018-00H	480 SC	1: Broadcast foliar; Flower buds visible from above	42 [395]	0.180 [0.201]	---	0.353 [0.394]
		2: Broadcast foliar; Full flowering, 50% of flowers on main raceme open	41 [381]	0.173 [0.193]	10	
Rosthern, SK; 2000 J6019-00H	480 SC	1: Broadcast foliar; Flower buds visible from above	12 [108]	0.178 [0.199]	---	0.359 [0.401]
		2: Broadcast foliar; Full flowering, 50% of flowers on main raceme open	12 [111]	0.181 [0.202]	13	
Blaine Lake, SK; 2000 J6020-00H	480 SC	1: Broadcast foliar; Flower buds raised above the youngest leaves	12 [110]	0.181 [0.202]	---	0.364 [0.407]
		2: Broadcast foliar; Full flowering, 50% of flowers on main raceme open	12 [112]	0.183 [0.205]	8	
Lacombe, AB; 2000 J6021-00H	480 SC	1: Broadcast foliar; 30% of flowers on main raceme open	22 [194]	0.175 [0.196]	---	0.358 [0.400]
		2: Broadcast foliar; Full flowering, 50% of flowers on main raceme open	22 [202]	0.183 [0.204]	9	
Kemnay, MB; 2000 J6022-00H	480 SC	1: Broadcast foliar; Flower buds visible from above	12 [115]	0.189 [0.211]	---	0.373 [0.417]
		2: Broadcast foliar; Full flowering, 50% flowers on main raceme open	12 [112]	0.184 [0.206]	17	

TABLE B.1.2. Study Use Pattern.

Location (City, State; Year)	EP[1]	Application				
		Method; Timing	Vol. (GPA[2]) [L/ha]	Rate (lb a.i./A) [kg a.i./ha]	RTI[3] (days)	Total Rate (lb a.i./A) [kg a.i./ha]
Carberry, MB; 2000 J6023-00HA	480 SC	1: Broadcast foliar; Flower buds raised above the youngest leaves	11 [106]	0.173 [0.193]	---	0.354 [0.396]
		2: Broadcast foliar; Full flowering, 50% of flowers on main raceme open	12 [110]	0.182 [0.203]	11	
Kipp, AB; 2000 J6024-00H	480 SC	1: Broadcast foliar; Flower buds visible from above	21 [196]	0.176 [0.197]	---	0.353 [0.394]
		2: Broadcast foliar; Full flowering, 50% of flowers on main raceme open	21 [196]	0.176 [0.197]	14	
Leduc, AB; 2000 J6025-00H	480 SC	1: Broadcast foliar; Individual flower buds (main infl.) visible	12 [111]	0.182 [0.203]	---	0.361 [0.404]
		2: Broadcast foliar; Full flowering, 50% of flowers on main raceme open	12 [109]	0.180 [0.201]	7	
Gwyne, AB; 2000 J6026-00H	480 SC	1: Broadcast foliar; 2 leaves unfolded	12 [108]	0.176 [0.197]	---	0.354 [0.396]
		2: Broadcast foliar; Flowering declining	12 [108]	0.178 [0.199]	44	
Glenboro, MB; 2000 J6027-00H	480 SC	1: Broadcast foliar; Flower buds free, level with the youngest leaves	12 [109]	0.179 [0.200]	---	0.354 [0.396]
		2: Broadcast foliar; Full flowering, 50% of flowers on main raceme open	11 [107]	0.175 [0.196]	15	
Chula, GA; 2000 J6125-00H	480 SC	1: Broadcast foliar; Flower buds free, level with the youngest leaves	29 [270]	0.180 [0.202]	---	0.359 [0.402]
		2: Broadcast foliar; Full flowering, 50% of flowers on main raceme open	26 [248]	0.179 [0.200]	16	
Northwood, ND; 2000 J6126-00H	480 SC	1: Broadcast foliar; Flower buds free, level with the youngest leaves	32 [298]	0.191 [0.215]	---	0.372 [0.417]
		2: Broadcast foliar; Full flowering, 50% of flowers on main raceme open	30 [279]	0.181 [0.202]	19	
New Rockford, ND; 2000 J6127-00H	480 SC	1: Broadcast foliar; Flower buds visible from above	30 [278]	0.182 [0.205]	---	0.369 [0.415]
		2: Broadcast foliar; Full flowering, 50% of flowers on main raceme open	30 [279]	0.187 [0.210]	21	
Jerome, ID; 2000 J6128-00D	480 SC	1: Broadcast foliar; 4 leaves unfolded	30 [280]	0.179 [0.200]	---	0.359 [0.402]
		2: Broadcast foliar; Full flowering, 50% of flowers on main raceme open	28 [264]	0.180 [0.202]	33	

TABLE B.1.2. Study Use Pattern.

Location (City, State; Year)	EP[1]	Application				
		Method; Timing	Vol. (GPA[2]) [L/ha]	Rate (lb a.i./A) [kg a.i./ha]	RTI[3] (days)	Total Rate (lb a.i./A) [kg a.i./ha]
Ashton, ID; 2000 J6129-00H	480 SC	1: Broadcast foliar; 4 leaves unfolded	16 [152]	0.177 [0.198]	---	0.357 [0.400]
		2: Broadcast foliar; Full flowering, 50% of flowers on main raceme open	17 [163]	0.180 [0.202]	24	
Ashton, ID; 2000 J6130-00HA	480 SC	1: Broadcast foliar; Flower buds free, level with the youngest leaves	18 [170]	0.173 [0.195]	---	0.356 [0.400]
		2: Broadcast foliar; Full flowering, 50% of flowers on main raceme open	19 [175]	0.183 [0.205]	17	
Branchton, ON; 2000 J6131-00D	480 SC	1: Broadcast foliar; 3 leaves unfolded	21 [201]	0.181 [0.202]	---	0.367 [0.410]
		2: Broadcast foliar; Full flowering, 50% of flowers on main raceme open	22 [204]	0.186 [0.208]	27	
Melfort, SK; 2000 J6132-00H	480 SC	1: Broadcast foliar; Flower buds raised above the youngest leaves	12 [110]	0.179 [0.200]	---	0.361 [0.403]
		2: Broadcast foliar; Full flowering, 50% of flowers on main raceme open	12 [111]	0.182 [0.203]	7	

[1] EP = End-use Product; 480 SC = 4 lb/gal (480 g/L) suspension concentrate (flowable concentrate; FlC) formulation
[2] GPA = Gallons per acre
[3] RTI = Retreatment Interval
No tank mix adjuvants were used.

TABLE B.1.3. Trial Numbers and Geographical Locations.

NAFTA Growing Region	Canola		
	Submitted	Requested	
		Canada	US
1			
1A			
2	1		1
3			
4			
5	2	1	2
5A			
5B			
6			
7	2	1	2
7A			
8			
9			
10			
11	3		3
12			
13			
14	14	14	
15			
16			
17			
18			
19			
20			
21			
Total	22	16	8

B.2. Sample Handling and Preparation

Samples of canola seed were collected 36-83 days (average of 56 days) after the last application. In two of the Canadian field trials, samples were cut and allowed to dry in the field for either 14 or 20 days prior to collection. The applicant noted that in Canada, canola is cut and allowed to dry in the field prior to harvesting seed as part of the normal agricultural practice. Samples were bagged and stored frozen within 4 hours of harvest. The samples were shipped frozen to Battelle-AgriFood Laboratories (Columbus, OH) for homogenization. At Battelle, the samples were homogenized with dry ice and stored frozen until shipment to Bayer Research Park (Stilwell, KS) for analysis.

B.3. Analytical Methodology

Samples of canola seed were analyzed for total prothioconazole-derived residues (prothioconazole and the metabolite prothioconazole-desthio) using LC-MS/MS method RPA JA/03/01 with modifications. Samples were extracted with a mixture of methanol, 30% hydrogen peroxide, and 0.05M aqueous sodium bicarbonate at 65°C for 2 hours. The oxidative extraction procedure converted prothioconazole residues to a mixture of prothioconazole-desthio and prothioconazole sulfonic acid. Residues of prothioconazole-desthio in the crop matrix were not changed by the extraction procedures. The cooled extract was spiked 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. Residue levels were quantitated from the daughter ion transitions of the 2 analytes and their respective internal standard analogs. The method was modified to use a different solvent for preparation of the spiking solutions, and to use slightly different m/z values for the quantitation ions. The validated LOQ for total prothioconazole-derived residues was 0.02 ppm.

Samples were analyzed for residues of 1*H*-1,2,4-triazole and the triazole conjugates (triazolylalanine and triazolylacetic acid) using an LC-MS/MS method (Bayer Report No. G200598) with modifications. It was noted that the applicant referred to the method as a "modified" method, but did not identify any modifications that had been made to the method. Briefly, crop matrices were extracted with aqueous methanol, and three separate aliquots of the extract were removed for determination of each of the three analytes. Isotopically labeled internal standard was added to each aliquot. For 1*H*-1,2,4-triazole, the aliquot was mixed with dansyl chloride to form the dansyl derivative of 1*H*-1,2,4-triazole, which was partitioned into ethyl acetate and then redissolved in acetonitrile (ACN)/water for LC-MS/MS analysis. For triazolylalanine, the aliquot was cleaned up by SPE, derivatized to the butyl ester using butanolic HCl, and then further derivatized using heptafluorobutyric anhydride (HFBA). The mixture was redissolved in ACN/water for LC-MS/MS analysis. For determination of triazolylacetic acid, the aliquot was cleaned up by SPE, derivatized to the butyl ester using butanolic HCl, and then redissolved in ACN/water for LC-MS/MS analysis. Residue levels were quantitated from the daughter ion transitions of the derivatives and their respective internal standard derivative analogs. The validated LOQs were 0.02 ppm for 1*H*-1,2,4-triazole and 0.025 ppm for the triazole conjugates.

C. RESULTS AND DISCUSSION

Concurrent method recovery data are presented in TABLE C.1. Samples of canola seed were analyzed for total prothioconazole-derived residues (prothioconazole and the metabolite prothioconazole-desthio) using LC-MS/MS method RPA JA/03/01 with modifications. The validated LOQ was 0.02 ppm for total prothioconazole-derived residues. Samples were analyzed for residues of 1*H*-1,2,4-triazole and the triazole conjugates (triazolylalanine and triazolylacetic acid) using an LC-MS/MS method (Bayer Report No. G200598) with modifications. The validated LOQs were 0.02 ppm for 1*H*-1,2,4-triazole, and 0.025 ppm for the triazole conjugates.

The methods were adequate for data collection based on acceptable concurrent method recovery data.

Apparent total prothioconazole-derived residues and residues of 1*H*-1,2,4-triazole were below the method LOQ (<0.02 ppm each) in/on all samples of untreated canola seed. Apparent residues of triazole conjugates were below the method LOQ (<0.025 ppm) in/on 11 samples of untreated canola seed; quantifiable apparent residues were observed in 22 samples of untreated seed (residue range of 0.027-1.06 ppm). The measurable control residues of the triazole conjugates may have resulted from the use of triazole fungicides from previous seasons or from naturally occurring triazole conjugates.

Sample storage conditions and intervals are summarized in TABLE C.2. The maximum storage interval of canola seed from harvest to analysis was 1265 days (41.6 months). Based on an interim report, residues of prothioconazole-desthio and prothioconazole-derived residues are stable for up to 12.7 months. The degree of loss of prothioconazole-desthio residues and prothioconazole-derived residues is not expected to exceed 30% after 41.6 months. Therefore, correction for dissipation of prothioconazole-derived residues during freezer storage will not be necessary.

The maximum storage interval of canola seed from harvest to analysis for 1*H*-1,2,4-triazole and triazole conjugate residues was 1131 days (37.2 months).

Residue data from the canola field trials are reported in TABLE C.3. A summary of prothioconazole residue data for canola seed is presented in TABLE C.4. Residues in/on canola harvested 36-83 days following the last of two broadcast foliar applications were <0.02-0.097 ppm for total prothioconazole-derived residues, less than the LOQ (<0.02 ppm) for 1*H*-1,2,4-triazole, and 0.064-0.848 ppm for the triazole conjugates.

In the residue decline trial conducted in ID, total prothioconazole-derived residues and residues of 1*H*-1,2,4-triazole were less than the LOQ (<0.02 ppm each) at all sampling intervals. Residues of the triazole conjugates remained stable at approximately 0.5-0.8 ppm, and so did not increase with increasing sampling intervals. The number of residue decline trials is in accordance with OPPTS Guideline 860.1500(e)(2)(vi)(A), and Directive 98-02; Section 9.

TABLE C.1. Summary of Concurrent Recoveries of Prothioconazole from Canola.

Matrix	Analyte	Spike level (ppm)	Sample size (n)	Recoveries (%)	Mean ± std dev
Seed	Prothioconazole	0.02	3	77, 81, 84	81 ± 3.5
	Prothioconazole-desthio	0.02	3	90, 90, 101	94 ± 6.4
	1*H*-1,2,4-triazole	0.1	7	71, 73, 77, 77, 83, 83, 86	79 ± 5.6
	Triazolylalanine	0.05	2	71, 92	82
		0.1	8	72, 74, 81, 84, 89, 95, 96, 102	87 ± 10.8
		1.5	3	79, 80, 81	80 ± 1.0
	Triazolylacetic acid	0.02	2	78, 81	80
		0.1	7	80, 84, 85, 86, 89, 90, 91	86 ± 3.9
		0.2	3	91, 91, 92	91 ± 0.6

TABLE C.2. Summary of Storage Conditions.

Matrix (RAC or Extract)	Storage Temp. (°C)	Actual Storage Duration[1]	Interval of Demonstrated Storage Stability
Total Prothioconazole-Derived Residues			
Canola, seed	-4.8 to -30.0	867-1265 days (28.5-41.6 months)	Stability demonstrated for up to 12.7 months (interim report). Awaiting final report as confirmatory data.
1*H*-1,2,4-triazole and Triazole Conjugate Residues			
Canola, seed	-4.8 to -30.0	730-1131 days (24.0-37.2 months)	Not applicable at this time. Awaiting final report.

[1] Actual storage duration from collection to analysis. All samples were analyzed within 4 days of extraction.

TABLE C.3. Residue Data from Crop Field Trials with Prothioconazole

Trial ID (City, State; Year)	Region	Canola Variety	Commodity or Matrix	Total Rate (lb a.i./A) [kg a.i./ha]	PHI (days) [1]	Total Prothioconazole-Derived Residues (ppm)	1*H*-1,2,4-triazole Residues[2] (ppm)	Triazole Conjugate Residues[2] (ppm)
Bethany, MB; 2000 J6014-00H	14	Quest	Seed	0.364 [0.407]	56	<0.02, <0.02	<0.02, <0.02	0.143, 0.150
Dundern, SK; 2000 J6015-00H	14	Exceed	Seed	0.359 [0.401]	54	<0.02, <0.02	<0.02, <0.02	0.194, 0.216
Brookdale, MB; 2000 J6016-00H	14	45A51	Seed	0.361 [0.404]	55	<0.02, <0.02	<0.02, <0.02	0.164, 0.189
Wakaw, SK; 2000 J6017-00H	14	45A71	Seed	0.361 [0.404]	59	<0.02, <0.02	<0.02, <0.02	0.149, 0.166
Hepburn, SK; 2000 J6018-00H	14	SW Legion	Seed	0.353 [0.394]	61	<0.02, <0.02	<0.02, <0.02	0.176, 0.184
Rosthern, SK; 2000 J6019-00H	14	46A76	Seed	0.359 [0.401]	63	<0.02, <0.02	<0.02, <0.02	0.307, 0.317
Blaine Lake, SK; 2000 J6020-00H	14	Quest	Seed	0.364 [0.407]	69	<0.02, <0.02	<0.02, <0.02	0.378, 0.380

TABLE C.3. Residue Data from Crop Field Trials with Prothioconazole

Trial ID (City, State; Year)	Region	Canola Variety	Commodity or Matrix	Total Rate (lb a.i./A) [kg a.i./ha]	PHI (days) [1]	Total Prothioconazole-Derived Residues (ppm)	1*H*-1,2,4-triazole Residues[2] (ppm)	Triazole Conjugate Residues[2] (ppm)
Lacombe, AB; 2000 J6021-00H	14	LG3235	Seed	0.358 [0.400]	48 (20)	<0.02, <0.02	<0.02, <0.02	0.306, 0.373
Kemnay, MB; 2000 J6022-00H	14	*Quest*	Seed	0.373 [0.417]	56	<0.02, <0.02	<0.02, <0.02	0.500, 0.501
Carberry, MB; 2000 J6023-00HA	14	2273 Invigor	Seed	0.354 [0.396]	71	<0.02, 0.029	<0.02, <0.02	0.275, 0.280
Kipp, AB; 2000 J6024-00H	14	Q2	Seed	0.353 [0.394]	36 (14)	0.022, 0.045	<0.02, <0.02	0.140, 0.133
Leduc, AB; 2000 J6025-00H	14	Agassiz	Seed	0.361 [0.404]	83	<0.02, <0.02	<0.02, <0.02	0.339, 0.388
Gwyne, AB; 2000 J6026-00H	14	Agassiz	Seed	0.354 [0.396]	73	<0.02, <0.02	<0.02, <0.02	0.475, 0.531
Glenboro, MB; 2000 J6027-00H	14	46A65	Seed	0.354 [0.396]	57	<0.02, <0.02	<0.02, <0.02	0.423, 0.452
Chula, GA; 2000 J6125-00H	2	Flint	Seed	0.359 [0.402]	78	<0.02, <0.02	<0.02, <0.02	0.624, 0.689
Northwood, ND; 2000 J6126-00H	5	Quantum	Seed	0.372 [0.417]	43	<0.02, <0.02	<0.02, <0.02	0.088, 0.108
New Rockford, ND; 2000 J6127-00H	7	Quantum	Seed	0.369 [0.415]	36	<0.02, <0.02	<0.02, <0.02	0.363, 0.427
Jerome, ID; 2000 J6128-00D	11	Phoenix	Seed	0.359 [0.402]	50	<0.02, <0.02	<0.02, <0.02	0.543, 0.547
					54	<0.02, <0.02	<0.02, <0.02	0.514, 0.561
					59	<0.02, <0.02	<0.02, <0.02	0.454, 0.463
					64	<0.02, <0.02	<0.02, <0.02	0.587, 0.588
Ashton, ID; 2000 J6129-00H	11	RaideRR	Seed	0.357 [0.400]	55	<0.02, <0.02	<0.02, <0.02	0.127, 0.130
Ashton, ID; 2000 J6130-00H	11	Chinook	Seed	0.356 [0.400]	37	0.074, 0.097	<0.02, <0.02	0.080, 0.064
Branchton, ON; 2000 J6131-00D	5	Invigor 2473 LL	Seed	0.367 [0.410]	41	<0.02, <0.02	<0.02, <0.02	0.583, 0.848
					41 (46)	<0.02, <0.02	<0.02, <0.02	0.556, 0.601
					41 (51)	<0.02, <0.02	<0.02, <0.02	0.651, 0.668
					41 (56)	<0.02, <0.02	<0.02, <0.02	0.598, 0.796
Melfort, SK; 2000 J6132-00H	7	46A74	Seed	0.361 [0.403]	58	<0.02, <0.02	<0.02, <0.02	0.314, 0.362

[1] *In selected field trials, canola was cut and left in the field to dry; days left in the field prior to collection are reported in* parentheses.

[2] Residue values are listed respectively for 1*H*-1,2,4-triazole and the triazole conjugates.

TABLE C.4. Summary of Residue Data from Crop Field Trials with Prothioconazole.									
Commodity	Total Applic. Rate (lb a.i./A) [kg a.i./ha]	PHI (days)	Residue Levels (ppm)[1]						
			n	Min.	Max.	HAFT[2]	Median (STMdR[3])	Mean (STMR[4])	Std. Dev.
Total Prothioconazole-Derived Residues									
Canola, seed	0.35-0.37 [0.39-0.42]	36-83	44	<0.020	0.097	0.086	0.010	0.015	0.0169
1*H*-1,2,4-triazole Residues									
Canola, seed	0.35-0.37 [0.39-0.42]	36-83	44	<0.020	<0.020	<0.020	0.010	0.010	0
Triazole Conjugate Residues									
Canola, seed	0.35-0.37 [0.39-0.42]	36-83	44	0.064	0.848	0.716	0.311	0.321	0.184

[1] For the determination of minimum, maximum, and HAFT values, the LOQ was used for residues reported as below the LOQ in TABLE C.3. For the determination of the median, mean, and standard deviation values, ½LOQ was used for residues reported as below the LOQ. Residue values from the appropriate harvest intervals from the residue decline trial (50-day PHI only, ID trial) were included in the summary table.
[2] HAFT = Highest Average Field Trial.
[3] STMdR = Supervised Trial Median Residue.
[4] STMR = Supervised Trial Mean Residue.

D. CONCLUSION

The study use pattern was 2 foliar applications of the 4 lb/gal (480 g/L) suspension concentrate formulation at a total seasonal rate of 0.35-0.37 lb a.i./A (0.39-0.42 kg a.i./ha) with an average 16 day retreatment interval. Canola seeds were harvested approximately 56 days after the last application. Maximum total prothioconazole-derived residues were 0.097 ppm. All residues of 1*H*-1,2,4-triazole were <LOQ (<0.02 ppm). Maximum residues of the triazole conjugates were 0.85 ppm. Acceptable methods were used for quantitation of residues in/on canola seed.

E. REFERENCES

Gould T. and Timberlake B. (2005) Storage stability of JAU6476 and desthio JAU6476 in canola, wheat, mustard greens, turnip root, and tomato fruit, processed products, and solutions, Bayer CropScience Interim Report.

F. DOCUMENT TRACKING

RDI: Date: Suzan Mathew (PMRA) xx/xx/2006; Henri Bietlot (PMRA) xx/xx/2006; Stephen Funk (IO)13/03/2006; Leung Cheng (RAB3).
Petition Number: PP#4F6830
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Template Version September 2003

Primary Evaluators	Stephen Funk, Ph.D., Chemist Immediate Office	Date: 13/03/2006
	Suzan Mathew, Evaluation Officer FREAS, HED	Date:23/01/2006
Approved by	Leung Cheng, Ph. D., Team Leader HED/RAB3	Date:
	Henri P. Bietlot, Acting Section Head FREAS, HED	Date:24/01/2006

In the absence of signatures, this document is considered to be a draft with deliberative material for internal use only.

This DER was originally prepared under contract by Dynamac Corporation (20440 Century Boulevard, Suite 100; Germantown, MD 20874; submitted 04/28/2005). The DER has been reviewed by the Health Effects Division (HED) and Health Canada's Pest Management Regulatory Agency (PMRA). The DER has been revised to reflect current Office of Pesticide Programs (OPP) policies and Directive 98-02.

STUDY REPORTS:

46246204 Moore, S.; Harbin, A. (2004) 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 (Version 2). Project Numbers: 200537, JA006-A04-02. Unpublished study prepared by Bayer CropScience. 131 p.

46246205 Justus, K.; Spiegel, K. (2004) (Triazole-UL-(^{14}C)) JAU6476: Extraction Efficiency of the Residue Analytical Method for the Determination of JAU6476, JAU6476-4-hydroxy and JAU 6476-desthio in Animal Tissues and Milk Using Aged Residues. Project Numbers: M9991256-1, MEF-020/04. Unpublished study prepared by Bayer CropScience AG. 58 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 Numbers: RAJAY004, Bayer-1517. Unpublished study prepared by Pyxant Labs Inc. 178 p.

Bayer CropScience has proposed a high performance liquid chromatography (with electrospray ionization) and tandem mass spectrometry (LC-MS/MS) method for the data gathering and enforcement of maximum residue limits for residues of prothioconazole, prothioconazole-desthio (JAU6476-desthio), prothioconazole-4-hydroxy (JAU6476-4-hydroxy), and conjugates that may be converted to these compounds by acid hydrolysis, in milk and cattle tissues.

Briefly, samples of bovine liver, kidney, and muscle are extracted with acetonitrile (ACN)/water and 25% aqueous L-cysteine HCl. An internal standard solution is added to the extract. The internal standard solution consists of a mixture of [triazole-$^{15}N_3$-$^{13}C_2$]-prothioconazole, [triazole-$^{15}N_3$-$^{13}C_2$]-prothioconazole-desthio, and [triazole-$^{15}N_3$-$^{13}C_2$]-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 directly. 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 range from 0.0007-0.0021 ppm for milk, 0.001-0.0019 ppm for skim milk, 0.0021-0.0035 ppm for cream, 0.0006-0.001 ppm for muscle, 0.0005-0.0029 ppm for liver, 0.0021-0.0025 ppm for kidney, and 0.0041-0.0115 ppm for fat.

Method validation data (and concurrent recovery data from the livestock feeding study) for the proposed enforcement method has demonstrated adequate method recoveries of prothioconazole, prothioconazole-desthio, and prothioconazole-4-hydroxy at 0.005 ppm (LOQ) and 0.010 ppm for milk; 0.010 ppm (LOQ) for skim milk, cream, and muscle; 0.010 ppm (LOQ) and 0.60 ppm for liver; 0.010 ppm (LOQ), 0.050 ppm, and 0.80 ppm for kidney; and 0.050 ppm (LOQ) and 0.080 ppm for fat. The range of recoveries (and CVs) are 77-115% (9.1%) for prothioconazole, 91-117% (6.0%) for prothioconazole-desthio, and 63-117% (14.7%) for prothioconazole-4-hydroxy over all matrices and spiking levels. The spiking levels and samples used in method validation and concurrent method recovery are adequate to bracket expected residue levels in milk and livestock tissues for residues of prothioconazole, prothioconazole-desthio, prothioconazole-4-hydroxy, and conjugates that may be converted to these compounds by acid hydrolysis.

Adequate extraction efficiency data have been submitted for the method using samples of goat milk, muscle, liver, and fat. Adequate independent laboratory validation data have been submitted for the method using samples of cattle milk and liver. Confirmatory analysis procedures have not been conducted for the proposed enforcement method due to the high specificity of the LC-MS/MS method.

STUDY/WAIVER ACCEPTABILITY/DEFICIENCIES/CLARIFICATIONS:

Under the conditions and parameters used in the study, the analytical method residue data are classified as scientifically acceptable. It is noted that an amendment has been provided to clarify that for all matrices internal standards are added to all samples after the extraction step, and not just the recovery samples. The proposed enforcement method will be forwarded to ACL for petition method validation, and to determine whether confirmatory analysis procedures are needed for the method.

The acceptability of this study for regulatory purposes is addressed in the forthcoming U.S. EPA Residue Chemistry Summary Document, [DP Barcode D303508], and in Canada's Regulatory Decision Document.

COMPLIANCE:

Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided. No deviations from regulatory requirements were reported which would have an impact on the validity of the study.

A. BACKGROUND INFORMATION

Prothioconazole is a broad-spectrum systemic fungicide intended for the control of many diseases in different crops such as cereals, oilseed rape or peanuts. The biochemical mode of action is the inhibition of the demethylation of lanosterol or 24-methylene-dihydrolanosterol, which are precursors of sterols in fungi. The chemical has been submitted for joint EPA and PMRA review by Bayer CropScience. The crops proposed for joint review include barley, the dried shell and bean subgroup, the oilseed crop group, and wheat. Uses on peanuts and rice are being proposed for the U.S. only.

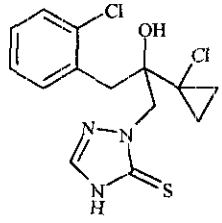
TABLE A.1. Test Compound Nomenclature.	
Chemical structure	
Common name	Prothioconazole (ISO accepted)
Company experimental name	JAU6476
IUPAC name	2-[(2*RS*)-2-(1-chlorocyclopropyl)-3-(2-chlorophenyl)-2-hydroxypropyl]-2*H*-1,2,4-triazole-3(4*H*)-thione
CAS name	2-[2-(1-chlorocyclopropyl)-3-(2-chlorophenyl)-2-hydroxypropyl]-1,2-dihydro-3*H*-1,2,4-triazole-3-thione
CAS registry number	178928-70-6
PC Code	113961
End-use product (EP)	Proline® 480 SC (4 lb/gal suspension concentrate), EPA File Symbol 264-IEL

TABLE A.2. Physicochemical Properties of the Technical Grade Test Compound.			
Parameter	Value		References
Melting range	139.1 to 144.5°C		MRID 46246003/CES
pH	5.8 (1% solution)		MRID 46246003/CES
Density at 20°C	1.36 g/mL		MRID 46246003/CES
Water solubility at 20°C	pH 4 8 9	mg/L 5 300 2000	MRID 46246003/CES
Solvent solubility at 20°C	Solvent Acetone Acetonitrile Dichloromethane Dimethysulfoxide Ethyl acetate n-Heptane 1-Octanol Polyethylene glycol 2-Propanol Xylene	g/L >250 69 88 126 >250 <0.1 58 >250 87 8	MRID 46246003/CES
Vapor pressure at 20 or 25°C	$<4 \times 10^{-7}$ Pa (calculated from determinations at 70°C)		MRID 46246003/CES
Dissociation constant, pK_a	6.9 (calculated from K_{OW})		MRID 46246003/CES
Octanol/water partition coefficient, at 20°C	pH unbuffered water 4 7 9	$Log(K_{OW})$ 4.05 4.16 3.82 2.00	MRID 46246003/CES
UV/visible absorption spectrum	Peak maxima at 257 nm. No absorption at > 300 nm.		MRID 46246003/CES

CES - Chemistry Evaluation Section of PMRA

B. MATERIALS AND METHODS

B.1. Data-Gathering Method

B.1.1. Principle of the Method:

Samples are first homogenized in a commercial food processor with dry ice. Bovine kidney, liver and muscle samples are extracted with acetonitrile (ACN) and water containing 250 mg/mL L-cysteine HCl (25% aqueous L-cysteine HCl). An internal standard solution (consisting of a mixture of [triazole-$^{15}N_3$-$^{13}C_2$]-prothioconazole, [triazole-$^{15}N_3$-$^{13}C_2$]-prothioconazole-desthio, and [triazole-$^{15}N_3$-$^{13}C_2$]-prothioconazole-4-hydroxy in ACN containing 50 µg/mL L-cysteine HCl) is then added to the extract. Fat samples are first extracted with n-hexane; then the filter cake is re-extracted with a mixture of ACN, 25% aqueous L-cysteine HCl, and acetone. These extracts are combined, and then allowed to separate before the internal standard solution is added to the aqueous phase. Samples of milk and cream are mixed with internal standard solution directly. All extracts are hydrolyzed using aqueous HCl. The resulting hydrolysate is

then partitioned with methylene chloride and acetone. The organic phase is concentrated, then combined with ACN and water before analysis by LC-MS/MS. For all matrices, the internal standard is added to all samples after the extraction step, not just the recovery samples. The method can analyse 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 range from 0.0007-0.0021 ppm for milk, 0.001-0.0019 ppm for skim milk, 0.0021-0.0035 ppm for cream, 0.0006-0.001 ppm for muscle, 0.0005-0.0029 ppm for liver, 0.0021-0.0025 ppm for kidney, and 0.0041-0.0115 ppm for fat. The LOQs and LODs are reported in TABLE C.1.2.

TABLE B.1.1. Summary Parameters for the Analytical Method Used for the Quantitation of Prothioconazole Residues in Bovine Matrices.

Method ID	None (Bayer Report No. 200537)
Analytes	Prothioconazole, prothioconazole-desthio, prothioconazole-4-hydroxy, plus any conjugate convertible to these compounds by acid hydrolysis
Extraction solvent/technique	Samples are homogenized in the presence of dry ice. For liver, kidney, and muscle samples, the sample is extracted twice with ACN:25% aqueous L-cysteine HCl (4:1, v:v), and the extract is isolated by filtration after the addition of Celite. A second extract is conducted, then combined with the first. The extract is diluted with ACN/25% aqueous L-cysteine HCl, and internal standard solution is added to all samples. Internal standard solution consists of a mixture of [triazole-$^{15}N_3$-$^{13}C_2$]-prothioconazole, [triazole-$^{15}N_3$-$^{13}C_2$]-prothioconazole-desthio, and [triazole-$^{15}N_3$-$^{13}C_2$]-prothioconazole-4-hydroxy in ACN containing 50 µg/mL L-cysteine HCl. The extract is concentrated to aqueous, mixed with 5N HCl and L-cysteine HCl, and hydrolyzed under reflux for 2 hours. The hydrolysate is diluted with water and partitioned twice with methylene chloride:acetone (3:2, v:v). The organic phase is mixed with 0.01% aqueous L-cysteine HCl, concentrated to aqueous, and mixed with ACN and water. For fat samples, the sample is extracted with n-hexane and then with a mixture of ACN:25% aqueous L-cysteine HCl (4:1, v:v) and acetone. The filter cake is re-extracted, then the extracts are combined and allowed to separate; the hexane phase is isolated and partitioned with ACN:25% aqueous L-cysteine HCl (4:1, v:v) and acetone. All aqueous phases are combined, internal standard solution is added to all samples, and the mixture is diluted with ACN:25% aqueous L-cysteine HCl (4:1, v:v). The extract is concentrated to aqueous, mixed with 5N HCl and L-cysteine HCl, and hydrolyzed under reflux for 2 hours. The hydrolysate is diluted with water and partitioned twice with methylene chloride:acetone (3:2, v:v). The organic phase is mixed with 0.01% aqueous L-cysteine HCl, concentrated to aqueous, and mixed with ACN and water. For milk, skim milk, and cream samples, internal standard solution is added directly to all samples, along with water, 5N HCl, and L-cysteine HCl. The mixture is hydrolyzed under reflux for 2 hours. The hydrolysate is diluted with water and partitioned twice with methylene chloride:acetone (3:2, v:v). The organic phase is mixed with 0.01% aqueous L-cysteine HCl, concentrated to aqueous, and mixed with ACN and water.
Cleanup strategies	The extracts are filtered through a Whatman GF/F filter prior to analysis.

TABLE B.1.1. Summary Parameters for the Analytical Method Used for the Quantitation of Prothioconazole Residues in Bovine Matrices.	
Instrument/Detector	HPLC utilizing a reverse-phase column and a gradient mobile phase of water and ACN, each containing 0.01% acetic acid, with tandem mass spectrometry (MS/MS) detection using electrospray ionization operating in the positive ion mode for prothioconazole-desthio, and negative ion mode for prothioconazole and prothioconazole-4-hydroxy. The ion transitions monitored are: Prothioconazole: 342 → 100 Prothioconazole internal standard: 347 → 105 Prothioconazole-desthio: 312 → 70 Prothioconazole-desthio internal standard: 317 → 75 Prothioconazole-4-hydroxy: 358 → 100 Prothioconazole-4-hydroxy internal standard: 363 → 105
Standardization method	External and internal standardization. Calibration standards, containing internal standards, are injected, and a calibration curve is constructed for each analyte of response factor (analyte peak area divided by internal standard peak area) versus concentration, using 1/x weighting. Response factors for samples are calculated, and residue levels are then determined from the calibration curves. The concentration of all calibration and spiking standards is calculated in prothioconazole equivalents. Therefore, results for prothioconazole-desthio and prothioconazole-4-hydroxy are reported in prothioconazole equivalents.
Stability of std solutions	Not addressed in the submission.
Retention times	Prothioconazole: ~7.9 minutes Prothioconazole-desthio: ~6.6 minutes Prothioconazole-4-hydroxy: ~5.1 minutes

B.2. Enforcement Method

The proposed enforcement method for livestock commodities is the same as the data-gathering method.

C. RESULTS AND DISCUSSION

C.1. Data-Gathering Method

The recovery data presented in TABLE C.1.1 below are included in the method submission. However, these data are the concurrent method recovery data conducted for the validation of the method with the cattle feeding study.

TABLE C.1.1. Recovery Results from Method Validation of Various Cattle Matrices using the Data-Gathering Analytical Method.[1]

Matrix	Analyte	Spiking Level (ppm)	Recoveries Obtained (%)	Recovery (%)		
				Mean	SD	CV
Milk	Prothioconazole	0.0050	101, 102, 103, 103, 104, 104, 104, 104, 104, 106, 106, 106,107, 111, 115	105	4	3
		0.010	99, 101, 104	102	3	2
	Prothioconazole-desthio	0.0050	91, 100, 101, 101, 101, 104, 104, 105, 107, 109, 109, 110, 110, 110, 116	105	6	6

TABLE C.1.1. Recovery Results from Method Validation of Various Cattle Matrices using the Data-Gathering Analytical Method.[1]

Matrix	Analyte	Spiking Level (ppm)	Recoveries Obtained (%)	Recovery (%)		
				Mean	SD	CV
		0.010	102, 106, 108	105	3	3
	Prothioconazole-4-hydroxy [2]	0.0050	69, 72, 76, 80, 80, 85, 87, 95, 99, 102, 105, 111, 113, 116, 117	94	17	18
		0.010	71, 77, 93	80	11	14
Skim milk	Prothioconazole	0.010	100, 103, 103	102	2	2
	Prothioconazole-desthio	0.010	97, 100, 100	99	2	2
	Prothioconazole-4-hydroxy [2]	0.010	83, 86, 89	86	3	3
Cream	Prothioconazole	0.010	98, 103, 104	102	3	3
	Prothioconazole-desthio	0.010	103, 106, 112	107	5	4
	Prothioconazole-4-hydroxy [2]	0.010	63, 65, 72	67	5	7
Muscle	Prothioconazole	0.010	91, 91, 92, 92	92	1	1
	Prothioconazole-desthio	0.010	100, 103, 104, 106	103	3	2
	Prothioconazole-4-hydroxy	0.010	98, 99, 99, 101	99	1	1
Liver	Prothioconazole	0.010	96, 98, 99, 101	99	2	2
		0.60	89, 91, 92	91	2	2
	Prothioconazole-desthio	0.010	105, 115, 116, 117	113	6	5
		0.60	104, 109, 113	109	5	4
	Prothioconazole-4-hydroxy	0.010	101, 102, 104, 104	103	2	1
		0.60	93, 95, 99	96	3	3
Kidney	Prothioconazole	0.010	77, 78, 84	80	4	5
		0.050	96, 101, 106, 109	103	6	6
		0.80	81, 83, 87	84	3	4
	Prothioconazole-desthio	0.010	106, 111, 112	110	3	3
		0.050	92, 101, 102, 106	100	6	6
		0.80	104, 104, 107	105	2	2
	Prothioconazole-4-hydroxy	0.010	93, 94, 99	95	3	3
		0.050	102, 106, 111, 112	108	5	4
		0.80	97, 101, 101	99	2	2
Fat	Prothioconazole	0.050	83, 86, 87, 93	87	4	5
		0.080	91, 92, 93	92	1	1
	Prothioconazole-desthio	0.050	91, 92, 93, 100	94	4	4
		0.080	102, 104, 104	103	1	1
	Prothioconazole-4-hydroxy	0.050	78, 79, 79, 86	81	4	5

TABLE C.1.1. Recovery Results from Method Validation of Various Cattle Matrices using the Data-Gathering Analytical Method.[1]

Matrix	Analyte	Spiking Level (ppm)	Recoveries Obtained (%)	Recovery (%)		
				Mean	SD	CV
		0.080	84, 85, 86	85	1	1

[1] Spiking standards were prepared in ACN containing 50 µg/mL L-cysteine HCl. Calibration standards were prepared in 50 µg/mL L-cysteine HCl in ACN/water (1:1, v:v). Recoveries were corrected for any apparent residues in controls.
[2] Residues of prothioconazole-4-hydroxy were quantitated using external standards only for milk, skim milk, and cream. An internal standard for prothioconazole-4-hydroxy was not available at the time the validation was conducted.

The spiking levels and samples used in method validation are adequate to bracket expected residue levels. A second transition (considered a confirmatory analysis) has not been conducted. Because of the specificity of LC-MS/MS analyses, an interference study has not been conducted. The applicant notes that for a compound to interfere, it would have to co-chromatograph with prothioconazole, prothioconazole-desthio, or prothioconazole-4-hydroxy and form an ion of the proper molecular weight (±1.0 amu from the analyte parent ions) in the ion source. The resulting ion would then have to fragment to a daughter ion of the proper molecular weight. Therefore, an additional confirmatory method is not required.

Prothioconazole-4-hydroxy residues in milk, cream and skim milk samples were quantitated using external standards because the method was developed before a prothioconazole-4-hydroxy internal standard was available. Higher recoveries of this analyte may have occurred if these samples were quantified by the internal standard.

TABLE C.1.2. Characteristics for the Data-Gathering Analytical Method Used for the Quantitation of Prothioconazole Residues in Livestock Matrices.

Analytes	Prothioconazole, prothioconazole-desthio, prothioconazole-4-hydroxy
Equipment ID	TSQ 7000 MS/MS with API II electrospray interface; SpectraSYSTEM AS3000 HPLC; Phenomenex Synergi MAX-RP, 4 µm (75 mm x 4.6 mm) column
Limit of quantitation (LOQ)	0.005 ppm for milk 0.010 ppm for skim milk, cream, muscle, liver, and kidney 0.050 ppm for fat (determined as lowest spiking level with adequate recovery)
Limit of detection (LOD)	Prothioconazole: 0.0007 ppm for milk; 0.001 ppm for skim milk; 0.0021 ppm for cream; 0.001 ppm for muscle; 0.0005 ppm for liver; 0.0025 ppm for kidney; 0.0115 ppm for fat Prothioconazole-desthio: 0.0008 ppm for milk; 0.0014 ppm for skim milk; 0.003 ppm for cream; 0.0007 ppm for muscle; 0.0029 ppm for liver; 0.0022 ppm for kidney; 0.0041 ppm for fat Prothioconazole-4-hydroxy: 0.0021 ppm for milk; 0.0019 ppm for skim milk; 0.0035 ppm for cream; 0.0006 ppm for muscle; 0.0007 ppm for liver; 0.0021 ppm for kidney; 0.0077 ppm for fat (determined for each analyte in each matrix by multiplying the standard deviation of the LOQ recoveries by the $t_{0.99}$ value for the number of measurements)

TABLE C.1.2. Characteristics for the Data-Gathering Analytical Method Used for the Quantitation of Prothioconazole Residues in Livestock Matrices.	
Accuracy/Precision	Percent recoveries and coefficients of variance (CVs) indicate acceptable accuracy/precision at 0.005 and 0.010 ppm for milk; 0.010 ppm for skim milk, cream, and muscle; 0.010 and 0.60 ppm for liver; 0.010, 0.050, and 0.80 ppm for kidney; and 0.050 and 0.080 ppm for fat. Recovery ranges (and CVs) from these matrices were 77-115% (9.1%) for prothioconazole, 91-117% (6.0%) for prothioconazole-desthio, and 63-117% (14.7%) for prothioconazole-4-hydroxy (See TABLE C.1.1).
Reliability of the Method (ILV)	An independent laboratory method validation (ILV) was conducted to verify the reliability of the LC-MS/MS method using samples of cattle liver and milk. The values obtained indicate that the method is reliable (see Section C.3).
Linearity	The method/detector response was linear (coefficient of determination, r^2, was 0.9986-0.9999 for prothioconazole; 0.9987-0.9999 for prothioconazole-desthio, and 0.9969-0.9999 for prothioconazole-4-hydroxy) within the range of 0.001-0.250 ppm for milk and 0.002-1.0 ppm for tissues, in both solvent and solvent plus matrix.
Specificity	The control chromatograms generally have no peaks above the chromatographic background, and the spiked sample chromatograms contain only the analyte peak of interest. Peaks are well defined and symmetrical.

The extraction solvents used in the LC-MS/MS method are similar to those used in the goat metabolism study provided with this submission. In the metabolism studies, the majority of the radioactivity are extracted using methanol (milk) or acetonitrile/water (tissues). Cysteine HCl is added to milk and to the extraction solvents for tissues. Although metabolites are identified in extracts that had not been subjected to acid hydrolysis; the extracts of milk, liver, kidney, and muscle are hydrolyzed with HCl and analyzed by HPLC. This indicates that acid hydrolysis results in cleavage of glucuronide and sulfate conjugates, with corresponding increases in the concentrations of prothioconazole and prothioconazole-4-hydroxy.

Bayer has submitted extraction efficiency data for this LC-MS/MS method. Samples of goat milk, liver, muscle, and fat dosed with [triazole-^{14}C]-prothioconazole are used from the metabolism study. Samples are extracted using the procedures of the LC-MS/MS method, as described in TABLE B.1.1. The radioactivity of liquid samples is determined by LSC, while solid samples undergo combustion/LSC. Total radioactive residues (TRRs) were not re-determined, as the values obtained in the metabolism study were used. HPLC analysis of the extracts is conducted using a C18 column, a gradient mobile phase of either ACN or water (each containing 1% acetic acid), a UV detector (254 nm), and a radioactivity detector. Analytes are identified by co-chromatography with reference standards of prothioconazole, prothioconazole-desthio, and prothioconazole-4-hydroxy. The results of the study indicated adequate extraction efficiency, as reported in TABLE C.1.3.

The methanolic extract of fat is not subjected to acid hydrolysis in the goat metabolism study. To allow determination of the extraction efficiency of the residue analytical method, the applicant calculated theoretical values of prothioconazole, prothioconazole-desthio, and prothioconazole-4-hydroxy that would have been found in acid-hydrolyzed fat extract (based on the metabolites found in the unhydrolyzed extract). For the calculation, the applicant assumed that JAU6476-S-glucuronide would be converted to prothioconazole, and JAU6476-hydroxy-glucuronides would be converted to prothioconazole-4-hydroxy.

TABLE C.1.3. Extraction Efficiency of the Enforcement Analytical Method Using Radiolabelled Samples from a Goat Metabolism Study.

Matrix	Metabolism study		Residue Method		Extraction Efficiency (%) [1]
	% TRR	ppm	% TRR	ppm	
Milk					
TRR	100.0	0.150	--	--	--
Extract before cleanup/hydrolysis [2]	83.76	0.126	--	--	--
Extract after hydrolysis [3]	21.92	0.033	26.33	0.040	121.2
Muscle					
TRR	100.0	0.117	--	--	--
Extract before cleanup/hydrolysis [2]	77.72	0.091	91.67	0.107	--
Extract after hydrolysis [3]	42.31	0.050	62.56	0.073	146.0
Liver					
TRR	100.0	6.248	--	--	--
Extract before cleanup/hydrolysis [2]	82.96	5.183	64.39	4.023	--
Extract after hydrolysis [3]	73.29	4.579	55.59	3.474	75.9
Fat					
TRR	100.0	0.174	--	--	--
Extract before cleanup/hydrolysis [2]	76.80	0.134	94.40	0.165	--
Extract after hydrolysis [4]	62.60	0.109	87.09	0.152	139.4

[1] Extraction efficiency = (ppm determined by the residue method) ÷ (ppm as determined in the metabolism study) x 100.
[2] Radioactivity in extract prior to cleanup (metabolism study only) and hydrolysis.
[3] Radioactivity in hydrolysate was measured for residue method samples. For the metabolism study samples, radioactivity in hydrolysate was assumed to be the same as in the extract prior to hydrolysis.
[4] The fat extract was not subjected to acid hydrolysis in the metabolism study. Therefore, the applicant calculated theoretical maximum amounts of prothioconazole, prothioconazole-desthio, and prothioconazole-4-hydroxy, and used the sum of the 3 analytes as the values for the metabolism study. This was based on the assumption that JAU6476-S-glucuronide would be converted to prothioconazole, and JAU6476-hydroxy-glucuronides would be converted to prothioconazole-4-hydroxy.

The submitted extraction efficiency data demonstrate that the extraction procedures of the LC-MS/MS adequately extract aged residues of prothioconazole, prothioconazole-desthio, prothioconazole-4-hydroxy, and metabolites hydrolysable to these compounds from goat matrices.

C.2. Enforcement Method

The proposed enforcement method for livestock commodities is the same as the data-gathering method.

C.3. Independent Laboratory Validation

An independent laboratory validation of the LC-MS/MS method has been conducted by Pyxant Labs Inc. (Colorado Springs, CO) using samples of cattle milk and liver. These represent difficult matrices to work with.

Samples of untreated cattle liver and milk were purchased commercially and spiked with a mixture of prothioconazole, prothioconazole-desthio, and prothioconazole-4-hydroxy at levels of 0.01 ppm (LOQ), 0.1 ppm, and 0.5 ppm of each analyte for liver; and 0.005 ppm (LOQ), 0.010 ppm, and 0.050 ppm of each analyte for milk. Liver samples are homogenized prior to spiking. Spiked and nonspiked (control) samples are analyzed using the LC-MS/MS method described in TABLE B.1.1.

The first ILV trial was successful. Recoveries of prothioconazole, prothioconazole-desthio, and prothioconazole-4-hydroxy from liver and milk samples are generally considered adequate, as reported in TABLE C.3.1. Residues of prothioconazole, prothioconazole-desthio, and prothioconazole-4-hydroxy are each nondetectable in two samples each of nonspiked milk and liver. The detector response is linear (r^2 = 0.9996-0.9998) for each of the 3 analytes over a range of 0.005-0.25 ppm standard concentration. The calculated LODs are 0.004 ppm for each analyte in liver, 0.0003 ppm for prothioconazole in milk, and 0.0002 ppm each for prothioconazole-desthio and prothioconazole-4-hydroxy in milk.

The laboratory reports that a set of 17 samples could be extracted and readied for analysis in an 8-hour day by one technician, with occasional help from a second technician. LC-MS/MS analyses requires approximately 8 hours, with an additional 4 hours required for data processing. The laboratory does not identify any critical steps or recommend any significant modifications to the method.

TABLE C.3.1. Recovery Results Obtained by an Independent Laboratory Validation of the Enforcement Method for the Determination of Prothioconazole Residues in Livestock Matrices.

Matrix	Spiking Analyte	Spiking Level (ppm)	Recoveries Obtained	Mean Recovery ± SD [CV]
Milk	Prothioconazole	0.005	98.1, 98.3, 98.5, 99.5, 102	99.3 ± 1.6 [1.6]
		0.01	102, 103, 103, 104, 105	103.4 ± 1.1 [1.1]
		0.05	98.3, 100, 101, 101, 101	100.3 ± 1.2 [1.2]
	Prothioconazole-desthio	0.005	96.0, 96.6, 97.9, 98.1, 98.3	97.4 ± 1.0 [1.0]
		0.01	100, 101, 103, 105, 106	103.0 ± 2.5 [2.5]
		0.05	100, 102, 102, 103, 103	102.0 ± 1.2 [1.2]
	Prothioconazole-4-hydroxy	0.005	98.4, 99.4, 99.5, 100, 101	99.7 ± 0.9 [1.0]
		0.01	103, 104, 104, 104, 104	103.8 ± 0.4 [0.4]
		0.05	101, 101, 101, 102, 102	101.4 ± 0.5 [0.5]

TABLE C.3.1. Recovery Results Obtained by an Independent Laboratory Validation of the Enforcement Method for the Determination of Prothioconazole Residues in Livestock Matrices.

Matrix	Spiking Analyte	Spiking Level (ppm)	Recoveries Obtained	Mean Recovery ± SD [CV]
Liver	Prothioconazole	0.01	69.3, 73.9, 82.0, 89.1, 93.0	81.5 ± 10.0 [12.2]
		0.1	78.1, 92.5, 93.9, 96.3, 97.5	91.7 ± 7.8 [8.5]
		0.5	80.7, 90.8, 94.8, 94.9, 102	92.6 ± 7.8 [8.4]
	Prothioconazole-desthio	0.01	70.8, 83.1, 92.2, 92.7, 99.0	87.6 ± 11.0 [12.5]
		0.1	89.7, 106, 106, 107, 112	104.1 ± 8.4 [8.1]
		0.5	87.7, 96.7, 103, 103, 106	99.3 ± 7.3 [7.4]
	Prothioconazole-4-hydroxy	0.01	67.2, 79.0, 82.4, 89.3, 92.0	82.0 ± 9.8 [11.9]
		0.1	80.9, 95.9, 98.9, 99.3, 99.3	94.9 ± 7.9 [8.4]
		0.5	85.5, 92.3, 96.6, 97.8, 100	94.4 ± 5.7 [6.1]

D. CONCLUSION

Method validation data have been submitted to determine residues of prothioconazole, prothioconazole-desthio, prothioconazole-4-hydroxy, and conjugates that may be converted to these compounds by acid hydrolysis in milk and livestock tissues. The data encompasses the expected residue levels, and adequately quantitates the analytes in the cattle matrices.

The method has been proposed for both data gathering and enforcement purposes. Adequate extraction efficiency data have been submitted for the method using samples of goat milk, muscle, liver, and fat. Adequate independent laboratory validation data have been submitted for the method using samples of cattle milk and liver.

E. REFERENCES

46246149 Weber, E.; Weber, H.; Spiegel, K. (2003) [Triazole-UL-(Carbon 14)]JAU6476: Absorption, Distribution, Excretion, and Metabolism in the Lactating Goat. Project Number: M51819114. MR/448/02. Unpublished study prepared by Bayer Ag, Institute of Product Info. 308 p.

46246150 Weber, H.; Spiegel, K. (2001) [Phenyl-UL-(Carbon 14)]JAU6476: Absorption, Distribution. Excretion, and Metabolism in the Lactating Goat. Project Number: M/91819082, MR/092/01. Unpublished study prepared by Bayer Ag Institut fuer Ruckstands-Analytik. 205 p.

46246213 Duah, F. (2004) A 28-Day Feeding Study with JAU6476 in Dairy Cattle. Lab Project Number: J6060401: 200715. Unpublished study prepared by Bayer CropScience and Genesis Midwest Laboratories. 498 p.

F. DOCUMENT TRACKING

RDI: Suzan Mathew (PMRA) 23/01/2006; Henri Bietlot (PMRA) 24/01/2006; Stephen Funk (IO) 13/03/2006; Leung Cheng (RAB3).
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In the absence of signatures, this document is considered to be a draft with deliberative material for internal use only.

This DER was originally prepared under contract by Dynamac Corporation (20440 Century Boulevard, Suite 100; Germantown, MD 20874; submitted 05/03/2005). The DER has been reviewed by the Health Effects Division (HED) and Health Canada's Pest Management Regulatory Agency (PMRA). The DER has been revised to reflect current Office of Pesticide Programs (OPP) policies and Directive 98-02.

STUDY REPORTS:

46246206 Gould, T.; Timberlake, 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 Numbers: J6111401, 200799 and RPA JA/03/01. Unpublished study prepared by Bayer CropScience. 238 p.

46246208 Gould, T.; Timberlake, B. (2004) Extraction Efficiency of JAU6476 and Metabolites by the Method JA/03/01. Project Number RAJAY011. Unpublished study prepared by Bayer CropScience. 59 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 Numbers: RAJAY020 and 48663. Unpublished study prepared by Analytical Bio-Chemistry Laboratories, Inc. 77 p.

Bayer CropScience has proposed the high performance liquid chromatography (with electrospray ionization) and tandem mass spectrometry (LC-MS/MS) method RPA JA/03/01 for data gathering and the enforcement of maximum residue limits (MRLs) for residues of prothioconazole (JAU6476) and the prothioconazole-desthio metabolite (JAU6476-desthio) in plant commodities.

In the method, crop matrices are extracted with a mixture of methanol, 30% hydrogen peroxide, and 5% aqueous sodium bicarbonate at 65°C for 2 hours. Prothioconazole is converted to both prothioconazole sulfonic acid and prothioconazole-desthio because of this oxidative extraction procedure. The prothioconazole-desthio metabolite remains unchanged after extraction. The cooled extract is spiked with an isotopically labeled internal standard, cleaned up by C18 solid-phase extraction (SPE), and mixed with either 0.1% formic acid or 1% 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 LOQs reported in the method are 0.02 ppm for canola seed, peanut nutmeat, and wheat grain; and 0.05 ppm for dried peas, wheat forage, wheat hay, and wheat straw. The calculated LODs range from 0.002-0.005 ppm for prothioconazole and prothioconazole-desthio in canola seed, dried peas, peanut nutmeat, and wheat (forage, hay, straw, and grain); and from 0.002-0.007 ppm for prothioconazole sulfonic acid in these same commodities.

Concurrent recovery data from the crop field trials, as well as data from the method validation study adequately bracket the expected residue levels. Method validation data demonstrate adequate method recoveries of prothioconazole, prothioconazole sulfonic acid, and prothioconazole-desthio at 0.020 ppm (LOQ) and 0.1 ppm for canola seed, peanut nutmeat, and wheat grain; and at 0.05 ppm (LOQ) and 1.0 ppm for dried peas, wheat forage, wheat hay, and wheat straw. The ranges of recoveries (and CVs) from these matrices are 71-91% (5.4%) for prothioconazole, 76-102% (5.8%) for prothioconazole sulfonic acid, and 85-106% (4.5%) for prothioconazole-desthio over all matrices and spiking levels.

Adequate extraction efficiency data have been submitted for the method using samples of sugar beet tops and wheat forage. Adequate independent laboratory validation data have been submitted for the method using samples of peanut nutmeat and wheat forage. Confirmatory analysis procedures were not conducted for the proposed enforcement method due to the high specificity of the LC-MS/MS method.

STUDY/WAIVER ACCEPTABILITY/DEFICIENCIES/CLARIFICATIONS:

Under the conditions and parameters used in the study, the analytical method residue data are classified as scientifically acceptable.

The proposed enforcement method has been forwarded to ACL for petition method validation, and to determine whether confirmatory analysis procedures are needed for the method.

The acceptability of this study for regulatory purposes is addressed in the forthcoming U.S. EPA Residue Chemistry Summary Document [DP Barcode D303508] and in Canada's Regulatory Decision Document.

COMPLIANCE:

Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided. No deviations from regulatory requirements were reported.

A. BACKGROUND INFORMATION

Prothioconazole is a broad-spectrum systemic fungicide intended for the control of many diseases in different crops such as cereals, oilseed rape or peanuts. The biochemical mode of action is the inhibition of the demethylation of lanosterol or 24-methylene-dihydrolanosterol, which are precursors of sterols in fungi. The chemical has been submitted for joint EPA and PMRA review by Bayer CropScience. The crops proposed for joint review include barley, the dried shell and bean subgroup, the oilseed crop group, and wheat. Uses on peanuts and rice are being proposed for the U.S. only.

TABLE A.1. Test Compound Nomenclature.

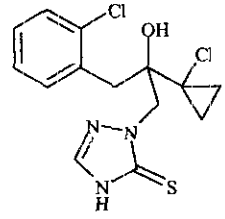
Chemical structure	
Common name	Prothioconazole (ISO accepted)
Company experimental name	JAU6476
IUPAC name	2-[(2*RS*)-2-(1-chlorocyclopropyl)-3-(2-chlorophenyl)-2-hydroxypropyl]-2*H*-1,2,4-triazole-3(4*H*)-thione
CAS name	2-[2-(1-chlorocyclopropyl)-3-(2-chlorophenyl)-2-hydroxypropyl]-1,2-dihydro-3*H*-1,2,4-triazole-3-thione
CAS registry number	178928-70-6
PC Code	113961
End-use product (EP)	Proline® 480 SC (4 lb/gal suspension concentrate), EPA File Symbol 264-IEL

TABLE A.2. Physicochemical Properties of the Technical Grade Test Compound.

Parameter	Value	References
Melting range	139.1 to 144.5°C	MRID 46246003/CES
pH	5.8 (1% solution)	MRID 46246003/CES

Parameter	Value	References
Density at 20°C	1.36 g/mL	MRID 46246003/CES
Water solubility at 20°C	pH / mg/L 4 / 5 8 / 300 9 / 2000	MRID 46246003/CES
Solvent solubility at 20°C	Solvent / g/L Acetone / >250 Acetonitrile / 69 Dichloromethane / 88 Dimethysulfoxide / 126 Ethyl acetate / >250 n-Heptane / <0.1 1-Octanol / 58 Polyethylene glycol / >250 2-Propanol / 87 Xylene / 8	MRID 46246003/CES
Vapor pressure at 20 or 25°C	<4 x 10^{-7} Pa (calculated from determinations at 70°C)	MRID 46246003/CES
Dissociation constant, pK_a	6.9 (calculated from K_{OW})	MRID 46246003/CES
Octanol/water partition coefficient, at 20°C	pH / Log(K_{OW}) unbuffered water / 4.05 4 / 4.16 7 / 3.82 9 / 2.00	MRID 46246003/CES
UV/visible absorption spectrum	Peak maxima at 257 nm. No absorption at > 300 nm.	MRID 46246003/CES

CES - Chemistry Evaluation Section of PMRA

B. MATERIALS AND METHODS

B.1. Data-Gathering Method

B.1.1. Principle of the Method:

Samples are homogenized with dry ice, then extracted with a combination of methanol, 30% hydrogen peroxide (H_2O_2), and 5% aqueous sodium bicarbonate ($NaCO_3$) in a water bath (65°C for 2 hours). After the extract has cooled, a mixture of 2 internal standards (prothioconazole sulfonic acid-$^{15}N_3$-$^{13}C_2$ and prothioconazole-desthio-$^{15}N_3$-$^{13}C_2$) is added. The sample is then cleaned up with a C-18 column preconditioned with methanol. The sample can be neutralized with either 1% acetic acid (as reported in the analytical method and ILV studies) or 0.1% formic acid (as reported in the method validation and extraction efficiency studies). The sample then undergoes LC-MS/MS analysis. 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 results for prothioconazole sulfonic acid and prothioconazole-desthio are reported in prothioconazole equivalents, then totaled to yield "total prothioconazole derived residues."

The reported LOQs for method RPA JA/03/01 to determine prothioconazole and prothioconazole-desthio residues are 0.02 ppm for canola seed, peanut nutmeat, and wheat grain; and 0.05 ppm for dried peas, wheat forage, wheat hay, and wheat straw. The LODs calculated for prothioconazole, prothioconazole sulfonic acid and prothioconazole-desthio vary from 0.002-0.005 ppm in canola seed, dried peas, peanut nutmeat, and wheat (forage, hay, straw, and grain); and from 0.002-0.007 ppm in the same commodities for prothioconazole sulfonic acid. The LOQs and LODs are reported in TABLE C.1.2.

TABLE B.1.1. Summary Parameters for the Analytical Method Used for the Quantitation of Prothioconazole Residues in Plant Matrices.	
Method ID	RPA JA/03/01
Analytes	Prothioconazole and prothioconazole-desthio
Extraction solvent/technique	Samples are homogenized in the presence of dry ice and then mixed with methanol, 30% hydrogen peroxide, and 5% aqueous sodium bicarbonate. The mixture is heated at 65 °C for 2 hours and then allowed to cool. A mixture of the internal standards of prothioconazole sulfonic acid-$^{15}N_3$-$^{13}C_2$ and prothioconazole-desthio-$^{15}N_3$-$^{13}C_2$ is added.
Cleanup strategies	The extract is cleaned up using C18 SPE and then mixed with either 1% acetic acid or 0.1% formic acid for analysis.
Instrument/Detector	HPLC utilizing a reverse-phase C8 column and a gradient mobile phase of ACN:water (1:9, v:v) and ACN, each containing 0.2% acetic acid, with tandem mass spectrometry (MS/MS) detection using electrospray ionization operating in the positive ion mode for prothioconazole-desthio and negative ion mode for prothioconazole sulfonic acid. The ion transitions monitored are: Prothioconazole sulfonic acid: 390.45-354.45 Prothioconazole sulfonic acid internal standard: 395.45-359.45 Prothioconazole-desthio: 312.35-125.52 Prothioconazole-desthio internal standard: 317.35-125.52
Standardization method	External and internal standardization. Five calibration standards, containing internal standards, are injected, and the relative responses (RRs; analyte peak area divided by internal standard peak area) and response factors (RR divided by standard concentration) are calculated; an average response factor for each analyte is calculated. RRs for samples are calculated and divided by the average response factor for the analyte to determine residue levels. Up to 20 samples may be injected before standards must be re-injected; standards should also be injected at the end of the analytical run. The concentration of all calibration and spiking standards is calculated in prothioconazole equivalents; therefore, results for prothioconazole sulfonic acid and prothioconazole-desthio are reported in prothioconazole equivalents.
Stability of std solutions	Standard solutions of prothioconazole-desthio and prothioconazole sulfonic acid are to be stored at 4 ± 3°C and are reportedly stable under these conditions for at least 6 months.
Retention times	Prothioconazole sulfonic acid: ~2.0 minutes Prothioconazole-desthio: ~4.2 minutes

B.2. Enforcement Method

The proposed enforcement method for plant commodities is the same as the data-gathering method.

C. RESULTS AND DISCUSSION

C.1. Data-Gathering Method

TABLE C.1.1. Recovery Results from Method Validation of Various Plant Matrices using the Data-Gathering Analytical Method.[1]

Matrix	Analyte	Spiking Level (ppm)	Recoveries Obtained (%)	Recovery		
				Mean	SD	CV
Canola seed	Prothioconazole	0.020	80, 83, 84, 84, 87, 87, 89	85	3	4
		0.100	84, 84, 85, 85, 86, 87, 87	85	1	1
	Prothioconazole sulfonic acid	0.020	94, 94, 97, 99, 100, 100, 102	98	3	3
		0.100	93, 94, 94, 94, 95, 95, 97	95	1	1
	Prothioconazole-desthio	0.020	90, 95, 95, 96, 97, 97, 99	96	3	3
		0.100	92, 92, 93, 93, 93, 93, 94	93	1	1
Dried peas	Prothioconazole	0.050	80, 82, 83, 84, 84, 87, 87	84	3	3
		1.000	81, 81, 82, 82, 82, 83, 84	82	1	1
	Prothioconazole sulfonic acid	0.050	89, 89, 91, 91, 91, 92, 94	91	2	2
		1.000	78, 83, 83, 84, 84, 84, 87	83	3	3
	Prothioconazole-desthio	0.050	92, 93, 94, 94, 94, 99, 99	95	3	3
		1.000	86, 92, 93, 94, 94, 96, 97	93	4	4
Peanut nutmeat	Prothioconazole	0.020	81, 82, 82, 82, 83, 84, 87	83	2	2
		0.100	83, 84, 85, 85, 85, 85, 89	85	2	2
	Prothioconazole sulfonic acid	0.020	90, 91, 92, 92, 93, 94, 98	93	3	3
		0.100	89, 89, 90, 90, 91, 91, 92	90	1	1
	Prothioconazole-desthio	0.020	90, 91, 94, 94, 95, 96, 97	94	3	3
		0.100	90, 90, 90, 91, 91, 92, 92	91	1	1
Wheat forage	Prothioconazole	0.050	79, 80, 81, 82, 83, 84, 85	82	2	3
		1.000	81, 81, 81, 82, 82, 82, 83	82	1	1
	Prothioconazole sulfonic acid	0.050	88, 89, 89, 90, 90, 91, 93	90	2	2
		1.000	84, 84, 85, 87, 87, 87, 88	86	2	2
	Prothioconazole-desthio	0.050	92, 94, 94, 95, 96, 96, 98	95	2	2
		1.000	97, 99, 100, 100, 100, 101, 106	100	3	3

TABLE C.1.1. Recovery Results from Method Validation of Various Plant Matrices using the Data-Gathering Analytical Method.[1]

Matrix	Analyte	Spiking Level (ppm)	Recoveries Obtained (%)	Recovery		
				Mean	SD	CV
Wheat hay	Prothioconazole	0.050	75, 76, 76, 78, 79, 80, 81	78	2	3
		1.000	71, 72, 73, 74, 76, 77, 77	74	2	3
	Prothioconazole sulfonic acid	0.050	76, 77, 78, 78, 84, 84, 86	80	4	5
		1.000	94, 94, 95, 95, 95, 96, 97	95	1	1
	Prothioconazole-desthio	0.050	93, 94, 95, 96, 98, 98, 98	96	2	2
		1.000	99, 102, 102, 102, 102, 105, 105	102	2	2
Wheat straw	Prothioconazole	0.050	87, 88, 88, 89, 89, 90, 91	89	1	2
		1.000	88, 89, 89, 90, 90, 91, 91	90	1	1
	Prothioconazole sulfonic acid	0.050	91, 93, 94, 97, 98, 98, 99	96	3	3
		1.000	88, 89, 90, 90, 90, 92, 93	90	2	2
	Prothioconazole-desthio	0.050	90, 91, 92, 94, 94, 95, 96	93	2	2
		1.000	94, 96, 97, 97, 97, 98, 98	97	1	1
Wheat grain	Prothioconazole	0.020	76, 76, 77, 78, 81, 82, 91	80	5	7
		0.100	79, 79, 80, 81, 82, 82, 82	81	1	2
	Prothioconazole sulfonic acid	0.020	88, 89, 90, 91, 92, 95, 99	92	4	4
		0.100	90, 91, 92, 92, 93, 96, 96	93	2	3
	Prothioconazole-desthio	0.020	89, 89, 89, 92, 92, 92, 93	91	2	2
		0.100	85, 86, 87, 87, 88, 88, 89	87	1	2

[1] Standards were prepared in methanol:1% acetic acid (1:2, v:v).

The expected residue levels are encompassed by both the method validation study and the concurrent recovery data from the crop field trials. A second transition (considered a confirmatory analysis) was not conducted. Because of the specificity of LC-MS/MS analyses, an interference study was not conducted. The applicant noted that for a compound to interfere, it would have to co-chromatograph with the internal standard, form an ion of the proper molecular weight (± 0.5 amu from the analyte parent ions), and then fragment to a daughter ion of the proper molecular weight. Therefore, an additional confirmatory method was not required.

TABLE C.1.2. Characteristics for the Data-Gathering Analytical Method Used for the Quantitation of Prothioconazole Residues in Plant Matrices.

Analytes	Prothioconazole and prothioconazole-desthio
Equipment ID	TSQ Quantum LC-MS/MS LiChroSpher RP Select B, 5 µm (100 mm x 2.1 mm)
Limit of quantitation (LOQ)	0.02 ppm for canola seed, peanut nutmeat, and wheat grain 0.05 ppm for dried peas and wheat - forage, hay, and straw (determined as lowest spiking level with adequate recovery)

TABLE C.1.2. Characteristics for the Data-Gathering Analytical Method Used for the Quantitation of Prothioconazole Residues in Plant Matrices.		
Limit of detection (LOD)	Prothioconazole:	0.002 ppm; canola seed, peanut nutmeat 0.003 ppm; wheat straw 0.004 ppm; wheat forage, hay, and grain 0.005 ppm; dried peas
	Prothioconazole sulfonic acid:	0.002 ppm; canola seed, peanut nutmeat 0.003 ppm; dried peas, wheat forage and grain 0.005 ppm; wheat straw 0.007 ppm; wheat hay
	Prothioconazole-desthio:	0.002 ppm; canola seed, peanut nutmeat, wheat grain 0.003 ppm; wheat forage 0.004 ppm, wheat hay, straw 0.005 ppm; dried peas
	(calculated for each analyte in each matrix by multiplying the standard deviation of the LOQ recoveries by the $t_{0.99}$ value for the number of measurements)	
Accuracy/Precision	Percent recoveries and coefficients of variance (CVs) indicate acceptable accuracy/precision at 0.020 and 0.1 ppm for canola seed, peanut nutmeat, and wheat grain, and at 0.05 and 1.0 ppm for dried peas and wheat forage, hay, and straw. Recovery ranges (and CVs) from these matrices were 71-91% (5.4%) for prothioconazole, 76-102% (5.8%) for prothioconazole sulfonic acid, and 85-106% (4.5%) for prothioconazole-desthio. See TABLE C.1.1 above.	
Reliability of the Method (ILV)	An independent laboratory method validation (ILV) was conducted to verify the reliability of method RPA JA/03/01 using samples of peanut nutmeat and wheat forage. The values obtained indicate that method RPA JA/03/01 is reliable; see Section C.3.	
Linearity	The method/detector response was linear (coefficient of determination, r^2, was 0.99962 for prothioconazole-desthio and 0.99994 for prothioconazole sulfonic acid) within the nominal range of 0.005-2.5 ppm.	
Specificity	The control chromatograms generally have no peaks above the chromatographic background, and the spiked sample chromatograms contain only the analyte peak of interest. Peaks were well defined and symmetrical.	

The extraction solvents used in method RPA JA/03/01 differ from those used in the peanut, sugar beet, and wheat metabolism studies conducted for this submission. In the metabolism studies, the majority of the radioactivity generally is extracted using acetonitrile/water containing cysteine HCl. Cysteine HCl is added to prevent oxidative decomposition of prothioconazole.

Bayer has submitted extraction efficiency data for method RPA JA/03/01. Samples of wheat forage that were treated with [triazole-3-5-^{14}C]-prothioconazole, and sugar beet tops that were treated with [phenyl-UL-^{14}C]-prothioconazole were used from their respective crop metabolism studies. The wheat forage and sugar beet top samples were chosen because both had measurable residues of parent prothioconazole. Samples are extracted using the method (as described in TABLE B.1.1) with the following exceptions: During the C18 SPE cleanup step, the cartridge is additionally eluted with methanol. The effluent of the cartridge is combined with the methanol eluate and the mixture concentrated and redissolved in methanol and 0.1% aqueous formic acid. Total radioactive residues (TRRs) are determined by summing the radioactivity in the extractable and nonextractable residues, and quantitated by LSC and combustion/LSC,

respectively. HPLC analysis of the extracts is conducted using a C8 column, a gradient mobile phase of ACN:water (1:9, v:v) and ACN, each containing 0.2% acetic acid, a UV detector (254 nm), and a radioactivity detector. Analytes are identified by comparison of retention times with those of radiolabeled standards of prothioconazole, prothioconazole-desthio, and prothioconazole sulfonic acid. The [phenyl-^{14}C]-prothioconazole sulfonic acid reference standard is prepared from [^{14}C]-prothioconazole. The detector response is linear (r^2 = 0.9995) over a nominal range of 600-500,000 dpm injected. The results of the study indicate adequate extraction efficiency, as reported in TABLE C.1.3.

TABLE C.1.3. Extraction Efficiency of the Enforcement Analytical Method (Method RPA JA/03/01) Using Radiolabeled Samples from Sugar Beet and Wheat Metabolism Studies.

Matrix	Metabolism study		Residue Method [1]		Extraction Efficiency (%) [2]
	% TRR	ppm	% TRR	ppm	
Wheat forage					
TRR	100.0	7.956	100.0	8.1523	--
Extract	88.4 [3]	7.032	93.5	7.6217	108.4
Sugar beet tops					
TRR	100.0	4.333	100.0	4.2517	--
Extract	92.9 [4]	4.025	96.0	4.0835	101.5

[1] Average of three replicate samples.
[2] Extraction efficiency = (ppm determined by the residue method) ÷ (ppm as determined in the metabolism study) x 100.
[3] Total of initial ACN/water extract and ACN/water extract resulting from accelerated solvent extraction.
[4] Initial ACN/water extract.

Two additional samples of untreated wheat forage spiked with [phenyl-^{14}C]-prothioconazole were analyzed as previously described. An average of 37% of the spiked radioactivity was detected as prothioconazole-desthio, and an average of 58% was detected as prothioconazole sulfonic acid, for a total average recovery of 95%.

The submitted extraction efficiency data demonstrate that the extraction procedures of method RPA JA/03/01 adequately extract aged residues of prothioconazole and the desthio metabolites from sugar beet top and wheat forage samples.

C.2. Enforcement Method

The proposed enforcement method for plant commodities is the same as the data-gathering method.

C.3. Independent Laboratory Validation

An independent laboratory validation of method RPA JA/03/01 was conducted by ABC Laboratories, Inc. (Columbia, MO) using samples of peanut nutmeat and wheat forage. These are considered the most difficult matrices to work with, and represent the range of matrices analysed for the crop field trials.

Samples of untreated peanut nutmeat and wheat forage (control samples supplied by Bayer) are homogenized and spiked with prothioconazole *per se*, or with a mixture of prothioconazole sulfonic acid and prothioconazole-desthio (each) at 0.02 ppm (LOQ) and 0.1 ppm for peanut nutmeat, and 0.05 ppm (LOQ) and 10 ppm for wheat forage. Spiked and unspiked (control) samples are analyzed using LC-MS/MS.

The first and second ILV trials failed due to errors made by the laboratory. In the first trial, after adding internal standard to the sample extract, the extract was not mixed prior to removing an aliquot for the next step. In the second trial, the wrong amount of internal standard was added to samples and standards. The third trial was successful. Recoveries of prothioconazole, prothioconazole sulfonic acid, and prothioconazole-desthio from peanut nutmeat and wheat forage samples are considered adequate, and are reported in TABLE C.3.1. Total prothioconazole-derived residues are nondetectable in two samples each of unspiked peanut nutmeat and wheat forage.

The laboratory reports that a set of 10 samples could be extracted with cleanup in approximately 5 person-hours, with an additional 3.6 hours required for the LC-MS/MS analyses. The laboratory did not identify any critical steps, but recommends the addition of specific instructions regarding mixing procedures and the calculation of method recoveries.

TABLE C.3.1. Recovery Results Obtained by an Independent Laboratory Validation of the Enforcement Method for the Determination of Prothioconazole Residues in Plant Matrices.

Matrix	Spiked Analyte	Spiking Level (ppm)	Recoveries Obtained	Mean Recoveries
Peanut nutmeat	Prothioconazole	0.02	90, 119	105
		0.1	69, 96	83
	Prothioconazole sulfonic acid [1]	0.02	83, 101	92
		0.1	78, 82	80
	Prothioconazole-desthio [1]	0.02	73, 81	77
		0.1	67, 76	72
Wheat forage	Prothioconazole	0.05	83, 89	86
		10	95, 100	98
	Prothioconazole sulfonic acid [1]	0.05	73, 73	73
		10	81, 83	82
	Prothioconazole-desthio [1]	0.05	87, 90	89
		10	88, 90	89

[1] Spiked with a mixture of prothioconazole sulfonic acid and prothioconazole-desthio.

D. CONCLUSION

Method validation data was submitted for the LC-MS/MS method RPA JA/03/01 to determine residues of prothioconazole and prothioconazole-desthio in various plant matrices. Overall, the

data encompass the expected residue levels, and adequately quantitate the analytes in the plant matrices.

The method is proposed for both data-gathering and enforcement purposes. The extraction efficiency data submitted is adequate for samples of sugar beet tops and wheat forage. The independent laboratory validation data submitted is adequate for peanut nutmeat and wheat forage.

E. REFERENCES

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46246141 Haas, M.; Bornatsch, W. (2000) Metabolism of JAU 6476 in Spring Wheat (after foliar application). Project Number: M/1730851/5, 110880, MR/198/99. Unpublished study prepared by Bayer Ag Institut fuer Ruckstands-Analytik. 149 p.

46246142 Haas, M. (2001) Metabolism of JAU 6476 in Spring Wheat after Seed Dressing. Project Number: M/1730885/2, 110881, MR/467/99. Unpublished study prepared by Bayer Ag Institut fuer Ruckstands-Analytik. 84 p.

46246143 Duah, F.; Lopez, R. (2004) The Metabolism of [Triazole-3, 5-(Carbon 14)] JAU6476 in Wheat. Project Number: J6041601, 200733. Unpublished study prepared by Bayer Corp. 197 p.

46246145 Haas, M. (2001) Metabolism of [Phenyl-UL-(Carbon 14)]JAU6476 in Peanuts. Project Number: M/1730984/2, MR/193/01. Unpublished study prepared by Bayer Ag Institut fuer Ruckstands-Analytik. 130 p.

46246146 Haas, M. (2003) Metabolism of [trizole-UL-(Carbon 14)]JAU6476 in Peanuts. Project Number: M1731145/2, MR/194/02. Unpublished study prepared by Bayer Ag, Institute of Product Info. 145 p.

46246147 Beedle, E.; Ying, S. (2004) The Metabolism of [Triazole-UL-(Carbon 14)]JAU6476 in Sugar Beets. Project Number: J6041603, 200467. Unpublished study prepared by Bayer Corp. 91 p.

46246148 Beedle, E.; Ying, S. (2004) The Metabolism of [Phenyl-UL-(Carbon 14)]JAU6476 in Sugar Beets. Project Number: J6041602, 200466. Unpublished study prepared by Bayer Corp. 86 p.

F. DOCUMENT TRACKING

RDI: Suzan Mathew (PMRA) 23/01/2006; Henri Bietlot (PMRA) 24/01/2006; Stephen Funk (IO)13/03/2006; Leung Cheng (RAB3).
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In the absence of signatures, this document is considered to be a draft with deliberative material for internal use only.

This DER was originally prepared under contract by Dynamac Corporation (20440 Century Boulevard, Suite 100; Germantown, MD 20874; submitted 05/25/2005). The DER has been reviewed by the Health Effects Division (HED) and Health Canada's Pest Management Regulatory Agency (PMRA). The DER has been revised to reflect current Office of Pesticide Programs (OPP) policies, and Directive 98-02.

STUDY REPORT:

46246218 Kraai, M. (2004) JAU6476 480 SC - Magnitude of the Residues in/on Wheat Grain, Wheat Aspirated Grain Fractions, and Wheat Processed Commodities. Lab Project Number: J619WH02: RCJAY004: 200521. Unpublished study prepared by Bayer CropScience. 744 p.

EXECUTIVE SUMMARY:

Bayer CropScience has submitted a processing study with wheat. In a single test conducted in KS during 2000, the 4 lb/gal (480 g/L) suspension concentrate formulation (flowable concentrate, FlC) was applied to wheat plants during flowering as two broadcast foliar applications with an 11-day retreatment interval at 0.564 lb a.i./A (0.632 kg a.i./ha) for the first application and 0.903 lb a.i./A (1.01 kg a.i./ha) for the second application, for a total application

rate of 1.467 lb a.i./A (1.64 kg a.i./ha; ~5.6 times the field trial application rate). Wheat grain was harvested at maturity 47 days after the last treatment. Samples of wheat grain (RAC) were collected, and the remaining bulk samples were processed into aspirated grain fractions, bran, middlings, shorts, flour, and germ using simulated commercial procedures.

Samples were analyzed for total prothioconazole-derived residues (prothioconazole and the metabolite prothioconazole-desthio), 1*H*-1,2,4-triazole and the triazole conjugates triazolylalanine and triazolylacetic acid using an LC-MS/MS method. The validated LOQs for prothioconazole-derived residues were 0.02 ppm for wheat grain, bran, flour, middlings, and shorts, and 0.25 ppm for aspirated grain fractions. The validated LOQ for 1*H*-1,2,4-triazole was 0.01 ppm for wheat grain and all processed commodities, and the validated LOQs for the triazole conjugates were 0.01 ppm for wheat grain, 0.20 ppm for aspirated grain fractions and bran, 0.30 ppm for wheat germ and shorts, and 0.25 ppm for middlings. The methods are adequate for data collection based on acceptable method validation and concurrent recovery data.

The maximum storage intervals from collection to analysis for total prothioconazole-derived residues were 1285 days (42 months) for wheat grain and 909 days (30 months) for processed wheat commodities. Prothioconazole-derived residues are relatively stable up to 1 year (interim report) in wheat matrices. Corrections due to apparent dissipation of prothioconazole-derived residues in samples stored beyond a year are not necessary due to the low absolute (ppm) and % residue levels in wheat matrices. Residues of prothioconazole-desthio are stable for up to 1 year and the degree of loss is not expected to exceed 30% after 42 months.

Residues of prothioconazole in/on wheat grain (RAC) from the processing study were 0.051 ppm for total prothioconazole-derived residues, less than the LOQ (<0.01 ppm) for 1*H*-1,2,4-triazole, and 1.33 ppm for residues of the triazole conjugates (average of triplicate analyses for each). Total prothioconazole-derived residues concentrated in aspirated grain fractions (245-fold), bran (2.4-fold), and germ (twofold). There was no concentration of residues in flour, middlings, and shorts (<0.4-fold, 0.6-fold, and onefold, respectively). Residues of the triazole conjugates concentrated in bran (3.1-fold), germ (3.6-fold), and shorts (1.5-fold), but did not concentrate in aspirated grain fractions, flour, and middlings (0.3-fold, 0.5-fold, and 0.6-fold). Residues of 1*H*-1,2,4-triazole were below the LOQ (<0.01 ppm) in all processed wheat commodities.

The reported processing factors do not exceed the theoretical concentration factors of 7.7-fold for wheat bran, 1.4-fold for wheat flour, and 8.3-fold for wheat shorts.

STUDY/WAIVER ACCEPTABILITY/DEFICIENCIES/CLARIFICATIONS:

Under the conditions and parameters used in the study, the wheat processing data are tentatively classified as scientifically acceptable. The final reports of the ongoing storage stability studies with prothioconazole, prothioconazole-desthio, 1*H*-1,2,4-triazole, triazolylalanine, and triazolylacetic acid will be submitted as confirmatory data.

The acceptability of this study for regulatory purposes is addressed in the forthcoming U.S. EPA Residue Chemistry Summary Document, DP Barcode D303508, and in Canada's Regulatory Decision Document.

COMPLIANCE:

Signed and dated GLP, quality assurance, and data confidentiality statements were provided. No deviations from regulatory requirements were noted that would impact the study results or their interpretation.

A. BACKGROUND INFORMATION

Prothioconazole is a broad-spectrum systemic fungicide intended for the control of many diseases in different crops such as cereals, oilseed rape or peanuts. The biochemical mode of action is the inhibition of the demethylation of lanosterol or 24-methylene-dihydrolanosterol, which are precursors of sterols in fungi. The chemical has been submitted for joint EPA and PMRA review by Bayer CropScience. The crops proposed for joint review include barley, the dried shell and bean subgroup, the oilseed crop group, and wheat. Uses on peanuts and rice are being proposed for the U.S. only.

TABLE A.1. Test Compound Nomenclature.

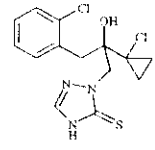
Chemical structure	
Common name	Prothioconazole (ISO accepted)
Company experimental name	JAU6476
IUPAC name	2-[(2*RS*)-2-(1-chlorocyclopropyl)-3-(2-chlorophenyl)-2-hydroxypropyl]-2*H*-1,2,4-triazole-3(4*H*)-thione
CAS name	2-[2-(1-chlorocyclopropyl)-3-(2-chlorophenyl)-2-hydroxypropyl]-1,2-dihydro-3*H*-1,2,4-triazole-3-thione
CAS registry number	178928-70-6
PC Code	113961
End-use product (EP)	Proline® 480 SC (4 lb/gal suspension concentrate), EPA File Symbol 264-IEL

TABLE A.2. Physicochemical Properties of Prothioconazole

Parameter	Value	Reference
Melting range	139.1 to 144.5°C	MRID 46246003 / CES[1]
pH	5.8 (1% solution)	MRID 46246003 / CES
Density at 20°C	1.36 g/mL	MRID 46246003 / CES
Water solubility at 20°C	pH: mg/L 4: 5 8: 300 9: 2000	MRID 46246003 / CES
Solvent solubility at 20°C	Solvent: g/L Acetone: >250 Acetonitrile: 69 Dichloromethane: 88 Dimethysulfoxide: 126 Ethyl acetate: >250 n-Heptane: <0.1 1-Octanol: 58 Polyethylene glycol: >250 2-Propanol: 87 Xylene: 8	MRID 46246003 / CES
Vapor pressure at 20 or 25°C	$<4 \times 10^{-7}$ Pa (calculated from determinations at 70 °C)	MRID 46246003 / CES
Dissociation constant, pK_a	6.9 (calculated from K_{OW})	MRID 46246003 / CES
Octanol/water partition coefficient, Log(K_{OW}), at 20°C	pH: Log Kow unbuffered water: 4.05 pH 4: 4.16 pH 7: 3.82 pH 9: 2.00	MRID 46246003 / CES
UV/visible absorption spectrum	Peak maxima at 257 nm	MRID 46246003 / CES

[1] CES: Chemistry Evaluation Section of PMRA

B. EXPERIMENTAL DESIGN

B.1. Application and Crop Information

TABLE B.1. Study Use Pattern on Wheat

Location (City, State, Year)	Application						
	EP[1]	Method; Timing	Volume (gal/A) [L/ha]	Single Rate (lb a.i./A) [kg a.i./ha]	No. of Appl.	RTI[2] (days)	Total Rate (lb a.i./A) [kg a.i./ha]
Stilwel, KS, 2000	480 SC	1: Broadcast foliar; 70% inflorescence emerge 2: Broadcast foliar; end of flowering	10 [95-96]	1: 0.564 [0.632] 2: 0.903 [1.01]	2	11	1.47 [1.64]

[1] EP = End-use Product; 480 SC = 4 lb/gal (480 g/L) suspension concentrate (flowable concentrate; FlC) formulation.
[2] RTI = Re-treatment interval.
No tank mix adjuvant was used

B.2. Processing Procedures

Wheat grain was harvested at maturity 47 days after the last treatment. After collection, wheat grain samples were placed in frozen storage at the field site within 1 hour and were stored frozen for 111 days, then shipped frozen via freezer truck to FPRDC at Texas A&M University (Bryan, TX). Grain samples were processed into aspirated grain fractions, bran, flour, middlings, shorts, and germ using simulated commercial procedures. The processed fractions and subsamples of grain were stored frozen, then shipped frozen to Battelle-Agrifood (Columbus, OH), where they were stored frozen (≤ -4.8°C) prior to shipment to Bayer Research Park (Stilwell, KS) for analysis.

Briefly, the wheat samples were cleaned, and the light impurities were collected by aspiration. Cleaned grain was moisture adjusted, broken into small pieces in a corrugated roller mill, and sieved to separate the bran and middlings. The middlings were reduced to flour in a smooth roller mill and sieved to separate into shorts, low-grade flour, and patent flour. Aspirated grain fractions (grain dust) were also collected.

The wheat processing procedures are summarized in the flow charts below, which were copied without alteration from the data package.

FIGURE B.2.1. Processing Procedures for Wheat Aspirated Grain Fractions.

MATERIAL BALANCE for ASPIRATED GRAIN FRACTION (GRAIN DUST) GENERATION

Sample # 2 (Treated) Code # J6065-00P-002

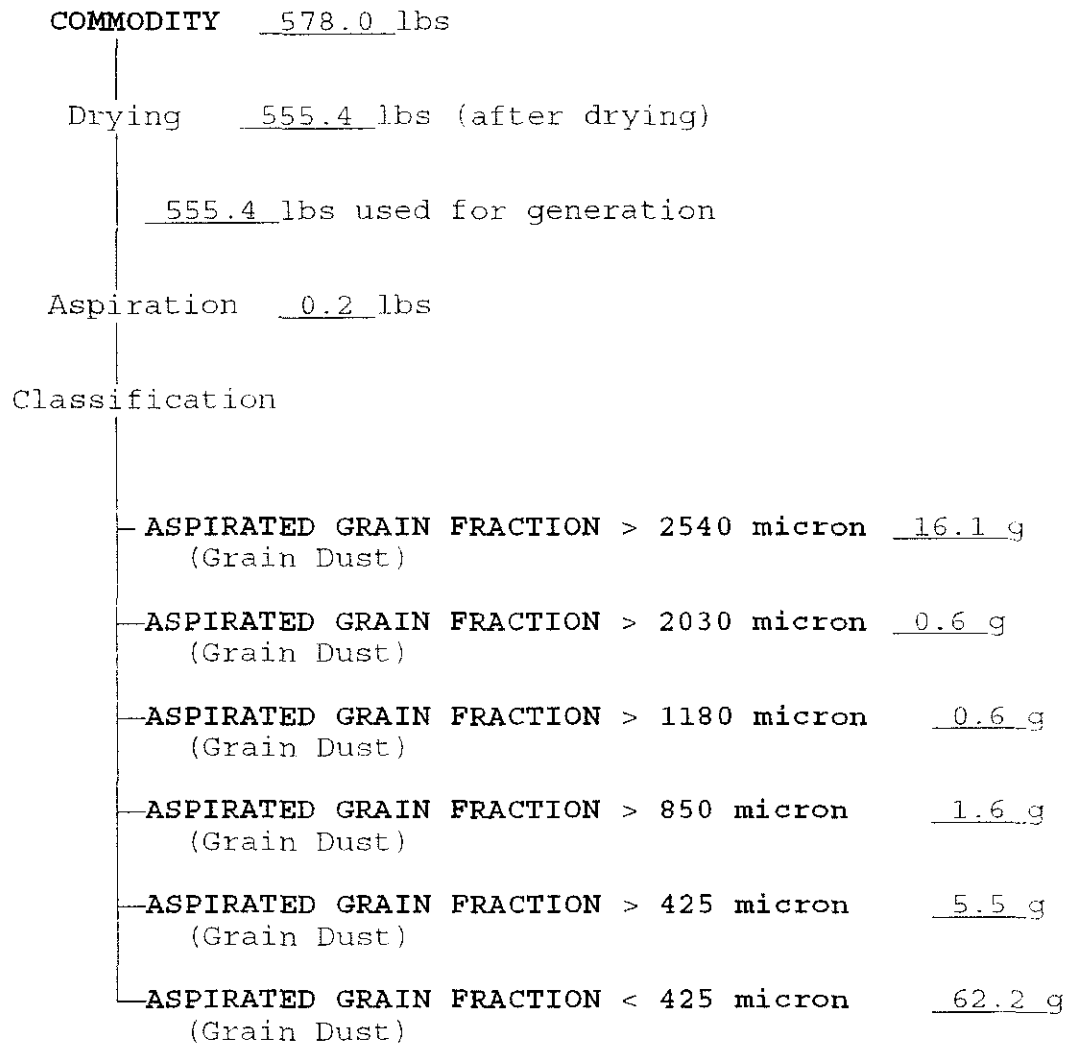
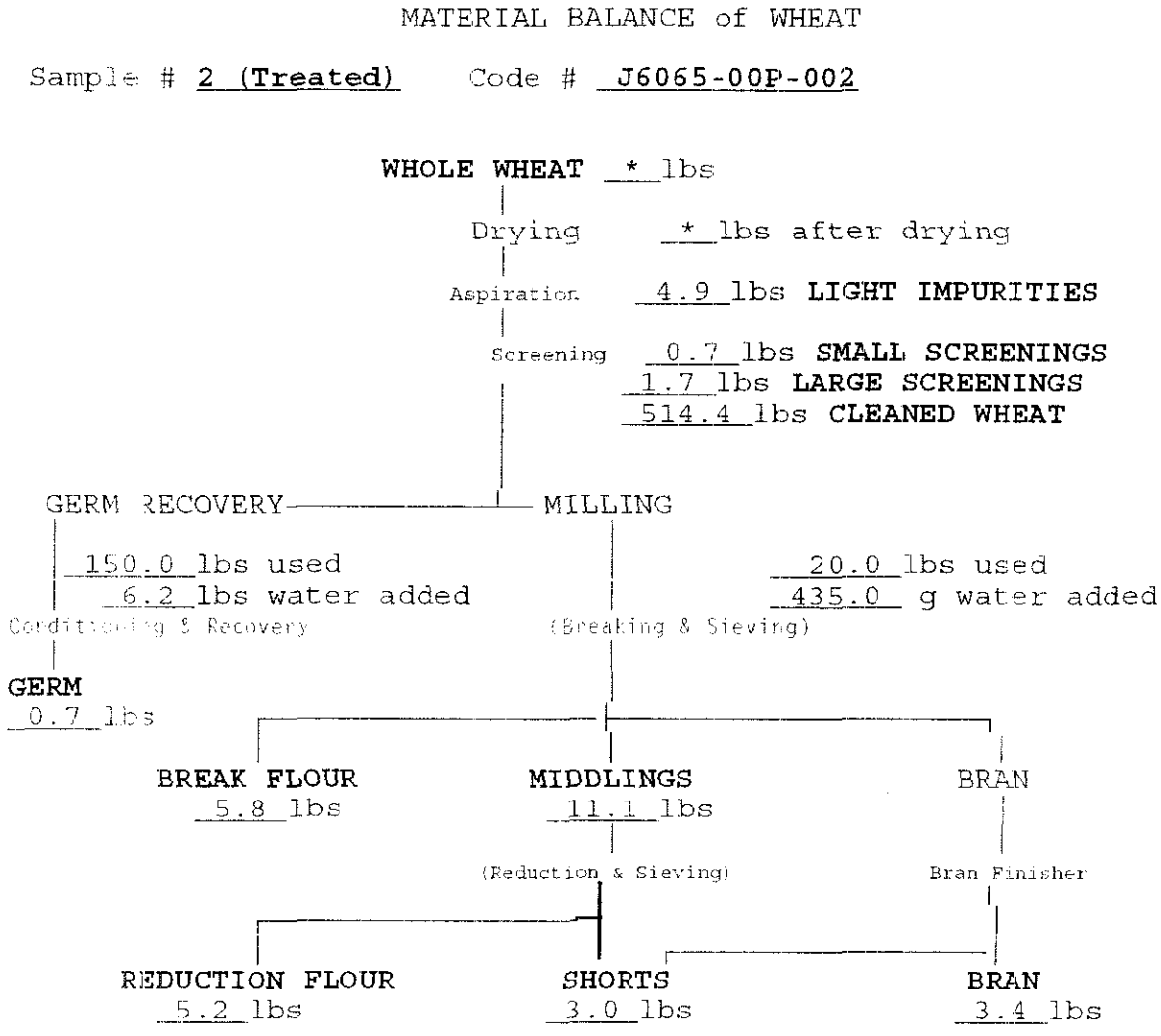


FIGURE B.2.2. Processing Procedures for Wheat Grain.



* Refer to form 300.21.

B.3. Analytical Methodology

Samples of wheat matrices were analyzed for total prothioconazole-derived residues (prothioconazole and the metabolite prothioconazole-desthio) using LC-MS/MS method RPA JA/03/01 with the following modifications: a different solvent was used to prepare the fortification standards and a smaller sample size was used in the extraction of the wheat aspirated grain fractions. Samples were 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 was spiked 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. Residue levels were quantitated from a calibration curve of mixed reference standards of prothioconazole, as the sulfonic acid, and prothioconazole-desthio. The validated LOQs were 0.02 ppm for wheat grain, bran, flour, middlings, and shorts, and 0.25 ppm for aspirated grain fractions.

Samples were analyzed for residues of 1*H*-1,2,4-triazole and the triazole conjugates (triazolylalanine and triazolylacetic acid) using an LC-MS/MS method (Bayer Report No. G200598) with modifications. Briefly, crop matrices were extracted with aqueous methanol, and three separate aliquots of the extract were removed for determination of each of the three analytes. Isotopically labeled internal standard was added to each aliquot. For 1*H*-1,2,4-triazole, the aliquot was mixed with dansyl chloride to form the dansyl derivative of 1*H*-1,2,4-triazole, which was partitioned into ethyl acetate and then redissolved in acetonitrile (ACN)/water for LC-MS/MS analysis. For triazolylalanine, the aliquot was cleaned up by SPE, derivatized to the butyl ester using butanolic HCl, and then further derivatized using heptafluorobutyric anhydride (HFBA). The mixture was redissolved in ACN/water for LC-MS/MS analysis. For determination of triazolylacetic acid, the aliquot was cleaned up by SPE, derivatized to the butyl ester using butanolic HCl, and then redissolved in ACN/water for LC-MS/MS analysis. Residue levels were quantitated from a calibration curve of reference standards of dansyl-1,2,4-triazole, triazolylalanine butyl ester HFBA, and triazolylacetic acid butyl ester (generated by derivatizing reference standard solutions of triazolylacetic acid). The validated LOQ for 1*H*-1,2,4-triazole was 0.01 ppm for wheat grain and all processed commodities, and the validated LOQs for the triazole conjugates were 0.01 ppm for wheat grain, 0.20 ppm for aspirated grain fractions and bran, 0.30 ppm for wheat germ and shorts, and 0.25 ppm for middlings for the triazole conjugates.

C. RESULTS AND DISCUSSION

Concurrent method recovery data are presented in TABLE C.1. Samples were analyzed for total prothioconazole-derived residues (prothioconazole and the metabolite prothioconazole-desthio) using LC-MS/MS method RPA JA/03/01. The validated LOQs were 0.02 ppm for wheat grain, bran, flour, middlings, and shorts, and 0.25 ppm for aspirated grain fractions. Samples were analyzed for residues of 1*H*-1,2,4-triazole and the triazole conjugates triazolylalanine and triazolylacetic acid using an LC-MS/MS method (Bayer Report No. G200598). The validated LOQ for 1*H*-1,2,4-triazole was 0.01 ppm for wheat grain and all processed commodities, and the validated LOQs for residues of the triazole conjugates were 0.01 ppm for wheat grain, 0.20 ppm for aspirated grain fractions and bran, 0.30 ppm for wheat germ and shorts, and 0.25 ppm for middlings. The methods are adequate for data collection based on acceptable method validation and concurrent recovery data.

Sample storage intervals and conditions are summarized in TABLE C.2. The maximum storage intervals from collection to analysis for total prothioconazole-derived residues were 1285 days

(42 months) for wheat grain samples and 909 days (30 months) for processed wheat commodities. Prothioconazole-derived residues and prothioconazole-desthio residues are stable for up to 1 year based on an interim report. Although, there is apparent instability of prothioconazole-derived residues in wheat bran (33%) after 12.7 months in freezer storage. However, the need to correct the results of the current study for decline of prothioconazole-derived residues will not be necessary taking into account the absolute (ppm) and relative % residue levels in wheat matrices. The degree of loss of prothioconazole-desthio residues is not expected to exceed 30% after 42 months.

The maximum storage intervals from collection to analysis for 1*H*-1,2,4-triazole and the triazole conjugate residues were 1286 days (42 months) for wheat grain samples and 914 days (30 months) for processed wheat commodities.

Residue data from the wheat processing study with prothioconazole are reported in TABLE C.3. Residues of prothioconazole in/on wheat grain (RAC) from the processing study were 0.051 ppm for total prothioconazole-derived residues, less than the LOQ (<0.01 ppm) for 1*H*-1,2,4-triazole, and 1.33 ppm for residues of the triazole conjugates (average of triplicate analyeses for each). The processing data for wheat (from wheat grain bearing quantifiable residues) indicated that total prothioconazole-derived residues concentrated in aspirated grain fractions (245-fold), bran (2.4-fold), and germ (twofold) and did not concentrate in flour, middlings, and shorts (respective processing factors of <0.4-fold, 0.6-fold, and onefold). Residues of the triazole conjugates concentrated in bran (3.1-fold), germ (3.6-fold), and shorts (1.5-fold), but did not concentrate in aspirated grain fractions, flour, and middlings (respective processing factors of 0.3-fold, 0.5-fold, and 0.6-fold). Residues of 1*H*-1,2,4-triazole were below the LOQ (<0.01 ppm) in all processed wheat commodities.

The reported processing factors did not exceed the theoretical concentration factors of 7.7-fold for wheat bran, 1.4-fold for wheat flour, and 8.3-fold for wheat shorts.

TABLE C.1. Summary of Method Validation and Concurrent Recoveries of Prothioconazole from Wheat Commodities.

Wheat matrix	Analyte	Spike level (ppm)	Sample size (n)	Recoveries (%)	Mean ± std dev
Method Validation Recoveries					
Grain	1*H*-1,2,4-triazole	0.01	3	71, 71, 72	71 ± 1
	Triazolylalanine	0.01	3	88, 90, 85	88 ± 3
		1.4	3	88, 90, 92	90 ± 2
	Triazolylacetic acid	0.01	3	84, 98, 105	96 ± 11
		0.7	3	79, 80, 80	80 ± 1
Aspirated Grain Fractions	1*H*-1,2,4-triazole	0.01	3	92, 88, 113	98 ± 13
	Triazolylalanine	0.1	3	81, 76, 82	80 ± 3
	Triazolylacetic acid	0.2	3	102, 98, 105	102 ± 4

TABLE C.1. Summary of Method Validation and Concurrent Recoveries of Prothioconazole from Wheat Commodities.

Wheat matrix	Analyte	Spike level (ppm)	Sample size (n)	Recoveries (%)	Mean ± std dev
Bran	1*H*-1,2,4-triazole	0.01	3	71, 84, 62	72 ± 11
	Triazolylalanine	3.2	3	70, 80, 73	74 ± 5
		4	3	87, 89, 88	88 ± 1
	Triazolylacetic acid	0.8	3	79, 81, 86	82 ± 4
Flour	1*H*-1,2,4-triazole	0.01	3	63, 78, 96	79 ± 17
	Triazolylalanine	0.1	3	91, 93, 93	92 ± 1
	Triazolylacetic acid	0.3	3	83, 75, 75	78 ± 5
Germ	1*H*-1,2,4-triazole	0.01	3	82, 81, 81	81 ± 1
	Triazolylalanine	4.5	3	93, 90, 84	89 ± 5
	Triazolylacetic acid	0.5	4	81, 77, 80, 97	84 ± 9
Middlings	1*H*-1,2,4-triazole	0.01	3	89, 85, 95	90 ± 5
		0.25	1	94	--
	Triazolylalanine	0.2	3	88, 67, 84	80 ± 11
	Triazolylacetic acid	0.3	5	98, 87, 92, 95, 83	91 ± 6
Shorts	1*H*-1,2,4-triazole	0.01	3	98, 93, 77	89 ± 11
	Triazolylalanine	1.5	3	81, 74, 81	79 ± 4
	Triazolylacetic acid	0.3	3	96, 78, 86	87 ± 9
Concurrent Recoveries					
Grain	Prothioconazole	0.02	1	75	--
		0.2	3	83, 81, 83	82 ± 1
	Prothioconazole-desthio	0.02	1	116	--
		0.2	3	108, 108, 109	108 ± 1
	1*H*-1,2,4-triazole	0.5	2	87, 92	90 ± 4
	Triazolylalanine	0.5	3	75, 77, 78	77 ± 2
	Triazolylacetic acid	0.5	2	74, 71	73 ± 2
Aspirated Grain Fractions	Prothioconazole	0.25	3	84, 85, 85	85 ± 1
		12.5	3	82, 83, 83	83 ± 1
	Prothioconazole-desthio	0.25	3	98, 98, 102	99 ± 2
		12.5	3	97, 96, 99	97 ± 2
	1*H*-1,2,4-triazole	0.5	3	92, 99, 101	97 ± 5
	Triazolylalanine	0.5	3	87, 80, 84	84 ± 4
	Triazolylacetic acid	0.5	3	97, 91, 92	93 ± 3

TABLE C.1. Summary of Method Validation and Concurrent Recoveries of Prothioconazole from Wheat Commodities.

Wheat matrix	Analyte	Spike level (ppm)	Sample size (n)	Recoveries (%)	Mean ± std dev
Bran	Prothioconazole	0.02	3	84, 79, 79	81 ± 3
		0.2	3	84, 85, 86	85 ± 1
	Prothioconazole-desthio	0.02	3	104, 103, 103	103 ± 1
		0.2	3	105, 105, 106	105 ± 1
	1*H*-1,2,4-triazole	0.5	4	88, 90, 93, 96	92 ± 4
	Triazolylalanine	0.5	4	91, 89, 72, 84	84 ± 9
	Triazolylacetic acid	0.5	4	89, 84, 78, 89	85 ± 5
Flour	Prothioconazole	0.02	3	74, 69, 73	72 ± 3
		0.2	3	84, 83, 84	84 ± 1
	Prothioconazole-desthio	0.02	3	101, 103, 104	103 ± 2
		0.2	3	107, 106, 106	106 ± 1
	1*H*-1,2,4-triazole	0.5	4	103, 97, 100, 87	97 ± 7
	Triazolylalanine	0.5	4	77, 93, 90, 103	91 ± 11
	Triazolylacetic acid	0.5	4	72, 84, 78, 82	79 ± 5
Germ	Prothioconazole	0.02	3	72, 81, 78	77 ± 5
		0.2	3	86, 83, 84	84 ± 2
	Prothioconazole-desthio	0.02	3	90, 96, 98	95 ± 4
		0.2	3	100, 100, 101	100 ± 1
	1*H*-1,2,4-triazole	0.5	4	87, 89, 83, 91	88 ± 3
	Triazolylalanine	0.5	4	89, 81, 83, 110	91 ± 13
	Triazolylacetic acid	0.3	3	93, 98, 91	94 ± 4
Middlings	Prothioconazole	0.02	3	77, 83, 78	79 ± 3
		0.2	3	90, 92, 93	92 ± 2
	Prothioconazole-desthio	0.02	3	102, 100, 102	101 ± 1
		0.2	3	106, 104, 110	107 ± 3
	1*H*-1,2,4-triazole	0.25	4	74, 82, 82, 89	82 ± 6
		0.5	3	97, 98, 100	98 ± 2
	Triazolylalanine	0.15	3	73, 119, 71	88 ± 27
		0.5	3	72, 77, 76	75 ± 3
		1	3	72, 79, 75	75 ± 4
	Triazolylacetic acid	0.25	3	80, 81, 81	81 ± 1
		0.5	3	73, 81, 71	75 ± 5

TABLE C.1. Summary of Method Validation and Concurrent Recoveries of Prothioconazole from Wheat Commodities.

Wheat matrix	Analyte	Spike level (ppm)	Sample size (n)	Recoveries (%)	Mean ± std dev
Shorts	Prothioconazole	0.02	3	90, 92, 86	89 ± 3
		0.2	3	91, 93, 90	91 ± 2
	Prothioconazole-desthio	0.02	3	103, 101, 104	103 ± 2
		0.2	3	111, 108, 101	107 ± 5
	1*H*-1,2,4-triazole	0.3	3	90, 98, 91	93 ± 4
		0.5	3	97, 95, 98	97 ± 2
	Triazolylalanine	0.3	3	73, 83, 72	76 ± 6
		0.5	3	86, 84, 90	87 ± 3
	Triazolylacetic acid	0.3	3	78, 82, 75	78 ± 4
		0.5	3	76, 76, 82	78 ± 3

TABLE C.2. Summary of Freezer Storage Conditions

Wheat Matrix	Storage Temp. (°C)	Actual Storage Duration [1]	Limit of Demonstrated Storage Stability
Total Prothioconazole-Derived Residues			
Grain	<-5	1285 days (42 months)	Stability demonstrated for up to 12.7 months (interim report). Awaiting final report as confirmatory data.
Aspirated grain fractions, bran, germ, flour, middlings, and shorts		909 days (30 months)	
1*H*-1,2,4-triazole and Triazole Conjugate Residues			
Grain	<-5	1286 days (42 months)	Not applicable at this time. Awaiting final report.
Aspirated grain fractions, bran, germ, flour, middlings, and shorts		914 days (30 months)	

[1] Extracts were stored frozen for <36 days prior to analysis.

TABLE C.3. Residue Data from Wheat Processing Study with Prothioconazole.

Processed Commodity	Total Rate (lb a.i./A) [kg a.i./ha]	PHI (days)	Total Prothioconazole-Derived		1*H*-1,2,4-triazole		Triazole Conjugate	
			Residues (ppm) [1]	Processing Factor	Residues (ppm) [1]	Processing Factor [2]	Residues (ppm) [1]	Processing Factor
Grain (RAC)	1.47 [1.64]	47	0.048, 0.049, 0.054 (**0.051**)	--	<0.01, <0.01, <0.01	--	1.27, 1.33, 1.38 (**1.33**)	--
Aspirated grain fractions			12.3, 12.5, 12.9 (**12.5**)	245-fold	<0.01	NC	**0.349**	0.3-fold
Bran			0.118, 0.119, 0.127 (**0.121**)	2.4-fold	<0.01, <0.01, <0.01	NC	4.08, 4.18, 4.29 (**4.18**)	3.1-fold
Flour			<0.02, <0.02, <0.02 (**<0.02**)	<0.4-fold	<0.01, <0.01, <0.01	NC	0.587, 0.634, 0.767 (**0.663**)	0.5-fold
Germ			0.092, 0.104, 0.106 (**0.100**)	2.0-fold	<0.01, <0.01, <0.01	NC	4.68, 4.88, 4.97 (**4.84**)	3.6-fold
Middlings			0.028, 0.028, 0.031 (**0.029**)	0.6-fold	<0.01, <0.01, <0.01	NC	0.54, 0.74, 0.76, 0.91 1.03 (**0.80**)	0.6-fold
Shorts			0.048, 0.051, 0.052 (**0.050**)	1.0-fold	<0.01, <0.01, <0.01	NC	2.00, 2.03, 2.04, (**2.03**)	1.5-fold

[1] Samples were analyzed in triplicate; average residues are **bolded** and reported in parentheses..
[2] NC = not calculated.

D. CONCLUSION

Total prothioconazole-derived residues concentrated in aspirated grain fractions (245-fold), bran (2.4-fold), and germ (twofold) and did not concentrate in flour, middlings, and shorts (<0.4-fold, 0.6-fold, and onefold, respectively). Residues of the triazole conjugates concentrated in bran (3.1-fold), germ (3.6-fold), and shorts (1.5-fold), but did not concentrate in aspirated grain fractions, flour, and middlings (0.3-fold, 0.5-fold, and 0.6-fold, respectively). Residues of 1*H*-1,2,4-triazole were below the LOQ (<0.01 ppm) in all processed wheat commodities. Acceptable methods were used for quantitation of residues in/on wheat grain and its processed commodities.

E. REFERENCES

Gould T. and Timberlake B. (2005) Storage stability of JAU6476 and desthio JAU6476 in canola, wheat, mustard greens, turnip root, and tomato fruit, processed products, and solutions, Bayer CropScience Interim Report.

F. DOCUMENT TRACKING

RDI: Louise Croteau, Suzan Mathew (PMRA) 23/01/2006; Henri Bietlot (PMRA) 24/01/2006; Stephen Funk (IO) 13/03/2006; Leung Cheng (RAB3).
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Date: Jan 24/06

In the absence of signatures, this document is considered to be a draft with deliberative material for internal use only.

This DER was originally prepared under contract by Dynamac Corporation (20440 Century Boulevard, Suite 100; Germantown, MD 20874; submitted 05/25/2005). The DER has been reviewed by the Health Effects Division (HED) and Health Canada's Pest Management Regulatory Agency (PMRA). The DER has been revised to reflect current Office of Pesticide Programs (OPP) policies, and Directive 98-02.

STUDY REPORT:

46246222 Harbin, A. (2004) JAU6476 480 SC - Magnitude of the Residues in/on Rice Grain and Rice Processed Commodities. Lab Project Number: J619RI02: RCJAY003: 200493. Unpublished study prepared by Bayer CropScience. 464 p.

EXECUTIVE SUMMARY:

Bayer CropScience has submitted a processing study with rice. In a single test conducted in MS during 2000, the 4 lb/gal (480 g/L) suspension concentrate formulation (flowable concentrate; FlC), was applied to rice plants during panicle formation as two broadcast foliar applications with a 13-day re-treatnent interval at 1.07 lb a.i./A (1.20 kg a.i./ha) for the first application and 0.75 lb a.i./A (0.84 kg a.i./ha) for the second application, for a total application rate of 1.82 lb

a.i./A (2.04 kg a.i./ha; ~5 times the field trial application rate). Rice grain was harvested at maturity 49 days after the last treatment. Sub-samples of rice grain (RAC) were collected, and the remaining bulk samples were processed into polished grain, bran, and hulls using simulated commercial procedures.

Samples were analyzed for total prothioconazole-derived residues (prothioconazole and the metabolite prothioconazole-desthio), 1*H*-1,2,4-triazole and the triazole conjugates triazolylalanine and triazolylacetic acid using an LC-MS/MS method. The validated LOQs for prothioconazole-derived residues were 0.02 ppm for rice grain, polished grain, and bran, and 0.01 ppm for rice hulls. The validated LOQ for 1*H*-1,2,4-triazole was 0.01 ppm, and the validated LOQs for the triazole conjugates were 0.05 ppm for rice grain, polished grain, and hulls, and 0.75 ppm for rice bran. The methods are adequate for data collection based on acceptable concurrent method recovery data.

The maximum storage intervals from collection to analysis for total prothioconazole-derived residues were 1222 days (40 months) for rice grain and 902 days (30 months) for processed rice commodities. Prothioconazole-derived residues are stable up to 1 year (interim report) in rice matrices. The degree of loss of prothioconazole-derived residues and prothioconazole-desthio residues is not expected to exceed 30% after 40 months in rice and its processed commodities.

Total prothioconazole-derived residues concentrated in rice hulls (4.4-fold) but not in polished grain or bran (<0.1-fold and 0.6-fold, respectively). Residues of the triazole conjugates concentrated in bran (6.9-fold), but did not concentrate in polished rice or hulls (0.5-fold and 0.3-fold, respectively). Residues of 1*H*-1,2,4-triazole were below the LOQ (<0.01 ppm) in polished rice, bran, and hulls. The reported processing factors did not exceed the theoretical concentration factors of 5.0-fold for rice hulls and 7.7-fold for rice bran.

STUDY/WAIVER ACCEPTABILITY/DEFICIENCIES/CLARIFICATIONS:

Under the conditions and parameters used in the study, the rice processing data are tentatively classified as scientifically acceptable. The final reports of the ongoing storage stability studies with prothioconazole, prothioconazole-desthio, 1*H*-1,2,4-triazole, triazolylalanine, and triazolylacetic acid will be submitted as confirmatory data.

The acceptability of this study for regulatory purposes is addressed in the forthcoming U.S. EPA Residue Chemistry Summary Document, DP Barcode D303508, and in Canada's Regulatory Decision Document.

COMPLIANCE:

Signed and dated GLP, quality assurance, and data confidentiality statements were provided. No deviations from regulatory requirements were noted that would impact the study results or their interpretation.

A. BACKGROUND INFORMATION

Prothioconazole is a broad-spectrum systemic fungicide intended for the control of many diseases in different crops such as cereals, oilseed rape or peanuts. The biochemical mode of action is the inhibition of the demethylation of lanosterol or 24-methylene-dihydrolanosterol, which are precursors of sterols in fungi. The chemical has been submitted for joint EPA and PMRA review by Bayer CropScience. The crops proposed for joint review include barley, the dried shell and bean subgroup, the oilseed crop group, and wheat. Uses on peanuts and rice are being proposed for the U.S. only.

TABLE A.1. Test Compound Nomenclature.

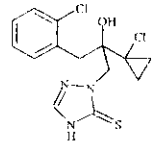
Chemical structure	
Common name	Prothioconazole (ISO accepted)
Company experimental name	JAU6476
IUPAC name	2-[(2*RS*)-2-(1-chlorocyclopropyl)-3-(2-chlorophenyl)-2-hydroxypropyl]-2*H*-1,2,4-triazole-3(4*H*)-thione
CAS name	2-[2-(1-chlorocyclopropyl)-3-(2-chlorophenyl)-2-hydroxypropyl]-1,2-dihydro-3*H*-1,2,4-triazole-3-thione
CAS registry number	178928-70-6
PC Code	113961
End-use product (EP)	Proline® 480 SC (4 lb/gal suspension concentrate), EPA File Symbol 264-IEL

TABLE A.2. Physicochemical Properties of Prothioconazole			
Parameter	Value		Reference
Melting range	139.1 to 144.5°C		MRID 46246003 / CES[1]
pH	5.8 (1% solution)		MRID 46246003 / CES
Density at 20°C	1.36 g/mL		MRID 46246003 / CES
Water solubility at 20°C	pH 4 8 9	mg/L 5 300 2000	MRID 46246003 / CES
Solvent solubility at 20°C	Solvent Acetone Acetonitrile Dichloromethane Dimethysulfoxide Ethyl acetate n-Heptane 1-Octanol Polyethylene glycol 2-Propanol Xylene	g/L >250 69 88 126 >250 <0.1 58 >250 87 8	MRID 46246003 / CES
Vapor pressure at 20 or 25°C	$<4 \times 10^{-7}$ Pa (calculated from determinations at 70 °C)		MRID 46246003 / CES
Dissociation constant, pK_a	6.9 (calculated from K_{OW})		MRID 46246003 / CES
Octanol/water partition coefficient, Log(K_{OW}), at 20°C	pH unbuffered water pH 4 pH 7 pH 9	Log Kow 4.05 4.16 3.82 2.00	MRID 46246003 / CES
UV/visible absorption spectrum	Peak maxima at 257 nm		MRID 46246003 / CES

[1] CES: Chemistry Evaluation Section of PMRA

B. EXPERIMENTAL DESIGN

B.1. Application and Crop Information

TABLE B.1. Study Use Pattern on Rice								
Location (City, State, Year)	Application							
	EP[1]	Method; Timing	Volume (gal/A) [L/ha]	Single Rate (lb a.i./A) [kg a.i./ha]	No. of Appl.	RTI[2] (days)	Total Rate (lb a.i./A)[3] [kg a.i./ha]	Tank Mix Adjuvants
Benoit, MS, 2000	480 SC	1: Broadcast foliar; Panicle initiation or green ring stage 2: Broadcast foliar; panicle formation	18-21 [172-199]	1: 1.07 [1.20] 2: 0.75 [0.84]	2	13	1.82 [2.04]	None

[1] EP = End-use Product; 480 SC = 4 lb/gal (480 g/L) suspension concentrate (flowable concentrate; FlC) formulation.
[2] RTI = Re-treatnent interval.

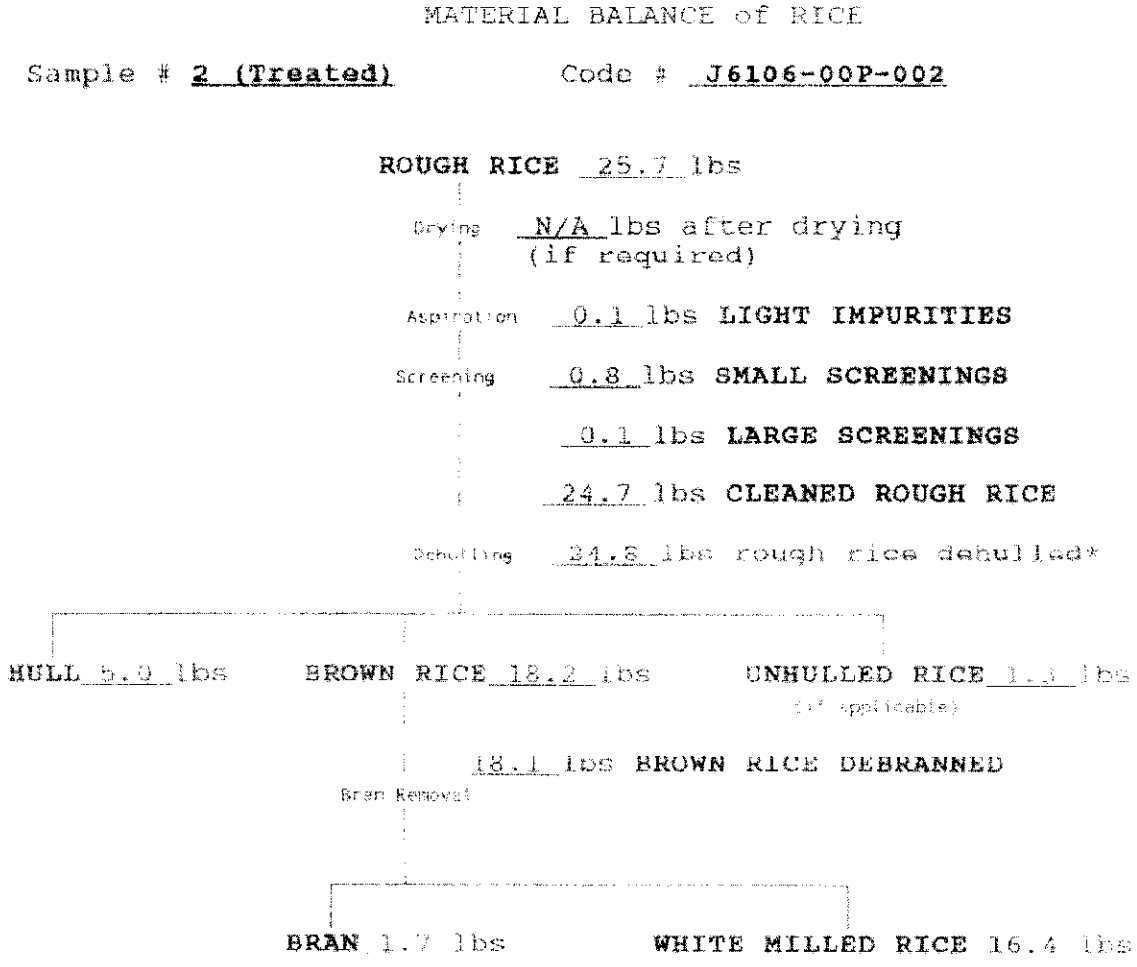
B.2. Processing Procedures

Rice grain was harvested at maturity 49 days after the last treatment. After collection, rice grain samples were placed in frozen storage at the field site within 5 minutes and were stored frozen for 8 days, then shipped frozen via freezer truck to FPRDC at Texas A&M University (Bryan, TX). Grain samples were processed into polished grain, bran, and hulls using simulated commercial procedures. The processed fractions and sub-samples of grain were stored frozen, then shipped frozen to Battelle-Agrifood Laboratories (Columbus, OH), where they were stored frozen (≤-4.8°C) prior to shipment to Bayer Research Park (Stilwell, KS) for analysis.

Briefly, unprocessed grain samples were cleaned by aspiration and mechanical screening. The aspirated fractions were further processed into grain dust by mechanical sifting and air-jet sieving. The cleaned grain sample that was caught on the 5/8-inch screen was milled two times to produce hulls, brown rice, and unhulled grain. The brown rice was processed through a commercial abrasion mill and screened to separate the polished rice (collected on the 1410-µm screen) and bran.

The rice processing procedures are summarized in the flow chart below, which was copied without alteration from the data package.

FIGURE B.2.1. Processing Procedures for Rice.



* Unexplained gain of 0.1 lb.

B.3. Analytical Methodology

Samples of rice matrices were analyzed for total prothioconazole-derived residues (prothioconazole and the metabolite prothioconazole-desthio) using LC-MS/MS method RPA JA/03/01 with the following modifications: a different solvent was used to prepare the spiking standards, slightly different quantitation ions were used, and different concentrations were used for the calibration standards. Samples were 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 was spiked 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. Residue levels were quantitated from a calibration curve of mixed reference standards of prothioconazole, as the sulfonic acid, and prothioconazole-desthio. The validated LOQs were 0.02 ppm for rice grain, polished grain, and bran, and 0.01 ppm for rice hulls.

Samples were analyzed for residues of 1*H*-1,2,4-triazole and the triazole conjugates (triazolylalanine and triazolylacetic acid) using an LC-MS/MS method (Bayer Report No. G200598) with modifications. Briefly, crop matrices were extracted with aqueous methanol, and three separate aliquots of the extract were removed for determination of each of the three analytes. Isotopically labeled internal standard was added to each aliquot. For 1*H*-1,2,4-triazole, the aliquot was mixed with dansyl chloride to form the dansyl derivative of 1*H*-1,2,4-triazole, which was partitioned into ethyl acetate and then redissolved in acetonitrile (ACN)/water for LC-MS/MS analysis. For triazolylalanine, the aliquot was cleaned up by SPE, derivatized to the butyl ester using butanolic HCl, and then further derivatized using heptafluorobutyric anhydride (HFBA). The mixture was redissolved in ACN/water for LC-MS/MS analysis. For determination of triazolylacetic acid, the aliquot was cleaned up by SPE, derivatized to the butyl ester using butanolic HCl, and then redissolved in ACN/water for LC-MS/MS analysis. Residue levels were quantitated from a calibration curve of reference standards of dansyl-1,2,4-triazole, triazolylalanine butyl ester HFBA, and triazolylacetic acid butyl ester (generated by derivatizing reference standard solutions of triazolylacetic acid). The validated LOQ for 1*H*-1,2,4-triazole was 0.01 ppm, and the validated LOQs for the triazole conjugates were 0.05 ppm for rice grain, polished grain, and hulls, and 0.75 ppm for rice bran.

C. RESULTS AND DISCUSSION

Concurrent method recovery data are presented in TABLE C.1. Samples were analyzed for total prothioconazole-derived residues (prothioconazole and the metabolite prothioconazole-desthio) using LC-MS/MS method RPA JA/03/01. The validated LOQs for total prothioconazole-derived residues were 0.02 ppm for rice grain, polished grain, and bran, and 0.01 ppm for rice hulls. Samples were analyzed for residues of 1*H*-1,2,4-triazole and the triazole conjugates triazolylalanine and triazolylacetic acid using an LC-MS/MS method (Bayer Report No. G200598). The validated LOQ for 1*H*-1,2,4-triazole was 0.01 ppm, and the validated LOQs for the triazole conjugates were 0.05 ppm for rice grain, polished grain, and hulls, and 0.75 ppm for rice bran. The methods are adequate for data collection based on acceptable concurrent method recovery data.

Sample storage intervals and conditions are summarized in TABLE C.2. The maximum storage intervals from collection to analysis for total prothioconazole-derived residues were 1222 days (40 months) for rice grain samples and 902 days (30 months) for processed rice commodities. Prothioconazole-derived residues are stable for up to 1 year based on an interim report. The need to correct the results of the current study for decline of prothioconazole-derived residues will not be necessary taking into account the absolute (ppm) and relative % residue levels in plant

matrices. The degree of loss of prothioconazole-desthio residues is not expected to exceed 30% after 40 months.

The maximum storage intervals from collection to analysis for 1*H*-1,2,4-triazole and triazole conjugate residues were 1232 days (40 months) for rice grain samples and 903 days (30 months) for processed rice commodities.

Residue data from the rice processing study with prothioconazole are reported in TABLE C.3. Residues of prothioconazole in/on rice grain (RAC) from the processing study were 0.176 ppm for total prothioconazole-derived residues, less than the LOQ (<0.01 ppm) for 1*H*-1,2,4-triazole, and 0.431 ppm for residues of the triazole conjugates. The processing data for rice indicated that in polished rice, bran, and hulls processed from rice grain bearing quantifiable residues, total prothioconazole-derived residues concentrated in rice hulls (4.4-fold) but did not concentrate in polished grain or bran (<0.1-fold and 0.6-fold, respectively). Residues of the triazole conjugates concentrated in bran (6.9-fold), but did not concentrate in polished rice or hulls (respective processing factors of 0.5-fold and 0.3-fold). Residues of 1*H*-1,2,4-triazole were below the LOQ (<0.01 ppm) in polished rice, bran, and hulls.

The reported processing factors did not exceed the theoretical concentration factors of 5.0-fold for rice hulls and 7.7-fold for rice bran.

TABLE C.1. Summary of Concurrent Recoveries of Prothioconazole from Rice Commodities.

Rice matrix	Analyte	Spike level (ppm)	Sample size (n)	Recoveries (%)	Mean ± std dev
Grain	Prothioconazole	0.02	1	86	--
	Prothioconazole-desthio	0.02	1	95	--
	1*H*-1,2,4-triazole	0.01	3	70, 72, 80	74 ± 5
		0.05	1	82	--
		0.1	1	95	--
	Triazolylalanine	0.05	4	86, 73, 87, 91	84 ± 8
		0.1	1	70	--
		0.5	3	100, 97, 100	99 ± 2
	Triazolylacetic acid	0.05	4	81, 70, 71, 74	74 ± 5
		0.1	1	86	--
		0.5	3	81, 83, 82	82 ± 1

TABLE C.1. Summary of Concurrent Recoveries of Prothioconazole from Rice Commodities.

Rice matrix	Analyte	Spike level (ppm)	Sample size (n)	Recoveries (%)	Mean ± std dev
Polished grain	Prothioconazole	0.02	3	81, 80, 79	80 ± 1
		0.2	3	86, 97, 98	94 ± 7
	Prothioconazole-desthio	0.02	3	100, 99, 101	100 ± 1
		0.2	3	104, 102, 104	103 ± 1
	1H-1,2,4-triazole	0.01	3	85, 99, 92	92 ± 7
		0.05	4	71, 71, 95, 98	84 ± 15
	Triazolylalanine	0.05	5	86, 70, 95, 71, 80	80 ± 10
		0.2	3	77, 79, 87	81 ± 5
	Triazolylacetic acid	0.05	4	80, 67, 86, 71	76 ± 9
		0.2	3	70, 73, 70	71 ± 2
Bran	Prothioconazole	0.02	3	76, 77, 80	78 ± 2
		0.2	3	82, 84, 85	84 ± 2
	Prothioconazole-desthio	0.02	3	99, 102, 100	100 ± 2
		0.2	3	103, 104, 102	103 ± 1
	1H-1,2,4-triazole	0.01	4	91, 87, 89, 100	92 ± 6
		0.05	3	93, 96, 85	91 ± 6
		0.5	1	98	--
	Triazolylalanine	0.5	1	81	--
		0.75	3	86, 89, 74	83 ± 8
		2	3	86, 81, 79	82 ± 4
	Triazolylacetic acid	0.5	1	97, 93 [1]	95
		0.75	3	103, 101, 100	101 ± 2
		0.75	3	97, 87, 91	92 ± 5
Hulls	Prothioconazole	0.1	3	75, 76, 79	77 ± 2
		1	3	79, 79, 79	79 ± 0
	Prothioconazole-desthio	0.1	3	105, 104, 104	104 ± 1
		1	3	98, 98, 100	99 ± 1
	1H-1,2,4-triazole	0.01	3	77, 77, 72	75 ± 3
		0.05	4	71, 82, 70, 86	77 ± 8
	Triazolylalanine	0.02	3	103, 116, 85	101 ± 16
		0.05	4	81, 64, 96, 79	80 ± 13
	Triazolylacetic acid	0.05	4	87, 108, 88, 119	100 ± 16
		0.20	3	96, 113, 108	106 ± 9

[1] Duplicate analysis of a single spiked sample.

TABLE C.2. Summary of Freezer Storage Conditions			
Rice Matrix	Storage Temp. (°C)	Actual Storage Duration [1]	Limit of Demonstrated Storage Stability
Total Prothioconazole-Derived Residues			
Grain	<-5	1222 days (40 months)	Stability demonstrated for up to 12.7 months (interim report). Awaiting final report as confirmatory data.
Polished grain, bran, and hulls		902 days (30 months)	
1*H*-1,2,4-triazole and Triazole Conjugate Residues			
Grain	<-5	1232 days (40 months)	Not applicable at this time. Awaiting final report.
Polished grain, bran, and hulls		903 days (30 months)	

[1] Extracts were stored frozen for ≤ 36 days prior to analysis.

TABLE C.3. Residue Data from Rice Processing Study with Prothioconazole (4 lb/gal FlC).								
Processed Commodity	Total Rate (lb a.i./A) [kg a.i./ha][1]	PHI (days)	Total Prothioconazole-Derived		1*H*-1,2,4-triazole		Triazole Conjugate	
			Residues (ppm)[1]	Processing Factor	Residues (ppm)[1]	Processing Factor[2]	Residues (ppm)[1]	Processing Factor
Grain (RAC)	1.82 [2.04]	49	0.174, 0.176, 0.179 (**0.176**)	--	<0.01, <0.01, <0.01	--	0.379, 0.452, 0.463 (**0.431**)	--
Polished Grain			<0.02, <0.02, <0.02 (**<0.02**)	<0.1-fold	<0.01, <0.01, <0.01	NC	0.153, 0.210, 0.276 (**0.213**)	0.5-fold
Bran			0.111, 0.114, 0.115 (**0.113**)	0.6-fold	<0.01, <0.01, <0.01	NC	2.94, 3.02 (**2.98**)[3]	6.9-fold
Hulls			0.780, 0.790, 0.794 (**0.788**)	4.4-fold	<0.01, <0.01, <0.01	NC	0.109, 0.119, 0.129 (**0.119**)	0.3-fold

[1] Samples were analyzed in triplicate; average residues are **bolded** and reported in parentheses.
[2] NC = not calculated.
[3] Only two values are reported because triplicate analysis was not performed for triazolylalanine.

D. CONCLUSION

Total prothioconazole-derived residues concentrated in rice hulls (4.4-fold) but did not concentrate in polished grain or bran (<0.1-fold and 0.6-fold, respectively). Residues of the triazole conjugates concentrated in bran (6.9-fold), but did not concentrate in polished rice or hulls (0.5-fold and 0.3-fold, respectively). Residues of 1*H*-1,2,4-triazole were below the LOQ (<0.01 ppm) in polished rice, bran, and hulls. Acceptable methods were used for quantitation of residues in/on rice grain and the processed commodities.

E. REFERENCES

Gould T. and Timberlake B. (2005) Storage stability of JAU6476 and desthio JAU6476 in canola, wheat, mustard greens, turnip root, and tomato fruit, processed products, and solutions, Bayer CropScience Interim Report.

F. DOCUMENT TRACKING

RDI: Louise Croteau, Suzan Mathew (PMRA) 23/01/2006; Henri Bietlot (PMRA) 24/01/2006; Stephen Funk (IO)13/03/2006; Leung Cheng (RAB3).
Petition Number: PP#4F6830
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Template Version September 2003

Primary Evaluators

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Date: Jan 24/06

In the absence of signatures, this document is considered to be a draft with deliberative material for internal use only.

This DER was originally prepared under contract by Dynamac Corporation (20440 Century Boulevard, Suite 100; Germantown, MD 20874; submitted 05/25/2005). The DER has been reviewed by the Health Effects Division (HED) and Health Canada's Pest Management Regulatory Agency (PMRA). The DER has been revised to reflect current Office of Pesticide Programs (OPP) policies, and Directive 98-02.

STUDY REPORT:

46246224 Beedle, E. (2004) JAU6476 480 SC - Magnitude of the Residues in/on Canola Seed and Canola Processed Commodities. Lab Project Numbers: J619CN02, RCJAY001, and 200953. Unpublished study prepared by Bayer CropScience. 309 p.

EXECUTIVE SUMMARY:

Bayer CropScience has submitted a processing study with canola. In a single test conducted in Ontario, Canada during 2000, the 4 lb/gal (480 g/L) suspension concentrate formulation (flowable concentrate; FlC), was applied to canola plants beginning at the two-leaf stage as two broadcast foliar applications with a 27-day retreatment interval at 0.912-0.919 lb a.i./A/application (1.02-1.03 kg a.i./ha/application), for a total application rate of 1.83 lb a.i./A (2.05 kg a.i./ha; ~5 times the field trial application rate). Canola plants were cut at maturity 47 days after the last treatment and were allowed to dry in the field for 5 days prior to collection of canola seed from treated and control plots. Samples of canola seed were collected, and the

remaining bulk samples were processed into meal and refined oil using simulated commercial procedures.

Samples were analyzed for total prothioconazole-derived residues (prothioconazole and the metabolite prothioconazole-desthio) using the LC-MS/MS method RPA JA/03/01. The validated LOQ for total prothioconazole-derived residues was 0.02 ppm for canola seed and processed commodities. Samples were analyzed for residues of 1*H*-1,2,4-triazole and the triazole conjugates triazolylalanine and triazolylacetic acid using an LC-MS/MS method with modifications. The validated LOQs for 1*H*-1,2,4-triazole were 0.02 ppm for canola seed and meal, and 0.01 ppm for canola oil. The validated LOQs for the triazole conjugates were 0.02 ppm for canola seed and oil, and 0.20 ppm for canola meal. The methods are adequate for data collection based on acceptable concurrent method recovery data.

The maximum storage intervals from collection to analysis for total prothioconazole-derived residues were 1261 days (41 months) for canola seed and 918 days (30 months) for processed canola commodities. Prothioconazole-derived residues and prothioconazole-desthio residues are stable up to 1 year (interim report) in canola matrices. The degree of loss of prothioconazole-derived residues and prothioconazole-desthio residues is not expected to exceed 30% after 41 months.

Total prothioconazole-derived residues did not concentrate in meal or refined oil (<0.7-fold each). Residues of the triazole conjugates concentrated in canola meal (2.9-fold), but not in refined oil (<0.02-fold). Processing factors could not be calculated for 1*H*-1,2,4-triazole in meal and refined oil, as residues were below the LOQ in these commodities.

STUDY/WAIVER ACCEPTABILITY/DEFICIENCIES/CLARIFICATIONS:

Under the conditions and parameters used in the study, the canola processing data are tentatively classified as scientifically acceptable. The final reports of the ongoing storage stability studies with prothioconazole, prothioconazole-desthio, 1*H*-1,2,4-triazole, triazolylalanine, and triazolylacetic acid will be submitted as confirmatory data.

The acceptability of this study for regulatory purposes is addressed in the forthcoming U.S. EPA Residue Chemistry Summary Document [DP Barcode D303508], and in Canada's Regulatory Decision Document.

COMPLIANCE:

Signed and dated GLP, quality assurance, and data confidentiality statements were provided. No deviations from regulatory requirements were noted that would impact the study results or their interpretation.

A. BACKGROUND INFORMATION

Prothioconazole is a broad-spectrum systemic fungicide intended for the control of many diseases in different crops such as cereals, oilseed rape or peanuts. The biochemical mode of action is the inhibition of the demethylation of lanosterol or 24-methylene-dihydrolanosterol, which are precursors of sterols in fungi. The chemical has been submitted for joint EPA and PMRA review by Bayer CropScience. The crops proposed for joint review include barley, the dried shell and bean subgroup, the oilseed crop group, and wheat. Uses on peanuts and rice are being proposed for the U.S. only.

TABLE A.1. Test Compound Nomenclature.

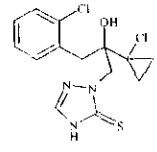
Chemical structure	
Common name	Prothioconazole (ISO accepted)
Company experimental name	JAU6476
IUPAC name	2-[(2*RS*)-2-(1-chlorocyclopropyl)-3-(2-chlorophenyl)-2-hydroxypropyl]-2*H*-1,2,4-triazole-3(4*H*)-thione
CAS name	2-[2-(1-chlorocyclopropyl)-3-(2-chlorophenyl)-2-hydroxypropyl]-1,2-dihydro-3*H*-1,2,4-triazole-3-thione
CAS registry number	178928-70-6
PC Code	113961
End-use product (EP)	Proline® 480 SC (4 lb/gal suspension concentrate), EPA File Symbol 264-IEL

TABLE A.2. Physicochemical Properties of the Technical Grade Test Compound.			
Parameter	Value		Reference
Melting point/range	139.1 to 144.5°C		MRID 46246003 / CES[1]
pH	5.8 (1% solution)		MRID 46246003 / CES
Density at 20°C	1.36 g/mL		MRID 46246003 / CES
Water solubility 20°C	pH 4 8 9	mg/L 5 300 2000	MRID 46246003 / CES
Solvent solubility at 20°C	Solvent Acetone Acetonitrile Dichloromethane Dimethylsulfoxide Ethyl acetate n-Heptane 1-Octanol Polyethylene glycol 2-Propanol Xylene	g/L >250 69 88 126 >250 <0.1 58 >250 87 8	MRID 46246003 / CES
Vapor pressure at 20 or 25°C	<4 x 10^{-7} Pa (calculated from determinations at 70 °C)		MRID 46246003 / CES
Dissociation constant, pK_a	6.9 (calculated from K_{OW})		MRID 46246003 / CES
Octanol/water partition coefficient, Log(K_{OW}) at 20°C	pH unbuffered water pH 4 pH 7 pH 9	Log K_{OW} 4.05 4.16 3.82 2.00	MRID 46246003 / CES
UV/visible absorption spectrum	Peak maxima at 257 nm		MRID 46246003 / CES

[1] CES: Chemistry Evaluation Section of PMRA

B. EXPERIMENTAL DESIGN

B.1. Application and Crop Information

TABLE B.1. Study Use Pattern on Canola								
Location (City, Province, Country, Year)	Application							
	EP[1]	Method; Timing	Volume (gal/A) [L/ha]	Single Rate (lb a.i./A) [kg a.i./ha]	No. of Appl.	RTI[2] (days)	Total Rate (lb a.i./A) [kg a.i./ha]	Tank Mix Adjuvants
Sheffield, Ontario, Canada, 2000	480 SC	1: Broadcast foliar; Two leaves unfolded 2: Broadcast foliar; Full flowering	19-23 [202-209]	0.912-0.919 [1.02-1.03]	2	27	1.83 [2.05]	None

[1] EP = End-use Product; 480 SC = 4 lb/gal (480 g/L) suspension concentrate (flowable concentrate; FlC) formulation
[2] RTI = Retreatment interval.

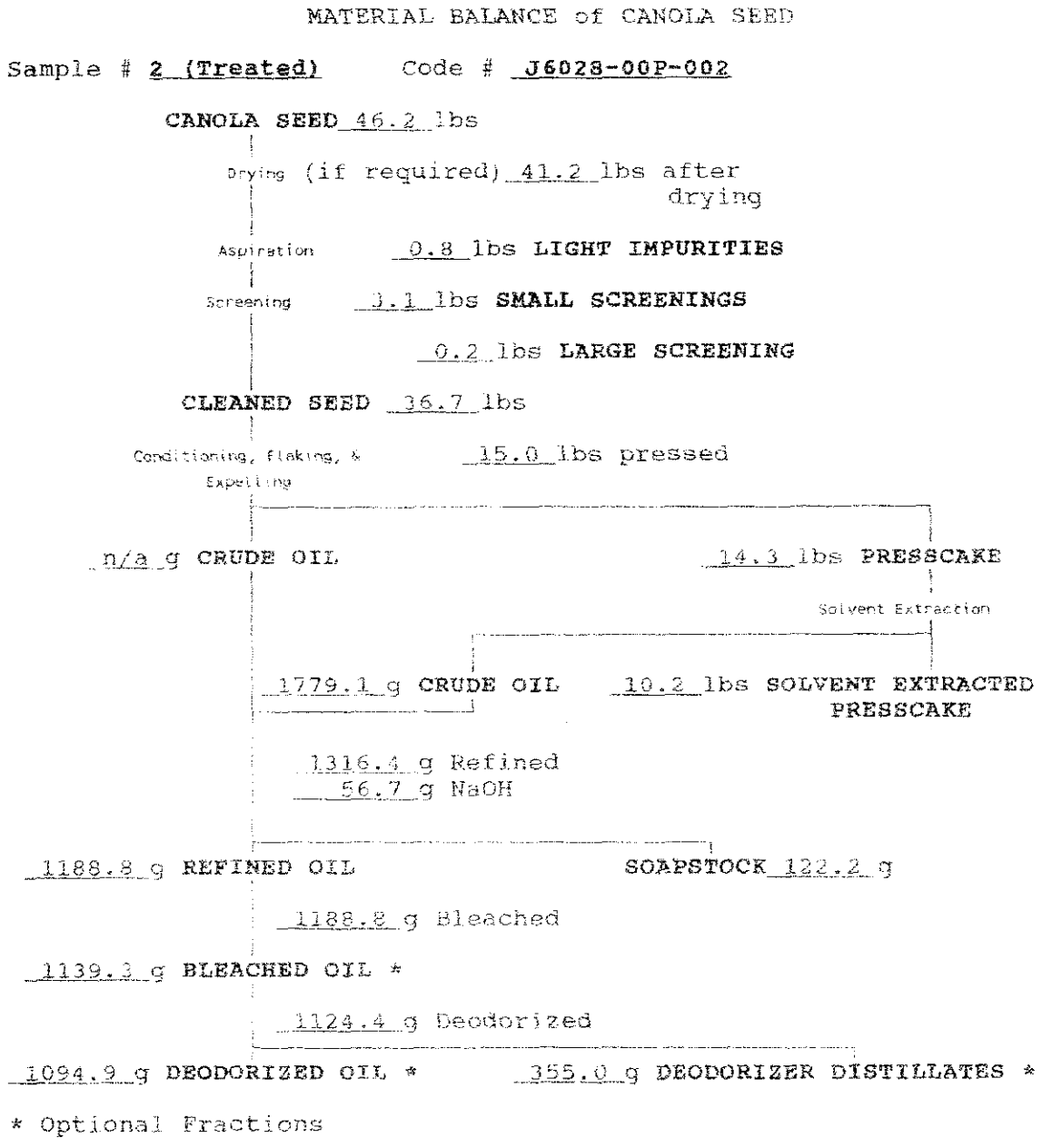
B.2. Processing Procedures

Canola plants were cut at maturity 47 days after the last treatment (47 DAT) and allowed to dry in the field. After 5 days, composite samples of treated and control seeds were collected. Canola seed samples were placed in frozen storage at the field site within 40 minutes of collection and were stored frozen for 31 days, then shipped frozen via freezer truck to the Food Protein Research and Development Center (FPRDC) at Texas A&M University (Bryan, TX). Seed samples were processed into meal and refined oil using simulated commercial procedures. The processed fractions and subsamples of seed were stored frozen, then shipped frozen to Battelle-Agrifood Laboratories (Columbus, OH) where seed samples were homogenized, and where all samples were stored frozen until shipment to Bayer Research Park (Stilwell, KS) for analysis.

Briefly, whole canola seeds were dried (54-71°C) until moisture was 7-10%. Whole seeds were flaked and heated (82-99°C) for 10-15 minutes. Flakes were pressed to extract crude oil from the presscake, then further extracted with hexane. This resulted in crude oil and solvent-extracted presscake (meal). Crude oil was heated (73-90°C) to remove hexane. The crude oil was then pretreated with phosphoric acid, and then separated into refined oil and soapstock.

The canola processing procedures are summarized in the flow chart below, which was copied without alteration from the data package.

FIGURE 1. Processing Procedures for Canola.



B.3. Analytical Methodology

Samples of canola matrices were analyzed for total prothioconazole-derived residues (prothioconazole and the metabolite prothioconazole-desthio) using LC-MS/MS method RPA JA/03/01, with the following modifications: a different solvent was used to prepare the spiking standards, and slightly different quantitation ions were used. Samples were 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 was spiked 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. Residue levels were quantitated from a calibration curve of mixed reference standards of prothioconazole, as the sulfonic acid, and prothioconazole-desthio. The validated LOQ for total prothioconazole-derived residues was 0.02 ppm for canola seed and processed commodities.

Samples were analyzed for residues of 1*H*-1,2,4-triazole and the triazole conjugates (triazolylalanine and triazolylacetic acid) using an LC-MS/MS method (Bayer Report No. G200598) with modifications. Briefly, crop matrices were extracted with aqueous methanol, and three separate aliquots of the extract were removed for determination of each of the three analytes. Isotopically labeled internal standard was added to each aliquot. For 1*H*-1,2,4-triazole, the aliquot was mixed with dansyl chloride to form the dansyl derivative of 1*H*-1,2,4-triazole, which was partitioned into ethyl acetate and then redissolved in acetonitrile (ACN)/water for LC-MS/MS analysis. For triazolylalanine, the aliquot was cleaned up by SPE, derivatized to the butyl ester using butanolic HCl, and then further derivatized using heptafluorobutyric anhydride (HFBA). The mixture was redissolved in ACN/water for LC-MS/MS analysis. For determination of triazolylacetic acid, the aliquot was cleaned up by SPE, derivatized to the butyl ester using butanolic HCl, and then redissolved in ACN/water for LC-MS/MS analysis. Residue levels were quantitated from a calibration curve of reference standards of dansyl-1,2,4-triazole, triazolylalanine butyl ester HFBA, and triazolylacetic acid butyl ester (generated by derivatizing reference standard solutions of triazolylacetic acid). The validated LOQs were 0.02 ppm for canola seed and meal, and 0.01 ppm for canola oil for 1*H*-1,2,4-triazole; and 0.02 ppm for canola seed and oil and 0.20 ppm for canola meal for the triazole conjugates.

C. RESULTS AND DISCUSSION

Concurrent method recovery data are presented in TABLE C.1. Samples were analyzed for total prothioconazole-derived residues (prothioconazole and the metabolite prothioconazole-desthio) using LC-MS/MS method RPA JA/03/01 with modifications. The validated LOQ for total prothioconazole-derived residues was 0.02 ppm for canola seed and processed commodities. Samples were analyzed for residues of 1*H*-1,2,4-triazole and the triazole conjugates triazolylalanine and triazolylacetic acid using an LC-MS/MS method (Bayer Report No. G200598) with modifications. The validated LOQs for 1*H*-1,2,4-triazole were 0.02 ppm for canola seed and meal, and 0.01 ppm for canola oil. The validated LOQs for the triazole conjugates were 0.02 ppm for canola seed and oil, and 0.20 ppm for canola meal. The methods are adequate for data collection based on acceptable concurrent method recovery data.

Sample storage intervals and conditions are summarized in TABLE C.2. The maximum storage intervals from collection to analysis for total prothioconazole-derived residues were 1261 days (41 months) for canola seed and 918 days (30 months) for processed canola meal and oil. Based on an interim report, residues of prothioconazole-desthio and prothioconazole-derived residues

are stable for up to 12.7 months. The degree of loss of prothioconazole-desthio residues and prothioconazole-derived residues is not expected to exceed 30% after 41 months.

The maximum storage intervals from collection to analysis for 1*H*-1,2,4-triazole and triazole conjugate residues were 1277 days (42 months) for canola seed and 933 days (30 months) for processed canola commodities.

Residue data from the peanut processing study with prothioconazole are reported in TABLE C.3. Residues of prothioconazole (average of triplicate analyses for each analyte) in/on canola seed (RAC) from the processing study were 0.028 ppm for total prothioconazole-derived residues, less than the LOQ (<0.02 ppm) for 1*H*-1,2,4-triazole, and 1.15 ppm for the triazole conjugates. The processing data for canola indicated that total prothioconazole-derived residues did not concentrate in meal (<0.7-fold) or refined oil (<0.7-fold) from canola seed bearing quantifiable residues. Residues of the triazole conjugates concentrated in canola meal (2.9-fold), but did not concentrate in refined oil (<0.02-fold). Residues of 1*H*-1,2,4-triazole were below the LOQs in both meal (<0.02 ppm) and refined oil (<0.01 ppm), so no processing factors could be calculated.

The reported processing factor of 2.9-fold for triazole conjugate residues in canola meal exceeded the theoretical concentration factor of 1.9-fold for canola meal, while the theoretical concentration factor was 3.0-fold for refined oil.

TABLE C.1. Summary of Concurrent Recoveries of Prothioconazole from Canola Commodities.

Canola matrix	Analyte	Spike level (ppm)	Sample size (n)	Recoveries (%)	Mean ± std dev
Seed	Prothioconazole	0.02	1	88	--
	Prothioconazole-desthio	0.02	1	100	--
	1*H*-1,2,4-triazole	0.02	3	76, 78, 83	79 ± 4
		0.20	1	100	--
	Triazolylalanine	0.01	3	79, 82, 91	84 ± 6
		0.20	4	72, 77, 72, 82	76 ± 5
	Triazolylacetic acid	0.02	3	109, 116, 95	107 ± 11
		0.20	1	96	--
Meal	Prothioconazole	0.02	3	82, 88, 82	84 ± 3
		0.10	3	84, 82, 85	84 ± 2
	Prothioconazole-desthio	0.02	3	97, 97, 100	98 ± 2
		0.10	3	95, 95, 93	94 ± 1
	1*H*-1,2,4-triazole	0.02	6	91, 86, 97, 98, 97, 87	93 ± 5
	Triazolylalanine	0.20	3	79, 79, 83	80 ± 2
		4.0	3	82, 78, 76	79 ± 3
	Triazolylacetic acid	0.01	3	105, 73, 85	88 ± 16
		0.02	6	88, 98, 88, 94, 98, 101	95 ± 6

TABLE C.1. Summary of Concurrent Recoveries of Prothioconazole from Canola Commodities.

Canola matrix	Analyte	Spike level (ppm)	Sample size (n)	Recoveries (%)	Mean ± std dev
Refined Oil	Prothioconazole	0.02	3	80, 82, 85	82 ± 3
	Prothioconazole-desthio	0.02	3	89, 89, 95	91 ± 3
	1*H*-1,2,4-triazole	0.01	3	67, 73, 76	72 ± 5
		0.02	4	105, 71, 117, 87	95 ± 20
		0.05	3	99, 86, 88	91± 7
	Triazolylalanine	0.02	3	69, 84, 89	81 ± 10
		0.05	3	79, 86, 97	87 ± 9
	Triazolylacetic acid	0.02	3	79, 77, 85	80 ± 4
		0.05	3	96, 101, 79	92 ± 12

TABLE C.2. Summary of Freezer Storage Conditions

Canola Matrix	Storage Temp. (°C)	Actual Storage Duration [1]	Limit of Demonstrated Storage Stability
Total Prothioconazole-Derived Residues			
Seed	-4.8 to -30.0	1261 days (41 months)	Stability demonstrated for up to 12.7 months (interim report). Awaiting final report as confirmatory data.
Meal and refined oil		918 days (30 months)	
1*H*-1,2,4-triazole and Triazole Conjugate Residues			
Seed	-4.8 to -30.0	1277 days (42 months)	Not applicable at this time. Awaiting final report.
Meal and refined oil		933 days (30 months)	

[1] All samples were analyzed within 2 days of extraction for total prothioconazole-derived residues and within 85 days of extraction for 1*H*-1,2,4-triazole and triazole conjugates.

TABLE C.3. Residue Data from Canola Processing Study with Prothioconazole.

Processed Commodity	Total Rate (lb a.i./A) [kg a.i./ha]	PHI[1] (days)	Total Prothioconazole-Derived		1*H*-1,2,4-triazole		Triazole Conjugate	
			Residues (ppm)[2]	Processing Factor	Residues (ppm)[2]	Processing Factor[3]	Residues (ppm)[2]	Processing Factor
Seed (RAC)	1.83 [2.05]	47	0.022, 0.027, 0.036 (**0.028**)	--	<0.02, <0.02, <0.02	--	0.97, 1.00, 1.48 (**1.15**)	--
Meal			<0.02, <0.02, <0.02 (**<0.02**)	<0.7-fold	<0.02, <0.02, <0.02	NC	3.21, 3.25, 3.42 (**3.29**)	2.9-fold
Refined oil			<0.02, <0.02, <0.02 (**<0.02**)	<0.7-fold	<0.01, <0.01, <0.01	NC	<0.02, <0.02, <0.02 (**<0.02**)	<0.02-fold

[1] Canola plants were cut 47 days after the second treatment and were left in the field to dry for 5 days prior to collection.
[2] Samples were analyzed in triplicate; average residues are **bolded** and reported in parentheses.
[3] NC = *not calculated.*

D. CONCLUSION

Total prothioconazole-derived residues did not concentrate in meal (<0.7-fold) or refined oil (<0.7-fold) from seed with quantifiable residues. Residues of the triazole conjugates concentrated in canola meal (2.9-fold), but not in refined oil (<0.02-fold). Residues of 1*H*-1,2,4-triazole were below the LOQs in/on canola seed (RAC) and meal (<0.02 ppm), and refined oil (<0.01 ppm), so no processing factors could be calculated. Acceptable methods were used for quantitation of residues in/on canola seed and the processed commodities.

E. REFERENCES

Gould T. and Timberlake B. (2005) Storage stability of JAU6476 and desthio JAU6476 in canola, wheat, mustard greens, turnip root, and tomato fruit, processed products, and solutions, Bayer CropScience Interim Report.

F. DOCUMENT TRACKING

RDI: Suzan Mathew (PMRA) xx/xx/2006; Henri Bietlot (PMRA) xx/xx/2006; Stephen Funk (IO) 13/03/2006; Leung Cheng (RAB3).
Petition Number: PP#4F6830
DP Barcode: D303508
PC Code: 113961

Template Version September 2003

Primary Evaluators	Stephen Funk, Ph.D., Senior Chemist Immediate Office	Date: Mar 13 2006
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	Suzan Mathew, Evaluation Officer FREAS, HED	Date: January 23/06
Approved by	Leung Cheng, Ph. D., Team Leader HED/RAB3	Date:
	Henri P Bietlot, Acting Section Head FREAS, HED	Date: Jan 24/06

This DER was originally prepared under contract by Dynamac Corporation (20440 Century Boulevard, Suite 100; Germantown, MD 20874; submitted 05/20/2005). The DER has been reviewed by the Health Effects Division (HED) and Health Canada's Pest Management Regulatory Agency (PMRA). The DER has been revised to reflect current Office of Pesticide Programs (OPP) policies and Directive 98-02.

STUDY REPORT:

46246216 Lenz, C. (2004) JAU6476 480 SC - Magnitude of the Residue In/On Rice. Project Number: J619RI01, RCJAY008, 200468. Unpublished study prepared by Bayer Corp. 501 p.

EXECUTIVE SUMMARY:

Bayer CropScience has submitted field trial data on rice. A total of 16 trials were conducted in Regions 4 (LA; 6 trials, AR; 4 trials, and MS; 1 trial), 5 (MI; 1 trial), 6 (TX; 2 trials), and 10 (CA; 2 trials) during the 2000 growing season. The number and locations of field trials are in accordance with OPPTS Guideline 860.1500 and Directive 98-02; Section 9.

At each test location, two broadcast foliar applications of the 4 lb/gal (480 g/L) suspension concentrate formulation (flowable concentrate; FlC) were made to rice at ~0.18 lb a.i./A (~0.20 kg a.i./ha) at 13- to 16-day retreatment intervals, for a total seasonal application rate of ~0.36 lb

a.i./A (~0.40 kg a.i./ha). Applications were made in ~12-23 gal/A of water using ground equipment. An adjuvant was not added to the spray mixture for any applications. Samples of rice were harvested at 14 test sites 40-67 days after the last application. At two locations, additional samples were collected to determine residue decline. Samples were harvested 49, 55, 58, and 65 days after treatment for the decline trial conducted in Benoit, MS (Region 4), and 64, 69, 74, and 80 days after treatment for the decline trial conducted in Glen, CA (Region 10).

Samples were analyzed for total prothioconazole-derived residues (prothioconazole and the metabolite prothioconazole-desthio) using LC-MS/MS method RPA JA/03/01. The validated LOQs for total prothioconazole-derived residues were 0.02 ppm for rice grain and 0.05 ppm for rice straw. Samples were analyzed for residues of 1*H*-1,2,4-triazole and the triazole conjugates triazolylalanine and triazolylacetic acid using an LC-MS/MS method (Bayer Report No. G200598). The validated LOQs were 0.01 ppm for 1*H*-1,2,4-triazole and 0.05 ppm for the triazole conjugates for rice grain and straw. The methods are adequate for data collection based on acceptable concurrent method recovery data.

The results from the rice field trials showed that the total prothioconazole-derived residues in/on rice matrices harvested 40-67 days following the last of two broadcast foliar applications were 0.222 ppm in/on rice grain and 1.277 ppm in/on rice straw. Residues of 1*H*-1,2,4-triazole were less than the LOQ (<0.01 ppm) in/on rice grain and straw. Maximum residues of the triazole conjugates were 0.571 ppm (rice grain) and 0.506 ppm (rice straw).

In the residue decline trials, total prothioconazole-derived residues in/on rice grain were <LOQ (0.02 ppm) in one trial, and did not increase with increasing sampling intervals in/on rice grain in the other trial. For rice straw, total prothioconazole-derived residues increased slightly with increasing sampling intervals in one trial. In the other trial, residues in/on straw increased slightly at the middle sampling intervals, and then decreased at the final sampling interval. Residues of the triazole conjugates in/on rice grain were <LOQ (<0.05 ppm) for one trial, and increased slightly in rice grain with increasing sampling intervals in the other trial. For rice straw, residues increased slightly with increasing sampling intervals in one trial, while residues did not increase in the other trial. Residues of 1*H*-1,2,4-triazole in/on rice grain and straw from both trials were less than the method LOQs (<0.02 ppm for total prothioconazole-derived residues, <0.05 ppm for the triazole conjugates, and <0.01 ppm for 1*H*-1,2,4-triazole) at all sampling intervals.

The maximum storage interval of crop samples from harvest to analysis for total prothioconazole-derived residues was 1240 days (40.8 months) for rice grain and straw. The degree of loss of prothioconazole-derived residues and prothioconazole-desthio residues is not expected to exceed 30% after 40.8 months in rice grain and straw.

STUDY/WAIVER ACCEPTABILITY/DEFICIENCIES/CLARIFICATIONS:

Under the conditions and parameters used in the study, the field trial residue data are tentatively classified as scientifically acceptable. The final reports of the ongoing storage stability studies

with prothioconazole, prothioconazole-desthio, 1*H*-1,2,4-triazole, triazolylalanine, and triazolylacetic acid will be submitted as confirmatory data.

The acceptability of this study for regulatory purposes is addressed in the forthcoming U.S. EPA Residue Chemistry Summary Document, DP Barcode D303508, and in Canada's Regulatory Decision Document.

COMPLIANCE:

Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided. No deviations from regulatory requirements were reported which would have an impact on the validity of the study.

A. BACKGROUND INFORMATION

Prothioconazole is a broad-spectrum systemic fungicide intended for the control of many diseases in different crops such as cereals, oilseed rape or peanuts. The biochemical mode of action is the inhibition of the demethylation of lanosterol or 24-methylene-dihydrolanosterol, which are precursors of sterols in fungi. The chemical has been submitted for joint EPA and PMRA review by Bayer CropScience. The crops proposed for joint review include barley, the dried shell and bean subgroup, the oilseed crop group, and wheat. Uses on peanuts and rice are being proposed for the U.S. only.

TABLE A.1. Test Compound Nomenclature.

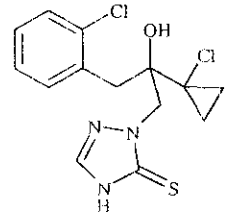
Chemical structure	
Common name	Prothioconazole (ISO accepted)
Company experimental name	JAU6476
IUPAC name	2-[(2*RS*)-2-(1-chlorocyclopropyl)-3-(2-chlorophenyl)-2-hydroxypropyl]-2*H*-1,2,4-triazole-3(4*H*)-thione
CAS name	2-[2-(1-chlorocyclopropyl)-3-(2-chlorophenyl)-2-hydroxypropyl]-1,2-dihydro-3*H*-1,2,4-triazole-3-thione
CAS registry number	178928-70-6
PC Code	113961
End-use product (EP)	Proline® 480 SC (4 lb/gal suspension concentrate), EPA File Symbol 264-IEL

TABLE A.2. Physicochemical Properties of the Technical Grade Test Compound.			
Parameter	Value		References
Melting range	139.1 to 144.5°C		MRID 46246003/CES
pH	5.8 (1% solution)		MRID 46246003/CES
Density at 20°C	1.36 g/mL		MRID 46246003/CES
Water solubility at 20°C	pH 4 8 9	mg/L 5 300 2000	MRID 46246003/CES
Solvent solubility at 20°C	Solvent Acetone Acetonitrile Dichloromethane Dimethysulfoxide Ethyl acetate n-Heptane 1-Octanol Polyethylene glycol 2-Propanol Xylene	g/L >250 69 88 126 >250 <0.1 58 >250 87 8	MRID 46246003/CES
Vapor pressure at 20 or 25°C	<4 x 10^{-7} Pa (calculated from determinations at 70°C)		MRID 46246003/CES
Dissociation constant, pK_a	6.9 (calculated from K_{OW})		MRID 46246003/CES
Octanol/water partition coefficient, at 20°C	pH unbuffered water 4 7 9	$Log(K_{OW})$ 4.05 4.16 3.82 2.00	MRID 46246003/CES
UV/visible absorption spectrum	Peak maxima at 257 nm. No absorption at > 300 nm.		MRID 46246003/CES

CES - Chemistry Evaluation Section of PMRA

B. EXPERIMENTAL DESIGN

B.1. Study Site Information

TABLE B.1.1. Trial Site Conditions

The information below was copied from the data report (MRID 46246216) without alteration.

Study location (city, state)	Trial Number	Year	Soil Characteristics[a]				Meterological Data[b]	
			Type	% OM*	pH*	CEC*	Total Rainfall (in)	Temp. range (°F)
Washington, Louisiana	J6092-00H	2000	NA	NA	NA	NA	11.07[c]	66-99
Ville Platte, Louisiana	J6093-00H	2000	NA	NA	NA	NA	7.42[c]	68-104
Benoit, Mississippi	J6094-00D	2000	NA	NA	NA	NA	2.40	52-106
Oberlin, Louisiana	J6095-00H	2000	NA	NA	NA	NA	15.68[c]	56-96
West Memphis, Arkansas	J6096-00H	2000	NA	NA	NA	NA	3.63[c]	65-107
Elton, Louisiana	J6097-00H	2000	NA	NA	NA	NA	15.68[c]	56-96
Turrell, Arkansas	J6098-00H	2000	NA	NA	NA	NA	0.54	59-105
Washington, Louisiana	J6099-00H	2000	NA	NA	NA	NA	11.35[c]	55-105
Washington, Louisiana	J6100-00H	2000	NA	NA	NA	NA	11.35[c]	55-105
Dexter, Missouri	J6101-00H	2000	NA	NA	NA	NA	3.87	56-101
East Bernard, Texas	J6102-00H	2000	NA	NA	NA	NA	2.62	61-108
East Bernard, Texas	J6103-00H	2000	NA	NA	NA	NA	2.52	63-108
Glenn, California	J6104-00D	2000	NA	NA	NA	NA	2.83	27-106
Escalon, California	J6105-00H	2000	NA	NA	NA	NA	0.13	51-104
Heth, Arkansas	J6106-00H	2000	NA	NA	NA	NA	4.79	65-107
Brickeys, Arkansas	J6107-00H	2000	NA	NA	NA	NA	1.99	60-105

* These parameters are optional except in cases where their value affects the use pattern for this chemical.

[a] NA = Not applicable since these parameters do not affect the use pattern of the chemical.
[b] The data is for the interval from the first application to the last sampling unless noted otherwise.
[c] The data is for the interval from the month of the first application through the month of the last sampling.

The actual temperature and rainfall recordings were within average historical values for the residue study period.

TABLE B.1.2. Study Use Pattern.

Location (City, State; Year)	EP[1]	Application				
		Method; Timing	Vol. (GPA[2])	Rate (lb a.i./A) [kg a.i./ha]	RTI[3] (days)	Total Rate (lb a.i./A) [g a.i./ha]
Washington, LA; 2000 J6092-00H	480 SC	1: Broadcast foliar; Panicle formation	22.9	0.18 [0.203]	---	0.36
		2: Broadcast foliar; 60% of panicle emerged	22.1	0.18 [0.200]	16	
Ville Platte, LA; 2000 J6093-00H	480 SC	1: Broadcast foliar; Panicle formation	13.6	0.18 [0.204]	—	0.36
		2: Broadcast foliar; 70% of panicle emerged	12.0	0.18 [0.205]	13	
Benoit, MS; 2000 J6094-00D	480 SC	1: Broadcast foliar; Panicle formation	19.9	0.20 [0.224]	—	0.34
		2: Broadcast foliar; Panicle formation	16.5	0.14 [0.153]	13	
Oberlin, LA; 2000 J6095-00H	480 SC	1: Broadcast foliar; Panicle formation	12.5	0.18 [0.203]	---	0.36
		2: Broadcast foliar; Panicle formation	12.9	0.18 [0.202]	14	
West Memphis, AR; 2000 J6096-00H	480 SC	1: Broadcast foliar; Panicle formation	18.3	0.18 [0.202]	—	0.36
		2: Broadcast foliar; Flag leaf stage	18.4	0.18 [0.202]	14	
Elton, LA; 2000 J6097-00H	480 SC	1: Broadcast foliar; Panicle formation	13.6	0.18 [0.205]	—	0.36
		2: Broadcast foliar; Mid boot stage	12.9	0.18 [0.206]	14	
Turrell, AR; 2000 J6098-00H	480 SC	1: Broadcast foliar; Panicle formation	18.9	0.18 [0.201]	—	0.36
		2: Broadcast foliar; Early boot stage	18.2	0.18 [0.201]	14	
Washington, LA; 2000 J6099-00H	480 SC	1: Broadcast foliar; Panicle formation	17.6	0.18 [0.200]	—	0.36
		2: Broadcast foliar; Internode elongation or jointing stage	19.2	0.18 [0.198]	14	
Washington, LA; 2000 J6100-00H	480 SC	1: Broadcast foliar; Panicle formation	17.6	0.18 [0.199]	—	0.36
		2: Broadcast foliar; Internode elongation or jointing stage	19.7	0.18 [0.204]	14	
Dexter, MI; 2000 J6101-00H	480 SC	1: Broadcast foliar; Panicle formation	19.0	0.18 [0.203]	—	0.36
		2: Broadcast foliar; Early boot stage	18.4	0.18 [0.202]	14	

TABLE B.1.2. Study Use Pattern.

Location (City, State; Year)	EP[1]	Application				
		Method; Timing	Vol. (GPA[2])	Rate (lb a.i./A) [kg a.i./ha]	RTI[3] (days)	Total Rate (lb a.i./A) [g a.i./ha]
East Bernard, TX; 2000 J6102-00H	480 SC	1: Broadcast foliar; Panicle formation	19.5	0.18 [0.202]	—	0.36
		2: Broadcast foliar; Flag leaf just visible, still rolled	22.9	0.18 [0.200]	14	
East Bernard, TX; 2000 J6103-00H	480 SC	1: Broadcast foliar; Panicle formation	22.8	0.18 [0.201]	---	0.36
		2: Broadcast foliar; Flag leaf just visible, still rolled	22.8	0.18 [0.200]	13	
Glen, CA; 2000 J6104-00D	480 SC	1: Broadcast foliar; Panicle formation	21.9	0.18 [0.201]	---	0.36
		2: Broadcast foliar; Early boot stage	23.2	0.18 [0.202]	14	
Escalon, CA; 2000 J6105-00H	480 SC	1: Broadcast foliar; Panicle formation	19.7	0.18 [0.199]	---	0.37
		2: Broadcast foliar; Internode elongation or jointing stage	20.8	0.18 [0.210]	14	
Heth, AR; 2000 J6106-00H	480 SC	1: Broadcast foliar; Panicle formation	18.9	0.18 [0.202]	---	0.36
		2: Broadcast foliar; Early boot stage	18.4	0.18 [0.203]	14	
Brickeys, AR; 2000 J6107-00H	480 SC	1: Broadcast foliar; Panicle formation	18.9	0.18 [0.202]	---	0.36
		2: Broadcast foliar; Early boot stage	18.3	0.18 [0.203]	14	

[1] EP = End-use Product; 480 SC = 4 lb/gal (480 g/L) suspension concentrate (flowable concentrate; FlC) formulation
[2] GPA = Gallons per acre
[3] RTI = Retreatment Interval
No tank mix adjuvants were used.

TABLE B.1.3. Trial Numbers and Geographical Locations.

NAFTA Growing Region	Rice		
	Submitted	Requested	
		Canada	US
1			
1A			
2			
3			
4	11		11
5	1		1
5A			
5B			
6	2		2
7			
7A			
8			
9			
10	2		2
11			
12			
13			
14			
15			
16			
17			
18			
19			
20			
21			
Total	16		16

B.2. Sample Handling and Preparation

Samples of rice grain and straw were collected 40-67 days (average = 49 days) after the last application. Samples were bagged and stored frozen within 4 hours of harvest. The samples were shipped frozen to Battelle-AgriFood Laboratories (Columbus, OH) for homogenization. At Battelle, the samples were homogenized with dry ice and stored frozen until shipment to Bayer Research Park (Stilwell, KS) for analysis.

B.3. Analytical Methodology

Samples of rice grain and straw were analyzed for total prothioconazole-derived residues (prothioconazole and the metabolite prothioconazole-desthio) using LC-MS/MS method RPA JA/03/01 with modifications. Samples were extracted with a mixture of methanol, 30% hydrogen peroxide, and 0.05M 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 was spiked 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. Residue levels were quantitated from a calibration curve of mixed reference standards of prothioconazole, as the sulfonic acid, and prothioconazole-desthio. The method was modified to use a different solvent for preparation of the spiking solutions and to use slightly different m/z values for the quantitation ions. The validated LOQs for total prothioconazole-derived residues were 0.02 ppm for rice grain and 0.05 ppm for straw.

Samples were analyzed for residues of 1*H*-1,2,4-triazole and the triazole conjugates (triazolylalanine and triazolylacetic acid) using an LC-MS/MS method (Bayer Report No. G200598) with modifications. Briefly, crop matrices were extracted with aqueous methanol, and three separate aliquots of the extract were removed for determination of each of the three analytes. Isotopically labeled internal standard was added to each aliquot. For 1*H*-1,2,4-triazole, the aliquot was mixed with dansyl chloride to form the dansyl derivative of 1*H*-1,2,4-triazole, which was partitioned into ethyl acetate and then redissolved in acetonitrile (ACN)/water for LC-MS/MS analysis. For triazolylalanine, the aliquot was cleaned up by SPE, derivatized to the butyl ester using butanolic HCl, and then further derivatized using heptafluorobutyric anhydride (HFBA). The mixture was redissolved in ACN/water for LC-MS/MS analysis. For determination of triazolylacetic acid, the aliquot was cleaned up by SPE, derivatized to the butyl ester using butanolic HCl, and then redissolved in ACN/water for LC-MS/MS analysis. Residue levels were quantitated from a calibration curve of reference standards of dansyl-1,2,4-triazole, triazolylalanine butyl ester HFBA, and triazolylacetic acid butyl ester (generated by derivatizing reference standard solutions of triazolylacetic acid). The validated LOQs for rice grain and straw were 0.01 ppm for 1*H*-1,2,4-triazole and 0.05 ppm for the triazole conjugates.

C. RESULTS AND DISCUSSION

Concurrent method recovery data are presented in TABLE C.1. Samples were analyzed for total prothioconazole-derived residues (prothioconazole and the metabolite prothioconazole-desthio) using LC-MS/MS method RPA JA/03/01 with modifications. The validated LOQs for total prothioconazole-derived residues were 0.02 ppm for rice grain and 0.05 ppm for rice straw. Samples were analyzed for residues of 1*H*-1,2,4-triazole and the triazole conjugates triazolylalanine and triazolylacetic acid using an LC-MS/MS method (Bayer Report No. G200598) with modifications. The validated LOQs were 0.01 ppm for 1*H*-1,2,4-triazole and 0.05 ppm for the triazole conjugates for rice grain and straw. The methods are adequate for data collection based on acceptable concurrent method recovery data.

Apparent total prothioconazole-derived residues and residues of 1*H*-1,2,4-triazole were below the method LOQs (<0.02 for grain and <0.05 ppm for straw for total prothioconazole-derived residues and <0.01 both matrices for 1*H*-1,2,4-triazole) in/on all samples of untreated rice grain and straw. Apparent residues of the triazole conjugates were below the method LOQ (<0.05 ppm) in/on samples of untreated grain (n =20) and straw (n =23). However, quantifiable apparent residues were observed in/on 4 samples of rice grain (residue range of 0.05 to 0.54 ppm) and 3 samples of rice straw (residue range of 0.10 to 0.20 ppm). The measurable control residues of the triazole conjugates may have resulted from the use of triazole fungicides from previous seasons or from naturally occurring triazole conjugates.

Sample storage conditions and intervals are summarized in TABLE C.2. The maximum storage interval of crop samples from harvest to analysis for total prothioconazole-derived residues was 1240 days (40.8 months) for rice grain and straw. Prothioconazole-derived residues are stable for up to 1 year based on an interim report. The need to correct the results of the current study for decline of prothioconazole-derived residues will not be necessary taking into account the absolute (ppm) and relative % residue levels in plant matrices. The degree of loss of prothioconazole-desthio residues is not expected to exceed 30% after 40.8 months.

The maximum storage interval of crop samples from harvest to analysis for 1*H*-1,2,4-triazole and triazole conjugate residues was 1077 days (35.4 months) for rice grain and rice straw.
Residue data from the rice field trials are reported in TABLE C.3. A summary of prothioconazole residue data for rice grain and straw is presented in TABLE C.4. Residues in/on rice harvested 40-67 days following the last of two broadcast foliar applications were <0.02-0.222 ppm in/on rice grain and <0.05-1.277 ppm in/on rice straw for total prothioconazole-derived residues, less than the LOQ (<0.01 ppm) in/on rice grain and straw for 1*H*-1,2,4-triazole, and <0.05-0.571 ppm in/on rice grain and <0.05-0.506 in/on rice straw for the triazole conjugates.

In the residue decline trials, total prothioconazole-derived residues in/on rice grain were <LOQ (0.02 ppm) in one trial, and did not increase with increasing sampling intervals in/on rice grain in the other trial. For rice straw, total prothioconazole-derived residues increased slightly with increasing sampling intervals in one trial. In the other trial, residues in/on straw increased slightly at the middle sampling intervals, and then decreased at the final sampling interval. Residues of the triazole conjugates in/on rice grain were <LOQ (<0.05 ppm) for one trial, and increased slightly in rice grain with increasing sampling intervals in the other trial. For rice straw, residues increased slightly with increasing sampling intervals in one trial, while residues did not increase in the other trial. Residues of 1*H*-1,2,4-triazole in/on rice grain and straw from both trials were less than the method LOQs (<0.02 ppm for total prothioconazole-derived residues, <0.05 ppm for the triazole conjugates, and <0.01 ppm for 1*H*-1,2,4-triazole) at all sampling intervals.

TABLE C.1. Summary of Concurrent Recoveries of Prothioconazole from Rice.

Matrix	Analyte	Spike level (ppm)	Sample size (n)	Recoveries (%)	Mean ± std dev
Grain	Prothioconazole	0.02	2	86, 88	87 ± 1.4
	Prothioconazole-desthio	0.02	2	95, 113	104 ± 12.7
	1*H*-1,2,4-triazole	0.02	1	87	Not applicable
		0.10	3	81, 90, 93	88 ± 6
	Triazolylalanine	0.10	3	71, 76, 87	78 ± 8.2
		0.50	1	88	Not applicable
	Triazolylacetic acid	0.10	3	72, 72, 74	73 ± 1.2
		0.50	1	78	Not applicable
Straw	Prothioconazole	0.10	2	74, 84	79 ± 7.1
	Prothioconazole-desthio	0.10	2	99, 105	102 ± 4.2
	1*H*-1,2,4-triazole	0.01	1	71	Not applicable
		0.10	3	76, 80, 91	82 ± 7.8
	Triazolylalanine	0.10	4	75, 91, 97, 97	90 ± 10.4
	Triazolylacetic acid	0.10	4	76, 76, 77, 91	80 ± 7.3

TABLE C.2. Summary of Storage Conditions.

Matrix (RAC or Extract)	Storage Temp. (°C)	Actual Storage Duration[1]	Interval of Demonstrated Storage Stability
Total Prothioconazole-Derived Residues			
Rice, grain	-4.8 to -30.0	1135-1240 days (37.3-40.8 months)	Stability demonstrated for up to 12.7 months (interim report). Awaiting final report as confirmatory data.
Rice, straw	-4.8 to -30.0	1120-1226 days (36.8-40.3 months)	
1*H*-1,2,4-triazole and Triazole Conjugate Residues			
Rice, grain	-4.8 to -30.0	973-1077 days (32.0-35.4 months)	Not applicable at this time. Awaiting final report.
Rice, straw	-4.8 to -30.0	971-1072 days (31.9-35.2 months)	

[1] Actual storage duration from collection to analysis. All samples were analyzed within 2 days of extraction.

TABLE C.3. Residue Data from Crop Field Trials with Prothioconazole.

Trial ID (City, State; Year)	Region	Rice Variety	Commodity or Matrix	Total Rate (lb a.i./A) [kg a.i./ha]	PHI (days)	Total Prothioconazole-Derived Residues (ppm)	*1H*-1,2,4-triazole Residues[1] (ppm)	Triazole Conjugate Residues[1] (ppm)
Washington, LA; 2000 J6092-00H	4	Cypress	Grain	0.36	40	0.159, 0.222	<0.01, <0.01	0.272, 0.281
			Straw	0.36	40	0.694, 0.850	<0.01, <0.01	0.056, 0.065
Ville Platte, LA; 2000 J6093-00H	4	Cocodrie	Grain	0.36	40	0.079, 0.086	<0.01, <0.01	0.073, 0.070
			Straw	0.36	40	0.363, 0.548	<0.01, <0.01	<0.05, <0.05
Benoit, MS; 2000 J6094-00D	4	Lemont	Grain	0.34	49	<0.02, <0.02	<0.01, <0.01	0.298, 0.337
					55	<0.02, <0.02	<0.01, <0.01	0.294, 0.334
					58	<0.02, <0.02	<0.01, <0.01	0.294, 0.312
					65	<0.02, <0.02	<0.01, <0.01	0.396, 0.457
			Straw	0.34	49	0.200, 0.219	<0.01, <0.01	0.506, 0.449
					55	0.184, 0.370	<0.01, <0.01	0.680, 0.991
					58	0.236, 0.313	<0.01, <0.01	0.721, 0.486
					65	0.118, 0.139	<0.01, <0.01	0.675, 0.832
Oberlin, LA; 2000 J6095-00H	4	Jackson	Grain	0.36	49	<0.02, <0.02	<0.01, <0.01	<0.05, <0.05
			Straw	0.36	49	0.497, 0.587	<0.01, <0.01	<0.05, <0.05
West Memphis, AR; 2000 J6096-00H	4	Lagrue	Grain	0.36	44	<0.02, <0.02	<0.01, <0.01	<0.05, <0.05
			Straw	0.36	44	0.700, 0.736	<0.01, <0.01	<0.05, <0.05
Elton, LA; 2000 J6097-00H	4	Jefferson	Grain	0.36	49	0.067, 0.075	<0.01, <0.01	<0.05, <0.05
			Straw	0.36	49	0.531, 0.650	<0.01, <0.01	<0.05, <0.05
Turrell, AR; 2000 J6098-00H	4	Cypress	Grain	0.36	47	<0.02, <0.02	<0.01, <0.01	<0.05, <0.05
			Straw	0.36	47	0.315, 0.360	<0.01, <0.01	<0.05, <0.05
Washington, LA; 2000 J6099-00H	4	Wells	Grain	0.36	67	<0.02, <0.02	<0.01, <0.01	0.060, 0.090
			Straw	0.36	67	<0.05, 0.071	<0.01, <0.01	0.101, 0.125
Washington, LA; 2000 J6100-00H	4	Cypress	Grain	0.36	60	<0.02, <0.02	<0.01, <0.01	<0.05, <0.05
			Straw	0.36	60	0.109, 0.121	<0.01, <0.01	0.053, 0.054
Dexter, MI; 2000 J6101-00H	5	Lagrue	Grain	0.36	43	<0.02, <0.02	<0.01, <0.01	<0.05, <0.05
			Straw	0.36	43	<0.05, <0.05	<0.01, <0.01	<0.05, <0.05
East Bernard, TX; 2000 J6102-00H	6	Lemont	Grain	0.36	43	<0.02, <0.02	<0.01, <0.01	0.075, 0.076
			Straw	0.36	43	0.526, 0.556	<0.01, <0.01	0.064, 0.072
East Bernard, TX; 2000 J6103-00H	6	Cypress	Grain	0.36	47	<0.02, <0.02	<0.01, <0.01	0.535, 0.571
			Straw	0.36	47	0.427, 0.437	<0.01, <0.01	0.283, 0.302

TABLE C.3. Residue Data from Crop Field Trials with Prothioconazole.

Trial ID (City, State; Year)	Region	Rice Variety	Commodity or Matrix	Total Rate (lb a.i./A) [kg a.i./ha]	PHI (days)	Total Prothioconazole-Derived Residues (ppm)	1*H*-1,2,4-triazole Residues[1] (ppm)	Triazole Conjugate Residues[1] (ppm)
Glen, CA; 2000 J6104-00D	10	CM-401	Grain	0.36	64	0.023, 0.031	<0.01, <0.01	<0.05, <0.05
					69	<0.02, 0.028	<0.01, <0.01	<0.05, <0.05
					74	<0.02, <0.02	<0.01, <0.01	<0.05, <0.05
					80	0.030, 0.034	<0.01, <0.01	<0.05, <0.05
			Straw	0.36	64	0.716, 1.031	<0.01, <0.01	<0.05, <0.05
					69	0.75, 0.869	<0.01, <0.01	<0.05, <0.05
					74	1.228, 1.438	<0.01, <0.01	0.077, <0.05
					80	0.851, 1.581	<0.01, <0.01	<0.05, <0.05
Escalon, CA, 2000 J6105-00H	10	M-103	Grain	0.37	48	<0.02, 0.025	<0.01, <0.01	<0.05, <0.05
			Straw	0.37	48	1.101, 1.277	<0.01, <0.01	<0.05, 0.052
Heth, AR; 2000 J6106-00H	4	Bengal	Grain	0.36	48	<0.02, <0.02	<0.01, <0.01	<0.05, <0.05
			Straw	0.36	48	0.194, 0.330	<0.01, <0.01	0.113, 0.105
Brickeys, AR 2000 J6107-00H	4	Drew	Grain	0.36	48	<0.02, <0.02	<0.01, <0.01	0.052, 0.057
			Straw	0.36	48	0.243, 0.349	<0.01, <0.01	<0.05, <0.05

[1] Residue values are listed respectively for 1*H*-1,2,4-triazole and the triazole conjugates.

TABLE C.4. Summary of Residue Data from Crop Field Trials with Prothioconazole.									
Commodity	Total Applic. Rate (lb a.i./A) [kg a.i./ha]	PHI (days)	Residue Levels (ppm)[1]						
			n	Min.	Max.	HAFT[2]	Median (STMdR[3])	Mean (STMR[4])	Std. Dev.
Total Prothioconazole-Derived Residues									
Rice, grain	0.34-0.37 [0.38-0.41]	40-67	32	<0.02	0.222	0.191	0.01	0.031	0.048
Rice, straw	0.34-0.37 [0.38-0.41]	40-67	32	<0.05	1.277	1.189	0.432	0.464	0.319
1*H*-1,2,4-triazole Residues									
Rice, grain	0.34-0.37 [0.38-0.41]	40-67	32	<0.01	<0.01	<0.01	0.005	0.005	0.0
Rice, straw	0.34-0.37 [0.38-0.41]	40-67	32	<0.01	<0.01	<0.01	0.005	0.005	0.0
Triazole Conjugate Residues									
Rice, grain	0.34-0.37 [0.38-0.41]	40-67	32	<0.05	0.571	0.553	0.025	0.103	0.148
Rice, straw	0.34-0.37 [0.38-0.41]	40-67	32	<0.05	0.506	0.478	0.025	0.088	0.122

[1] For the determination of minimum, maximum, and HAFT values, the LOQ was used for residues reported as below the LOQ in TABLE C.3. For the determination of the median, mean, and standard deviation values, ½LOQ was used for residues reported as below the LOQ. Residue values from the appropriate harvest intervals from the residue decline trials (49-day PHI for the MS trial and 64-day PHI for the CA trial were included in the summary table.
[2] HAFT = Highest Average Field Trial.
[3] STMdR = Supervised Trial Median Residue.
[4] STMR = Supervised Trial Mean Residue.

D. CONCLUSION

The study use pattern was two foliar applications of the 4 lb/gal FlC formulation for a total seasonal rate of 0.34-0.37 lb a.i./A (0.38-0.41 kg a.i./ha), with a 13- to 16-day re-treatment interval. Rice grain and straw were harvested 40 to 67 days after the last application. The maximum total prothioconazole-derived residues were 0.22 ppm (rice grain), and 1.3 ppm (rice straw). Residues of 1,2,4-triazole were less than the LOQ (<0.01 ppm) in/on rice grain and straw. Maximum residues of the triazole conjugates were 0.57 ppm, and 0.51 ppm in/on rice grain and straw, respectively. Acceptable methods were used for quantitation of residues in/on rice grain, and straw.

E. REFERENCES

Gould T. and Timberlake B. (2005) Storage stability of JAU6476 and desthio JAU6476 in canola, wheat, mustard greens, turnip root, and tomato fruit, processed products, and solutions, Bayer CropScience Interim Report.

F. DOCUMENT TRACKING

RDI: Louise Croteau, Suzan Mathew (PMRA) 23/01/2006; Henri Bietlot (PMRA) 24/01/2006; Stephen Funk (IO)13/03/2006; Leung Cheng (RAB3).
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This DER was originally prepared under contract by Dynamac Corporation (20440 Century Boulevard, Suite 100; Germantown, MD 20874; submitted 05/20/2005). The DER has been reviewed by the Health Effects Division (HED) and Health Canada's Pest Management Regulatory Agency (PMRA). The DER has been revised to reflect current Office of Pesticide Programs (OPP) policies and Directive 98-02.

STUDY REPORT:

46246217 Fischer, D. (2004) JAU6476 480 SC - Magnitude of the Residue In/On Peanuts. Project Number: J619PE01, RCJAY007, 200508. Unpublished study prepared by Bayer Corp., Battelle and Bayer Research Farm. 490 p.

EXECUTIVE SUMMARY:

Bayer CropScience has submitted field trial data on peanuts. Twelve trials were conducted in Regions 2 (AL; 1 trial, GA; 3 trials, NC; 3 trials, and VA; 1 trial), 3 (FL; 1 trial), 6 (TX; 2 trials), and 8 (OK; 1 trial) during the 2000 growing season. The number and locations of field trials are in accordance with OPPTS Guideline 860.1500 and Directive 98-02; Section 9.

At each test location, four broadcast foliar applications of the 4 lb/gal (480 g/L) suspension concentrate formulation (flowable concentrate; FlC) were made to peanuts at ~0.18 lb a.i./A (~0.20 kg a.i./ha) at 12- to 14-day retreatment intervals, for a total seasonal application rate of ~0.72 lb a.i./A (~0.80 kg a.i./ha). Applications were made in ~13-37 gal/A (~119-349 L/ha) of water using ground equipment. An adjuvant was not added to the spray mixture for any applications. Peanut plants were dug up at all test sites 13-15 days after treatment, and were left in the field for 2-8 days prior to collection of peanuts and peanut hay. In one field trial (GA), additional samples were dug up at 7, 14, 21, and 28 days following the last application to evaluate residue decline.

Samples were analyzed for total prothioconazole-derived residues (prothioconazole and the metabolite prothioconazole-desthio) using LC-MS/MS method RPA JA/03/01. The validated LOQs for total prothioconazole-derived residues were 0.02 ppm for peanut nutmeat and 0.05 ppm for hay. Samples were analyzed for residues of 1*H*-1,2,4-triazole and the triazole conjugates triazolylalanine and triazolylacetic acid using an LC-MS/MS method (Bayer Report No. G200598). The validated LOQ for 1*H*-1,2,4-triazole was 0.02 ppm for peanut nutmeat and hay, and the validated LOQs for the triazole conjugates were 0.125 ppm for peanut nutmeat and 0.10 ppm for hay. The methods were adequate for data collection based on acceptable concurrent method recovery data.

The results from the peanut field trials indicated that the maximum residues of prothioconazole in/on peanut matrices harvested 13-15 days following the last of four broadcast foliar applications at a total seasonal rate of 0.707-0.734 lb a.i./A (0.792-0.823 kg a.i./ha) were <0.02 ppm in/on nutmeat and 4.458 ppm in/on hay for total prothioconazole-derived residues; 0.02 ppm in/on nutmeat and less than the LOQ (<0.02 ppm) in/on hay for 1*H*-1,2,4-triazole; and 3.903 ppm in/on nutmeat and 1.278 ppm in/on hay for the triazole conjugates.

In the residue decline trial, residues of 1*H*-1,2,4-triazole were less than the method LOQ (<0.02 ppm) at all sampling intervals for peanut nutmeat and hay, and total prothioconazole-derived residues were less than the method LOQ (<0.02 ppm) at all sampling intervals for nutmeat. The average total prothioconazole-derived residues in hay increased slightly from the 7-day sampling interval to the 14-day sampling interval and then decreased by the 28-day sampling interval. Residues of the triazole conjugates increased slightly in nutmeat (from an average of 0.868 ppm to an average of 0.964 ppm) with increasing sampling intervals; a greater increase was observed in peanut hay (from an average of 0.117 ppm to an average of 0.355 ppm).

The maximum storage interval of crop samples from harvest to analysis for total prothioconazole-derived residues was 1214 days (39.9 months) for peanut nutmeat and hay. Prothioconazole-derived residues and prothioconazole-desthio residues are stable up to 12.7 months (interim report). The degree of loss of prothioconazole-derived residues and prothioconazole-desthio residues is not expected to exceed 30% after 39.9 months.

STUDY/WAIVER ACCEPTABILITY/DEFICIENCIES/CLARIFICATIONS:

Under the conditions and parameters used in the study, the field trial residue data are tentatively classified as scientifically acceptable. The final reports of the ongoing storage stability studies with prothioconazole, prothioconazole-desthio, 1*H*-1,2,4-triazole, triazolylalanine, and triazolylacetic acid will be submitted as confirmatory data.

The acceptability of this study for regulatory purposes is addressed in the forthcoming U.S. EPA Residue Chemistry Summary Document, DP Barcode D303508, and in Canada's Regulatory Decision Document.

COMPLIANCE:

Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided. No deviations from regulatory requirements were reported which would have an impact on the validity of the study.

A. BACKGROUND INFORMATION

Prothioconazole is a broad-spectrum systemic fungicide intended for the control of many diseases in different crops such as cereals, oilseed rape or peanuts. The biochemical mode of action is the inhibition of the demethylation of lanosterol or 24-methylene-dihydrolanosterol, which are precursors of sterols in fungi. The chemical has been submitted for joint EPA and PMRA review by Bayer CropScience. The crops proposed for joint review include barley, the dried shell and bean subgroup, the oilseed crop group, and wheat. Uses on peanuts and rice are being proposed for the U.S. only.

TABLE A.1. Test Compound Nomenclature.

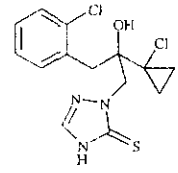
Chemical structure	
Common name	Prothioconazole (ISO accepted)
Company experimental name	JAU6476
IUPAC name	2-[(2*RS*)-2-(1-chlorocyclopropyl)-3-(2-chlorophenyl)-2-hydroxypropyl]-2*H*-1,2,4-triazole-3(4*H*)-thione
CAS name	2-[2-(1-chlorocyclopropyl)-3-(2-chlorophenyl)-2-hydroxypropyl]-1,2-dihydro-3*H*-1,2,4-triazole-3-thione
CAS registry number	178928-70-6
PC Code	113961
End-use product (EP)	Proline® 480 SC (4 lb/gal suspension concentrate), EPA File Symbol 264-IEL

TABLE A.2. Physicochemical Properties of the Technical Grade Test Compound.			
Parameter	Value		References
Melting range	139.1 to 144.5°C		MRID 46246003/CES
pH	5.8 (1% solution)		MRID 46246003/CES
Density at 20°C	1.36 g/mL		MRID 46246003/CES
Water solubility at 20°C	pH 4 8 9	mg/L 5 300 2000	MRID 46246003/CES
Solvent solubility at 20°C	Solvent Acetone Acetonitrile Dichloromethane Dimethysulfoxide Ethyl acetate n-Heptane 1-Octanol Polyethylene glycol 2-Propanol Xylene	g/L >250 69 88 126 >250 <0.1 58 >250 87 8	MRID 46246003/CES
Vapor pressure at 20 or 25°C	$<4 \times 10^{-7}$ Pa (calculated from determinations at 70°C)		MRID 46246003/CES
Dissociation constant, pK_a	6.9 (calculated from K_{OW})		MRID 46246003/CES
Octanol/water partition coefficient, at 20°C	pH unbuffered water 4 7 9	$Log(K_{OW})$ 4.05 4.16 3.82 2.00	MRID 46246003/CES
UV/visible absorption spectrum	Peak maxima at 257 nm. No absorption at > 300 nm.		MRID 46246003/CES

CES - Chemistry Evaluation Section of PMRA

B. EXPERIMENTAL DESIGN

B.1. Study Site Information

TABLE B.1.1. Trial Site Conditions

The table below was copied from the data report (MRID 46246217) without alteration.

Study Location (City, State)	Trial Number	Year	Soil Characteristics[a]				Meteorological Data[b]	
			Type	% OM	pH	CEC	Total Rainfall (in)	Temp. Range (°F)
Tifton, Georgia	J6029-00D	2000	NA	NA	NA	NA	12.89	40 - 100
Suffolk, Virginia	J6030-00H	2000	NA	NA	NA	NA	9.33	30 - 93
Jamesville North Carolina	J6031-00H	2000	NA	NA	NA	NA	15.72	50 - 96
Roper, North Carolina	J6032-00H	2000	NA	NA	NA	NA	15.72	50 - 96
Inaha, Georgia	J6033-00H	2000	NA	NA	NA	NA	10.42[c]	52 - 102
Herod, Georgia	J6034-00H	2000	NA	NA	NA	NA	10.67[c]	35 - 105
Columbia, Alabama	J6035-00H	2000	NA	NA	NA	NA	8.92[c]	52 - 103
Knightdale, North Carolina	J6036-00H	2000	NA	NA	NA	NA	7.60	48 - 99
Vero Beach, Florida	J6037-00H	2000	NA	NA	NA	NA	15.95[c]	54 - 92
Vernon, Texas	J6038-00H	2000	NA	NA	NA	NA	21.3[c]	27 - 107
Vernon, Texas	J6039-00H	2000	NA	NA	NA	NA	22.8[c]	27 - 107
Eakly, Oklahoma	J6040-00H	2000	NA	NA	NA	NA	5.35[c]	28 - 108

* *These parameters are optional except in cases where their value affects the use pattern for this chemical.*

[a] NA = Not Applicable, since these parameters do not affect the use pattern of the chemical.
[b] Data is for the interval from first application to last sampling.
[c] Overhead irrigation values added to rainfall values.

The actual temperature and rainfall recordings were within average historical values for the residue study period. The applicant indicated that irrigation was used to supplement as needed at seven trial sites.

TABLE B.1.2. Study Use Pattern.

Location (City, State; Year)	EP[1]	Application				
		Method; Timing	Vol. (GPA[2]) [L/ha]	Rate (lb a.i./A) [g a.i./ha]	RTI[3] (days)	Total Rate (lb a.i./A) [g a.i./ha]
Tifton, GA; 2000 J6029-00D	480 SC	1: Broadcast foliar; Main phase pod developed, continuation of pod filling	16 [147]	0.180 [0.202]	---	0.720 [0.808]
		2: Broadcast foliar; Main phase pod developed, continuation of pod filling	15 [143]	0.180 [0.202]	13	
		3: Broadcast foliar; About 70% of pods developed to final size are ripe	15 [143]	0.180 [0.202]	15	
		4: Broadcast foliar; About 70% of pods developed to final size are ripe	15 [136]	0.180 [0.202]	14	
Suffolk, VA; 2000 J6030-00H	480 SC	1: Broadcast foliar; Foliage of 90% of plants meet between rows	20 [190]	0.181 [0.203]	---	0.734 [0.822]
		2: Broadcast foliar; Foliage of 90% of plants meet between rows	23 [214]	0.186 [0.208]	14	
		3: Broadcast foliar; Foliage of 90% of plants meet between rows	22 [202]	0.183 [0.205]	15	
		4: Broadcast foliar; Foliage of 90% of plants meet between rows	23 [214]	0.184 [0.206]	13	
Jamesville, NC; 2000 J6031-00H	480 SC	1: Broadcast foliar; Tip of 1st carpophore(s) (peg(s)) swollen	31 [286]	0.181 [0.203]	---	0.722 [0.809]
		2: Broadcast foliar; Main phase pod developed, continuation of pod filling	31 [289]	0.181 [0.203]	14	
		3: Broadcast foliar; Main phase pod developed, continuation of pod filling	31 [287]	0.180 [0.202]	14	
		4: Broadcast foliar; Advanced pod filling	28 [260]	0.180 [0.201]	14	
Roper, NC; 2000 J6032-00H	480 SC	1: Broadcast foliar; Begin. pod filling on 1st pods attained final size	30 [277]	0.178 [0.200]	---	0.707 [0.792]
		2: Broadcast foliar; Main phase pod developed, continuation of pod filling	29 [275]	0.177 [0.198]	14	
		3: Broadcast foliar; Main phase pod developed, continuation of pod filling	30 [279]	0.176 [0.197]	14	
		4: Broadcast foliar; Advanced pod filling	27 [253]	0.176 [0.197]	14	

TABLE B.1.2. Study Use Pattern.

Location (City, State; Year)	EP[1]	Application				
		Method; Timing	Vol. (GPA[2]) [L/ha]	Rate (lb a.i./A) [g a.i./ha]	RTI[3] (days)	Total Rate (lb a.i./A) [g a.i./ha]
Inaha, GA; 2000 J6033-00H	480 SC	1: Broadcast foliar; Main phase pod developed, continuation of pod filling	14 [133]	0.176 [0.197]	---	0.716 [0.803]
		2: Broadcast foliar; Advanced pod filling	14 [127]	0.179 [0.201]	14	
		3: Broadcast foliar; About 50% of pods developed to final size are ripe	13 [125]	0.180 [0.202]	14	
		4: Broadcast foliar; About 60% of pods developed to final size are ripe	14 [129]	0.181 [0.203]	14	
Herod, GA; 2000 J6034-00H	480 SC	1: Broadcast foliar; Main phase pod developed, continuation of pod filling	14 [129]	0.181 [0.203]	---	0.722 [0.810]
		2: Broadcast foliar; Advanced pod filling	13 [124]	0.180 [0.202]	14	
		3: Broadcast foliar; About 20% of pods developed to final size are ripe	13 [123]	0.182 [0.204]	15	
		4: Broadcast foliar; About 50% of pods developed to final size are ripe	13 [123]	0.179 [0.201]	13	
Columbia, AL; 2000 J6035-00H	480 SC	1: Broadcast foliar; Fresh seed fill cavity of pods attained fin. size	14 [130]	0.180 [0.202]	---	0.721 [0.809]
		2: Broadcast foliar; Advanced pod filling	14 [130]	0.179 [0.201]	14	
		3: Broadcast foliar; About 50% of pods developed to final size are ripe	13 [126]	0.181 [0.203]	14	
		4: Broadcast foliar; About 60% of pods developed to final size are ripe	13 [119]	0.181 [0.203]	13	
Knightdale, NC; 2000 J6036-00H	480 SC	1: Broadcast foliar; Main phase pod developed, continuation of pod filling	37 [345]	0.185 [0.207]	---	0.728 [0.816]
		2: Broadcast foliar; Flowering declining	35 [323]	0.182 [0.204]	14	
		3: Broadcast foliar; Main phase pod devel., continuation of pod filling	32 [299]	0.179 [0.201]	14	
		4: Broadcast foliar; About 50% of pods developed to final size are ripe	33 [306]	0.182 [0.204]	15	
Vero Beach, FL; 2000 J6037-00H	480 SC	1: Broadcast foliar; First carpophore(s) (peg(s)) visibly elongated	15 [143]	0.180 [0.202]	---	0.722 [0.810]
		2: Broadcast foliar; Tip of 1st carpophore(s) (peg(s)) swollen	15 [145]	0.180 [0.202]	13	
		3: Broadcast foliar; Main phase pod devel., continuation of pod filling	16 [146]	0.182 [0.204]	13	
		4: Broadcast foliar; Advanced pod	16	0.180	15	

TABLE B.1.2. Study Use Pattern.

Location (City, State; Year)	EP[1]	Application				
		Method; Timing	Vol (GPA[2]) [L/ha]	Rate (lb a.i./A) [g a.i./ha]	RTI[3] (days)	Total Rate (lb a.i./A) [g a.i./ha]
Vernon TX; 2000 J6038-00H	480 SC	1: Broadcast foliar; Advanced pod filling	34 [317]	0.182 [0.204]	---	0.726 [0.813]
		2: Broadcast foliar; Advanced pod filling	37 [348]	0.179 [0.201]	13	
		3: Broadcast foliar; About 80% of pods developed to final size are ripe	37 [344]	0.181 [0.202]	14	
		4: Broadcast foliar; About 80% of pods developed to final size are ripe	37 [347]	0.184 [0.206]	14	
Vernon TX; 2000 J6039-00H	480 SC	1: Broadcast foliar; Advanced pod filling	34 [315]	0.181 [0.203]	---	0.722 [0.810]
		2: Broadcast foliar; Advanced pod filling	37 [349]	0.179 [0.201]	13	
		3: Broadcast foliar; About 80% of pods developed to final size are ripe	37 [345]	0.181 [0.203]	14	
		4: Broadcast foliar; About 80% of pods developed to final size are ripe	37 [343]	0.181 [0.203]	14	
Eakly, OK; 2000 J6040-00H	480 SC	1: Broadcast foliar; Fresh seeds fill cavity of pods attained fin. size	15 [137]	0.188 [0.211]	---	0.734 [0.823]
		2: Broadcast foliar; Fresh seeds fill cavity of pods attained fin. size	14 [132]	0.181 [0.203]	15	
		3: Broadcast foliar; About 10% of pods developed to final size are ripe	14 [129]	0.185 [0.207]	13	
		4: Broadcast foliar; About 60% of pods developed to final size are ripe	14 [129]	0.180 [0.202]	12	

[1] EP = End-use Product; 480 SC = 4 lb/gal (480 g/L) suspension concentrate (flowable concentrate; FlC) formulation
[2] GPA = Gallons per acre
[3] RTI = Retreatment Interval
No tank mix adjuvants were used.

TABLE B.1.3. Trial Numbers and Geographical Locations.

NAFTA Growing Region	Peanut		
	Submitted	Requested	
		Canada	US
1			
1A			
2	8		8
3	1		1
4			
5			
5A			
5B			
6	2		2
7			
7A			
8	1		1
9			
10			
11			
12			
13			
14			
15			
16			
17			
18			
19			
20			
21			
Total	12		12

B.2. Sample Handling and Preparation

Peanut plants were dug up from each site and left in the field to dry. Samples of peanuts with shells and peanut hay were collected 2-8 days after the plants were dug up. Samples were frozen within 3.5 hours of collection and were shipped frozen to Battelle-AgriFood Laboratories (Columbus, OH) for homogenization. At Battelle, the samples were homogenized with dry ice and stored frozen until shipment to Bayer Research Park (Stilwell, KS) for analysis.

B.3. Analytical Methodology

Samples of peanut nutmeat and hay were analyzed for total prothioconazole-derived residues (prothioconazole and the metabolite prothioconazole-desthio) using LC-MS/MS method RPA JA/03/01 with modifications. Samples were extracted with a mixture of methanol, 30% hydrogen peroxide, and 0.05M 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 was spiked 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. Residue levels were quantitated from a calibration curve of mixed reference standards of prothioconazole, as the sulfonic acid, and prothioconazole-desthio. The method was modified to use a different solvent for preparation of the spiking solutions and to use slightly different m/z values for the quantitation ions. The validated LOQs for total prothioconazole-derived residues were 0.02 ppm for peanut nutmeat and 0.05 ppm for peanut hay.

Samples were analyzed for residues of 1*H*-1,2,4-triazole and the triazole conjugates (triazolylalanine and triazolylacetic acid) using an LC-MS/MS method (Bayer Report No. G200598) with modifications. Briefly, crop matrices were extracted with aqueous methanol, and three separate aliquots of the extract were removed for determination of each of the three analytes. Isotopically labeled internal standard was added to each aliquot. For 1*H*-1,2,4-triazole, the aliquot was mixed with dansyl chloride to form the dansyl derivative of 1*H*-1,2,4-triazole, which was partitioned into ethyl acetate and then redissolved in acetonitrile (ACN)/water for LC-MS/MS analysis. For triazolylalanine, the aliquot was cleaned up by SPE, derivatized to the butyl ester using butanolic HCl, and then further derivatized using heptafluorobutyric anhydride (HFBA). The mixture was redissolved in ACN/water for LC-MS/MS analysis. For determination of triazolylacetic acid, the aliquot was cleaned up by SPE, derivatized to the butyl ester using butanolic HCl, and then redissolved in ACN/water for LC-MS/MS analysis. Residue levels were quantitated from a calibration curve of reference standards of dansyl-1,2,4-triazole, triazolylalanine butyl ester HFBA, and triazolylacetic acid butyl ester (generated by derivatizing reference standard solutions of triazolylacetic acid). The validated LOQ for 1*H*-1,2,4-triazole was 0.02 ppm for peanut nutmeat and hay, and the validated LOQs for the triazole conjugates were 0.125 ppm for peanut nutmeat and 0.10 ppm for peanut hay.

C. RESULTS AND DISCUSSION

Concurrent method recovery data are presented in TABLE C.1. Samples were analyzed for total prothioconazole-derived residues (prothioconazole and the metabolite prothioconazole-desthio) using LC-MS/MS method RPA JA/03/01. The validated LOQs for total prothioconazole-derived residues were 0.02 ppm for peanut nutmeat and 0.05 ppm for hay. Samples were analyzed for residues of 1*H*-1,2,4-triazole and the triazole conjugates triazolylalanine and triazolylacetic acid using an LC-MS/MS method (Bayer Report No. G200598). The validated LOQ for 1*H*-1,2,4-triazole was 0.02 ppm for peanut nutmeat and hay, and the validated LOQs for the triazole

conjugates were 0.125 ppm for peanut nutmeat and 0.10 ppm for hay. The methods are adequate for data collection based on acceptable concurrent method recovery data.

Apparent total prothioconazole-derived residues were below the method LOQs in/on all samples of untreated peanut nutmeat and hay (<0.02 ppm and <0.05 ppm, respectively). Apparent residues of 1*H*-1,2,4-triazole were below the method LOQ (<0.02 ppm) in/on all samples of untreated peanut hay and 17 samples of nutmeat; quantifiable apparent residues at 0.03 ppm were observed in two samples of nutmeat. Apparent residues of the triazole conjugates were below the method LOQs (<0.125 ppm for nutmeat and <0.1 ppm for hay) in/on samples of untreated nutmeat (n=10) and hay (n=12); quantifiable apparent residues were observed in/on 13 samples of peanut nutmeat (residue range of 0.13 to 2.55 ppm) and 6 samples of peanut hay (residue range of 0.17 to 1.17 ppm). The measurable control residues of the triazole conjugates may have resulted from the use of triazole fungicides from previous seasons or from naturally occurring triazole conjugates.

Sample storage conditions and intervals are summarized in TABLE C.2. The maximum storage interval of crop samples from harvest to analysis for total prothioconazole-derived residues was 1214 days (39.9 months) for peanut nutmeat and hay. Based on an interim report, residues of prothioconazole-desthio and prothioconazole-derived residues are stable for up to 12.7 months. The degree of loss of prothioconazole-desthio residues and prothioconazole-derived residues is not expected to exceed 30% after 39.9 months in peanut nutmeat and hay.

The maximum storage interval of crop samples from harvest to analysis for 1*H*-1,2,4-triazole and triazole conjugate residues was 1223 days (40.2 months) for peanut nutmeat and hay.

Residue data from the peanut field trials are reported in TABLE C.3. A summary of prothioconazole residue data for peanut nutmeat and hay is presented in TABLE C.4. Residues in/on peanuts harvested 13-15 days following the last of four broadcast foliar applications at a total seasonal rate of 0.707-0.734 lb a.i./A (0.792-0.823 kg a.i./ha) were less than the LOQ (<0.02 ppm) in/on peanut nutmeat and 0.989-4.458 ppm in/on peanut hay for total prothioconazole-derived residues; <0.02-0.02 ppm in/on peanut nutmeat and less than the LOQ (<0.02 ppm) in/on peanut hay for 1*H*-1,2,4-triazole; and 0.162-3.903 ppm in/on peanut nutmeat and <0.10-1.278 ppm in/on peanut hay for the triazole conjugates.

In the residue decline trial, residues of 1*H*-1,2,4-triazole were less than the method LOQ (<0.02 ppm) at all sampling intervals for peanut nutmeat and hay, and total prothioconazole-derived residues were less than the method LOQ (<0.02 ppm) at all sampling intervals for nutmeat. The average total prothioconazole-derived residues in hay increased slightly from the 7-day sampling interval to the 14-day sampling interval and then decreased by the 28-day sampling interval. Residues of the triazole conjugates increased slightly in nutmeat (from an average of 0.868 ppm to an average of 0.964 ppm) with increasing sampling intervals; a greater increase was observed in peanut hay (from an average of 0.117 ppm to an average of 0.355 ppm).

TABLE C.1. Summary of Concurrent Recoveries of Prothioconazole from Peanuts.

Matrix	Analyte	Spike level (ppm)	Sample size (n)	Recoveries (%)	Mean ± std dev
Nutmeat	Prothioconazole	0.02	2	81, 89	85 ± 5.7
	Prothioconazole-desthio	0.02	2	96, 102	99 ± 4.2
	1*H*-1,2,4-triazole	0.02	2	86, 95	90.5 ± 6.4
		0.05	3	83, 92, 92	89 ± 5.2
		0.5	1	80	80
	Triazolylalanine	0.5	2	91, 93	92 ± 1.4
	Triazolylacetic acid	0.1	2	85, 90	87.5 ± 3.5
		0.5	1	71	71
Hay	Prothioconazole	0.01	3	79, 82, 84	81.7 ± 2.5
		5	3	79, 81, 82	80.7 ± 1.5
	Prothioconazole-desthio	0.01	3	94, 94, 102	96.7 ± 4.6
		5	3	98, 98, 99	98.3 ± 0.6
	1*H*-1,2,4-triazole	0.02	2	88, 99	98.3 ± 0.6
		0.5	1	100	100
	Triazolylalanine	0.03	2	73, 77	75 ± 2.8
		0.5	2	88, 89	88.5 ± 0.7
	Triazolylacetic acid	0.1	2	71, 89	80 ± 12.7
		0.5	1	97	97

TABLE C.2. Summary of Storage Conditions.

Matrix (RAC or Extract)	Storage Temp. (°C)	Actual Storage Duration [1]	Interval of Demonstrated Storage Stability
Total Prothioconazole-Derived Residues			
Peanut, nutmeat	-4.8 to -30.0	1175-1214 days (38.6-39.9 months)	Stability demonstrated for up to 12.7 months (interim report). Awaiting final report as confirmatory data.
Peanut, hay	-4.8 to -30.0	1173-1212 days (38.6-39.8 months)	
1*H*-1,2,4-triazole and Triazole Conjugate Residues			
Peanut, nutmeat	-4.8 to -30.0	1059-1223 days (34.8-40.2 months)	Not applicable at this time. Awaiting final report.
Peanut, hay	-4.8 to -30.0	1060-1222 days (34.8-40.2 months)	

[1] Actual storage duration from collection to analysis; samples were collected 2-8 days after plants were dug up and left in the field. All samples were analyzed within 3 days of extraction.

TABLE C.3. Residue Data from Crop Field Trials with Prothioconazole

Trial ID (City, State; Year)	Region	Peanut Variety	Commodity or Matrix	Total Rate (lb a.i./A) [kg a.i./ha]	PHI (days)[1]	Total Prothioconazole-Derived Residues (ppm)	1*H*-1,2,4-triazole Residues[2] (ppm)	Triazole Conjugate Residues[2] (ppm)
Tifton, GA; 2000 J6029-00D	2	Georgia Greens	Nutmeat	0.720 [0.808]	7	<0.02, <0.02	<0.02, <0.02	0.867, 0.868
					14	<0.02, <0.02	<0.02, <0.02	0.797, 0.857
					21	<0.02, <0.02	<0.02, <0.02	0.833, 0.841
					28	<0.02, <0.02	<0.02, <0.02	0.963, 0.965
			Hay	0.720 [0.808]	7	2.359, 2.420	<0.02, <0.02	0.123, 0.110
					14	2.908, 4.350	<0.02, <0.02	0.276, 0.221
					21	3.385, 3.958	<0.02, <0.02	0.242, 0.167
					28	2.066, 3.781	<0.02, <0.02	0.382, 0.327
Suffolk, VA; 2000 *J6030-00H*	2	VA 98R	Nutmeat	0.734 [0.822]	14	<0.02, <0.02	<0.02, <0.02	0.938, 1.131
			Hay	0.734 [0.822]	14	2.787, 3.741	<0.02, <0.02	<0.10, <0.10
Jamesville, NC; 2000 J6031-00H	2	NC 12C, Lot G-2204	Nutmeat	0.722 [0.809]	13	<0.02, <0.02	<0.02, <0.02	0.162, 2.563
			Hay	0.722 [0.809]	13	1.645, 2.921	<0.02, <0.02	0.588, 0.384
Roper, NC; 2000 *J6032-00H*	2	VA-C-92R, CV92R54 399	Nutmeat	0.707 [0.792]	13	<0.02, <0.02	<0.02, <0.02	2.876, 3.903
			Hay	0.707 [0.792]	13	2.289, 3.831	<0.02, <0.02	1.278, 1.209
Inaha, GA; 2000 J6033-00H	2	Georgia Green	Nutmeat	0.716 [0.803]	15	<0.02, <0.02	<0.02, <0.02	0.201, 0.229
			Hay	0.716 [0.803]	15	0.989, 1.249	<0.02, <0.02	0.328, 0.261
Herod, GA; 2000 J6034-00H	2	AgraTech 201	Nutmeat	0.722 [0.810]	14	<0.02, <0.02	<0.02, <0.02	0.603, 0.610
			Hay	0.722 [0.810]	14	2.036, 2.740	<0.02, <0.02	<0.10, <0.10
Columbia, AL; 2000 J6035-00H	2	Georgia Green	Nutmeat	0.721 [0.809]	15	<0.02, <0.02	<0.02, <0.02	2.450, 3.544
			Hay	0.721 [0.809]	15	2.574, 3.044	<0.02, <0.02	0.597, 0.452
Knightdale, NC; 2000 J6036-00H	2	VA98R	Nutmeat	0.728 [0.816]	15	<0.02, <0.02	<0.02, <0.02	0.992, 1.244
			Hay	0.728 [0.816]	15	1.863, 2.342	<0.02, <0.02	0.113, 0.130
Vero Beach, FL; 2000 J6037-00H	3	Georgia Green	Nutmeat	0.722 [0.810]	14	<0.02, <0.02	<0.02, <0.02	0.248, 0.255
			Hay	0.722 [0.810]	14	1.709, 2.235	<0.02, <0.02	<0.10, <0.10

TABLE C.3. Residue Data from Crop Field Trials with Prothioconazole

Trial ID (City, State; Year)	Region	Peanut Variety	Commodity or Matrix	Total Rate (lb a.i./A) [kg a.i./ha]	PHI (days)[1]	Total Prothioconazole-Derived Residues (ppm)	1*H*-1,2,4-triazole Residues[2] (ppm)	Triazole Conjugate Residues[2] (ppm)
Vernon TX; 2000 J6038-00H	6	TAMRun	Nutmeat	0.726 [0.813]	14	<0.02, <0.02	<0.02, 0.02	0.199, 0.203
			Hay	0.726 [0.813]	14	2.801, 4.458	<0.02, <0.02	<0.10, <0.10
Vernon TX; 2000 J6039-00H	6	TAMRun	Nutmeat	0.722 [0.810]	14	<0.02, <0.02	<0.02, <0.02	1.640, 1.692
			Hay	0.722 [0.810]	14	2.564, 3.025	<0.02, <0.02	<0.10, <0.10
Eakly, OK; 2000 J6040-00H	8	Spanco	Nutmeat	0.734 [0.823]	15	<0.02, <0.02	<0.02, <0.02	0.219, 0.246
			Hay	0.734 [0.823]	15	1.797, 2.799	<0.02, <0.02	0.630, 0.776

[1] Residue values are listed respectively for 1*H*-1,2,4-triazole and the triazole conjugates.

TABLE C.4. Summary of Residue Data from Crop Field Trials with Prothioconazole.

Commodity	Total Applic. Rate (lb a.i./A) [kg a.i./ha]	PHI (days)	Residue Levels (ppm) [1]						
			n	Min.	Max.	HAFT[2]	Median (STMdR[3])	Mean (STMR[4])	Std. Dev.
Total Prothioconazole-Derived Residues									
Peanut, nutmeat	0.707-0.734 [0.792-0.823]	13-15	24	<0.02	<0.02	<0.02	0.01	0.01	0
Peanut, hay	0.707-0.734 [0.792-0.823]	13-15	24	0.989	4.458	3.63	2.657	2.612	0.884
1*H*-1,2,4-triazole Residues									
Peanut, nutmeat	0.707-0.734 [0.792-0.823]	13-15	24	<0.02	<0.02	<0.02	0.01	0.01	0
Peanut, hay	0.707-0.734 [0.792-0.823]	13-15	24	<0.02	<0.02	<0.02	0.01	0.01	0
Triazole Conjugate Residues									
Peanut, nutmeat	0.707-0.734 [0.792-0.823]	13-15	24	0.162	3.903	3.39	0.827	1.158	1.127
Peanut, hay	0.707-0.734 [0.792-0.823]	13-15	24	<0.10	1.278	1.244	0.176	0.323	0.361

[1] For the calculation of minimum, maximum, and HAFT values, the LOQ value was used for residues reported as below the LOQ in TABLE C.3. For calculation of the median, mean and standard deviation, ½LOQ was used for residues reported as below the LOQ.
[2] HAFT = Highest Average Field Trial.
[3] STMdR = Supervised Trial Median Residue.
[4] STMR = Supervised Trial Mean Residue.

D. CONCLUSION

The study use pattern was four foliar applications of the 4 lb/gal FlC formulation for a total seasonal rate of 0.707-0.734 lb a.i./A (0.792-0.823 kg a.i./ha), with a 12- to 14-day re-treatment interval. Peanut nutmeat and hay were harvested 13 to 15 days after the last application. The maximum total prothioconazole-derived residues were <0.02 ppm (nutmeat), 4.5 ppm (hay). Residues of 1,2,4-triazole were less than the LOQ (<0.02 ppm) in/on peanut nutmeat and hay. Maximum residues of the triazole conjugates were 3.9 ppm, and 1.3 ppm in/on peanut nutmeat and hay, respectively. Acceptable methods were used for quantitation of residues in/on peanut nutmeat and hay.

E. REFERENCES

Gould T. and Timberlake B. (2005) Storage stability of JAU6476 and desthio JAU6476 in canola, wheat, mustard greens, turnip root, and tomato fruit, processed products, and solutions, Bayer CropScience Interim Report.

F. DOCUMENT TRACKING

RDI: Louise Croteau, Suzan Mathew (PMRA) 23/01/2006; Henri Bietlot (PMRA) 23/01/2006; Stephen Funk (IO) 13/03/2006; Leung Cheng (RAB3).
Petition Number: PP#4F6830
DP Barcode: D303508
PC Code: 113961

Template Version September 2003

Primary Evaluator _____ Date: Mar 13 2006
Stephen Funk, Ph.D., Senior Chemist
Immediate Office

Approved by _____ Date:
Leung Cheng, Ph. D., Team Leader
HED/RAB3

In the absence of signatures, this document is considered to be a draft with deliberative material for internal use only.

This DER was originally prepared under contract by Dynamac Corporation (20440 Century Boulevard, Suite 100; Germantown, MD 20874; submitted 04/28/2005). The DER has been reviewed by the Health Effects Division (HED). The DER has been revised to reflect current Office of Pesticide Programs (OPP) policies.

END-USE PRODUCTS:

TABLE 1. Summary of End-Use Products.

Trade Name	Reg. No.	ai (% of formulation)	Formulation Type	Target Crops	Target Pests	Label Date
U.S. Label						
Proline® 480 SC Fungicide	264-IEL	4 lb/gal (41%)	Suspension concentrate	Barley; canola; chickpea; dried shelled peas and beans *subgroup; lentils;* oilseed crop subgroup; peanut; rice; wheat	*Ascomycetes*, *Basidiomycetes*, and *Deuteromycetes* diseases	3/31/04 (draft)

Use directions for the label (EPA File Symbol 264-IEL) are from a draft label dated 3/31/04 for the 4 lb/gal suspension concentrate formulation (equivalent to a flowable concentrate formulation). Use directions are summarized in TABLE 2.

TABLE 2. Summary of Directions for Use of Prothioconazole.						
Trade Name	Applic. Timing, Type, and Equip.	Applic. Rate (lb a.i./A) [g a.i./ha]	Max. No. Applic. per Season	Max. Seasonal Applic. Rate (lb a.i./A) [g a.i./ha]	PHI (days)	Use Directions and Limitations
Barley						
Proline® 480 SC Fungicide	Postemergence Broadcast foliar Ground or aerial	0.134-0.178 [150-200]	2	0.293 [328]	32	Apply during Feekes stages 10.3-10.5 (70-100% heads on the main stem fully emerged); applications may be made up to Feekes stage 10.52 (heads in full flower). A 7- to 14-day retreatment interval is proposed. Use of a spray surfactant is recommended.
Proline® 480 SC Fungicide	Postemergence Broadcast foliar Ground or aerial	0.088-0.134 [100-150]	2	293 [328]	32	Apply when earliest disease symptoms appear on leaves or stems. A 7- to 14-day retreatment interval is proposed. Use of a spray surfactant is recommended.
Canola						
Proline® 480 SC Fungicide	Postemergence Broadcast foliar Ground or aerial	0.134-0.178 [150-200]	2	0.356 [400]	36	Apply during the 20% up to the 50% bloom stage. A 5- to 7-day retreatment interval is proposed. Use of a spray surfactant is recommended.
Chickpea and Lentils						
Proline® 480 SC Fungicide	Postemergence Broadcast foliar Ground or aerial	0.134-0.178 [150-200]	3	0.534 [600]	7	Apply at early flower (lentil) or first sign of disease. A 10- to 14-day retreatment interval is proposed. Use of a spray surfactant is recommended.
Dried Shelled Peas and Beans Subgroup : *Lupinius* spp. (grain, sweet, white, and white sweet lupin); *Phaseolus* spp. (field, kidney, dry lima, navy, pinto, and tepary beans); *Vigna* spp. (adzuki bean, blackeyed pea, catjang, cowpea, Crowder pea, moth bean, mung bean, rice bean, southern pea, and urd bean); dry broad bean; guar; lablab bean; and *Pisum* spp. (pea (including field pea), and pigeon pea)						
Proline® 480 SC Fungicide	Postemergence Broadcast foliar Ground or aerial	0.134-0.178 [150-200]	3	0.534 [600]	7	Apply at first sign of disease. A 5- to 14-day retreatment interval is proposed. Use of a spray surfactant is recommended.
Oilseed Crop Subgroup: Rapeseed (canola varieties only - see specific instructions above for canola); rapeseed (*Brassica napus* and *Brassica rapa*; Indian rapeseed (*Brassica rapa)*; Indian mustard (*Brassica juncea*); field mustard (*Brassica rapa)*; black mustard (*Brassica nigra*); flax (*Linum usitatissimum*); crambe (*Crambe abyssinica)*; and borage (*Borago officinalis*)						
Proline® 480 SC Fungicide	Postemergence Broadcast foliar Ground or aerial	0.134-0.178 [150-200]	2	0.356 [400]	36	Apply during the 20% up to the 50% bloom stage. A 5- to 7-day retreatment interval is proposed. Use of a spray surfactant is recommended.

TABLE 2. Summary of Directions for Use of Prothioconazole.						
Trade Name	Applic. Timing, Type, and Equip.	Applic. Rate (lb a.i./A) [g a.i./ha]	Max. No. Applic. per Season	Max. Seasonal Applic. Rate (lb a.i./A) [g a.i./ha]	PHI (days)	Use Directions and Limitations
Peanut						
Proline® 480 SC Fungicide	Postemergence Broadcast soil Ground or aerial	0.178 [200]	4	0.713 [800]	14	Apply as 4 consecutive applications with a 14-day retreatment interval. The feeding of hay or threshings or grazing of livestock in treated areas is prohibited.
Proline® 480 SC Fungicide	Postemergence Broadcast foliar Ground or aerial	0.156-0.178 [175-200]	4	0.713 [800]	14	A 14- to 21-day retreatment interval is proposed. Use of a spray surfactant is recommended. The feeding of hay or threshings or grazing of livestock in treated areas is prohibited.
Rice						
Proline® 480 SC Fungicide	Postemergence Broadcast foliar Ground or aerial	0.143 [160]	2	0.285 [320]	40	Apply at first sign of disease; usually from panicle differentiation to late boot. A second application may be made up to 70% panicle emergence from the boot. A retreatment interval is not specified. Use of a spray surfactant is recommended.
Wheat						
Proline® 480 SC Fungicide	Postemergence Broadcast foliar Ground or aerial	0.134-0.178 [150-200]	2	0.293 [328]	30	Apply during Feekes stages 10.4-10.52 (at least 75% of wheat heads on main stem fully emerged to when 50% of heads on main stem in flower); applications may be made up to Feekes stage 10.52 (heads in full flower). A 7- to 14-day retreatment interval is proposed. Use of a spray surfactant is recommended.
Proline® 480 SC Fungicide	Postemergence Broadcast foliar Ground or aerial	0.134-0.156 [150-175]	2	0.293 [328]	30	Apply when earliest disease symptoms appear on leaves or stems. A 7- to 14-day retreatment interval is proposed. Use of a spray surfactant is recommended.

The general use directions for the label specify that ground applications are to be made in a minimum of 10 gal/A, and aerial applications are to be made in a minimum of 5 gal/A. For all uses except soil application in peanuts, the label specifies that the lowest labeled rate of a non-ionic spray surfactant should be tank-mixed with the product. For soil application in peanuts, the

label notes that the product must be carried by rainfall or irrigation into the root and pod zone of the plants. Application through any type of irrigation system is prohibited. A restricted entry interval of 24 hours is specified.

The label provides mixing procedures for tank mixes, and notes that the product is compatible with most insecticide, fungicide, herbicide, and foliar nutrient products. It states that physical compatibility of product with tank-mix partners should be tested using a jar test before use.

The following rotational crop restrictions are proposed: crops listed on the label may be planted as soon as practical after last application; all other crops may be planted 30 days following last application.

CONCLUSION

The proposed label is adequate to allow evaluation of the residue data submitted in support of this petition.

Although the label specifies use of a spray adjuvant for all uses except soil application to peanuts, the only crops for which surfactants were used in the field trials were those in the dried pea/bean crop group. In the absence of data supporting their use, the label must be modified to remove the recommendation regarding spray adjuvants for all crops except chickpea, lentils, and the dried shelled peas and beans subgroup.

The applicant has proposed use on an "Oilseed Crop Subgroup" which consists of the members of the Oilseed Crop Group 20 with the exception of safflower seed and sunflower seed. The representative crops of Crop Group 20 are canola and sunflower. Currently, no crop subgroups have been defined by HED for Crop Group 20. The applicant has submitted crop field trial data for canola but not for sunflower. Therefore, in the absence of crop field trial data for sunflower, the applicant must modify the Oilseed Crop Subgroup listing to remove all commodities except canola and crambe.

We note that the use directions for barley and wheat specify that the maximum single application rate is 0.178 lb a.i./A (200 g a.i./ha) and that a maximum of two applications may be made. The maximum seasonal application rate for barley and wheat is 0.293 lb a.i./A (328 g a.i./ha) which is less than two times the maximum single application rate. For wheat and barley, the applicant may wish to note on the product label that the maximum seasonal rate would be exceeded if two applications were made at the maximum single application rate.

REFERENCES

None.

the applicant must modify the Oilseed Crop Subgroup listing to remove all commodities except canola and crambe.

We note that the use directions for barley and wheat specify that the maximum single application rate is 0.178 lb a.i./A (200 g a.i./ha) and that a maximum of two applications may be made. The maximum seasonal application rate for barley and wheat is 0.293 lb a.i./A (328 g a.i./ha) which is less than two times the maximum single application rate. For wheat and barley, the applicant may wish to note on the product label that the maximum seasonal rate would be exceeded if two applications were made at the maximum single application rate.

REFERENCES

None.

DOCUMENT TRACKING

RDI: Stephen Funk (IO)15/03/2006; Leung Cheng (RAB3).
Petition Number: PP#4F6830
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PC Code: 113961

Template Version September 2003

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In the absence of signatures, this document is considered to be a draft with deliberative material for internal use only.

This DER was originally prepared under contract by Dynamac Corporation (20440 Century Boulevard, Suite 100; Germantown, MD 20874; submitted 05/05/2005). The DER has been reviewed by the Health Effects Division (HED) and Health Canada's Pest Management Regulatory Agency (PMRA). The DER has been revised to reflect current Office of Pesticide Programs (OPP) policies, and PMRA Directive 98-02.

STUDY REPORT:

46246147 Beedle, E.; Ying, S. (2004) The Metabolism of [Triazole-UL-(Carbon 14)]JAU6476 in Sugar Beets. Project Number: J6041603, 200467. Unpublished study prepared by Bayer Corp. 91 p.

46246148 Beedle, E.; Ying, S. (2004) The Metabolism of [Phenyl-UL-(Carbon 14)]JAU6476 in Sugar Beets. Project Number: J6041602, 200466. Unpublished study prepared by Bayer Corp. 86 p.

EXECUTIVE SUMMARY:

Phenyl-label Study

Bayer CropScience submitted studies investigating the metabolism of [phenyl-UL-^{14}C]-prothioconazole in sugar beet. The radiolabeled test substances were formulated as suspension concentrates and applied as four foliar broadcast sprays at 14-day re-treatment intervals to sugar beet plants. The rate applied for the phenyl-label study was 0.228 lb a.i./A/application (255 g a.i./ha) for a total of 1.028 lb a.i./A/season (1152 g a.i./ha/season). Sugar beet roots and tops were harvested 7 days following the final application.

Following foliar application of [phenyl-UL-^{14}C]-prothioconazole to sugar beets, total radioactive residues (TRRs), determined by combustion and liquid scintillation counting (LSC), were 4.333 ppm in tops and 0.119 ppm in roots. Solvent extraction with acetonitrile/water (4:1) released the majority of the TRRs in tops (92.9 % of the TRRs) and root (69.9 % of the TRRs). Cysteine HCl was added to all extracting solvents to prevent oxidative decomposition of prothioconazole. Additional radioactivity was released from sugar beet matrices by: reflux with methanol/water (4:1) at 60-70°C for 8-9 hours, accelerated solvent extraction with methanol/water (1:1), and acid and base hydrolysis with 0.1% TFA and *1N* NaOH. Non-extractable residues remaining following extraction/hydrolysis accounted for 1.3% of the TRRs (0.056 ppm) in tops, and 8.4 % of the TRRs (0.010 ppm) in root. The applicant normalized the extraction results for accountabilites of 100%. However, the reported accountability in tops prior to normalization was 96%. Residues were identified and quantitated primarily by HPLC co-chromatography with confirmatory analysis and/or structure elucidation by *TLC, LC-MS, and LC-MS/MS*. These methods successfully identified the predominant residues in sugar beet tops and roots. The interval of storage from harvest to analysis for both the RAC and extracts was less than 28 days for both studies.

Approximately 65% of the TRRs were identified in sugar beet tops, and 60% of the TRRs were identified in roots. Prothioconazole was identified at 7.4% of the TRRs (0.322 ppm) in tops, but was not identified in roots. Metabolite JAU6476-desthio was the major identified residue in both tops and roots, accounting for 28.8% of the TRRs (1.248 ppm) and 57.6% of the TRRs (0.068 ppm), respectively. JAU6476-triazolinone was identified in both tops and roots, at 2.0-2.4% of the TRRs (0.003-0.088 ppm). JAU6476-desthio-hydroxy-dieneyl- cysteine isomers were identified in sugar beet tops only at 10.5% of the TRRs (0.454 ppm). The following additional minor metabolites were identified in sugar beet tops only: JAU6476-OH-sulfonic acid glucoside isomers (8.1% of the TRRs; 0.351 ppm), a JAU6476-OH-desthio glucoside isomer (5.1% of the TRRs; 0.222 ppm), JAU6476-α-OH-desthio and JAU6476-OH-di-sulfonic acid glucoside (≤1.9% of the TRRs each; ≤0.083 ppm). Remaining radioactivity in sugar beet matrices was characterized as: (1) multicomponent and minor unknowns, accounting for 31.4% of the TRRs in tops (>13 components, each present at ≤4.3% of the TRRs, ≤0.188 ppm); (2) methanol-extractable residues (10.8% of the TRRs in root); and (3) acid- and base-hydrolyzable residues (1.6% of the TRRs in tops and 11.0% of the TRRs in root).

Triazole-label Study

Bayer CropScience submitted studies investigating the metabolism of [triazole-UL-^{14}C]-prothioconazole (specific activity 18.6 mCi/mmol [MBq/mg]) in sugar beet. The radiolabeled test substances were formulated as suspension concentrates and applied as four foliar broadcast sprays at 14-day re-treatment intervals to sugar beet plants. The rate applied for the triazole-label study was 0.286 lb a.i./A for a total of 1.032 lb a.i./A/season (1157 g a.i./ha). Sugar beet roots and tops were harvested 7 days following the final application.

Following foliar application of [triazole-UL-^{14}C]-prothioconazole to sugar beets, total radioactive residues (TRRs), determined by combustion and liquid scintillation counting (LSC), were 5.154 ppm in tops and 0.130 ppm in roots. Solvent extraction with acetonitrile/water (4:1) released the majority of the TRRs in tops (90.0% of the TRRs) and root (70.2 % of the TRRs). Cysteine HCl was added to all extracting solvents to prevent oxidative decomposition of prothioconazole. Additional radioactivity was released from sugar beet matrices by: reflux with methanol/water (4:1) at 60-70°C for 8-9 hours, accelerated solvent extraction with methanol/water (1:1), and acid and base hydrolysis with 0.1% TFA and *1N* NaOH. Non-extractable residues remaining following extraction/hydrolysis accounted for 1.9 % of the TRRs (0.099 ppm) in tops, and 6.0% of the TRRs (0.008 ppm) in root. The applicant normalized the extraction results for accountabilities of 100%. However, the reported accountabilities prior to normalization ranged from 85-101%. Residues were identified and quantitated primarily by HPLC co-chromatography with confirmatory analysis and/or structure elucidation by TLC, LC-MS, and LC-MS/MS. These methods successfully identified the predominant residues in sugar beet tops and roots. The interval of storage from harvest to analysis for both the RAC and extracts was less than 28 days for both studies.

Approximately 69% of the TRRs were identified in sugar beet tops, and 61% of the TRRs were identified in roots. Prothioconazole was identified at 5.1% of the TRRs (0.265 ppm) in tops, but was not identified in roots. Metabolite JAU6476-desthio was a major identified residue in both tops and roots, accounting for 19.2% of the TRRs (0.988 ppm) and 25.5% of the TRRs (0.033 ppm), respectively. JAU6476-triazolinone was identified in both tops and roots, at 1.6-2.0% of the TRRs (0.003-0.105 ppm). In the triazole-label study, the triazole-specific metabolite, triazolylalanine, was the major identified residue in sugar beet roots at 28.9% of the TRRs (0.038 ppm) and was identified in tops at 1.6% of the TRRs (0.084 ppm). Accordingly, in the triazole-label study, JAU6476-desthio-hydroxy-dieneyl- cysteine isomers were identified in both tops and roots, at 9.9% of the TRRs (0.512 ppm) and 5.4% of the TRRs (0.007 ppm), respectively. The following additional minor metabolites were identified in sugar beet tops only: JAU6476-OH-sulfonic acid glucoside isomers (6.1% of the TRRs; 0.316 ppm), a JAU6476-OH-desthio glucoside isomer (6.5% of the TRRs; 0.334 ppm), triazolyl-sulfonic acid-ethanol glucoside and triazolyl-ethanol-glucoside (together at 5.1% of the TRRs; 0.263 ppm), triazolylhydroxypropionic acid (THPA) and triazolyl-ethanol (3.8-4.0% of the TRRs; 0.194-0.207 ppm), JAU6476 sulfonic acid (4.0% of the TRRs; 0.205 ppm), JAU6476-OH-desthio (1.2% of the TRRs; 0.063 ppm). Remaining radioactivity in sugar beet matrices was characterized as: (1) multicomponent and minor unknowns, accounting for 21.4% of the TRRs in tops (9 components, each present at ≤4.6% of the TRRs, ≤0.236 ppm); (2) methanol-

extractable residues (5.3% of the TRRs in tops and 16.1% of the TRRs in root); and (3) acid- and base-hydrolyzable residues (2.7% of the TRRs in tops and 7.7% of the TRRs in root). In the triazole-label study, strong anion exchange (SAX) and strong cation exchange (SCX) solid phase extraction cartridges (SPE) eluate/effluents accounted for 3.8% of the TRRs in sugar beet root.

Prothioconazole was extensively metabolized in sugar beet via: (1) oxidation of the sulfur of the triazolinethione ring to the corresponding sulfonic acid and subsequent elimination of the sulfonic acid group to form JAU6476-desthio; and (2) hydroxylation of the phenyl ring or the benzyl carbon to form multiple isomers, with subsequent conjugation with glucose or further reaction to produce JAU6476-desthio-hydroxy-dieneyl-cysteine. Observed in the triazole-label study only was the release of the triazole moiety to form triazolylalanine (TA) and triazolylhydroxypropionic acid (THPA) and elimination of the phenyl ring. Free triazole (1*H*-1,2,4-triazole) was not identified in any of the sugar beet matrices.

STUDY/WAIVER ACCEPTABILITY/DEFICIENCIES/CLARIFICATIONS:

The acceptability of this study for regulatory purposes is addressed in the forthcoming U.S. EPA Residue Chemistry Summary Document, DP Barcode D303508, PP#4F6830, and in Canada's Regulatory Decision Document.

COMPLIANCE:

Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided. No deviations from regulatory requirements were reported.

A. BACKGROUND INFORMATION

Prothioconazole is a broad-spectrum systemic fungicide intended for the control of many diseases in different crops such as cereals, oilseed rape or peanuts. The biochemical mode of action is the inhibition of the demethylation of lanosterol or 24-methylene-dihydrolanosterol, which are precursors of sterols in fungi. The chemical has been submitted for joint EPA and PMRA review by Bayer CropScience. The crops proposed for joint review include barley, the dried shell and bean subgroup, the oilseed crop group, and wheat. Uses on peanuts and rice are being proposed for the U.S. only.

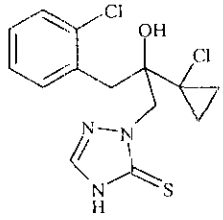
TABLE A.1. Test Compound Nomenclature.	
Chemical structure	
Common name	Prothioconazole (ISO approved)
Company experimental name	JAU6476
IUPAC name	2-[(2*RS*)-2-(1-chlorocyclopropyl)-3-(2-chlorophenyl)-2-hydroxypropyl]-2*H*-1,2,4-triazole-3(4*H*)-thione
CAS name	2-[2-(1-chlorocyclopropyl)-3-(2-chlorophenyl)-2-hydroxypropyl]-1,2-dihydro-3*H*-1,2,4-triazole-3-thione
CAS registry number	178928-70-6
PC Code	113961
End-use product (EP)	Proline® 480 SC (4 lb/gal suspension concentrate), EPA File Symbol 264-IEL

TABLE A.2. Physicochemical Properties of the Technical Grade Test Compound.			
Parameter	Value		Reference
Melting range	139.1 to 144.5°C		MRID 46246003 / CES
pH	5.8 (1% solution)		MRID 46246003 / CES
Density at 20°C	1.36 g/mL		MRID 46246003 / CES
Water solubility at 20°C	pH	mg/L	MRID 46246003 / CES
	4	5	
	8	300	
	9	2000	
Solvent solubility at 20°C	Solvent	g/L	MRID 46246003 / CES
	Acetone	>250	
	Acetonitrile	69	
	Dichloromethane	88	
	Dimethysulfoxide	126	
	Ethyl acetate	>250	
	n-Heptane	<0.1	
	1-Octanol	58	
	Polyethylene glycol	>250	
	2-Propanol	87	
	Xylene	8	
Vapor pressure at 20 or 25°C	$<4 \times 10^{-7}$ Pa (calculated from determinations at 70 °C)		MRID 46246003 / CES
Dissociation constant, pK_a	6.9 (calculated from K_{OW})		MRID 46246003 / CES
Octanol/water partition coefficient, $Log(K_{OW})$, at 20°C	pH	Log Kow	MRID 46246003 / CES
	unbuffered water	4.05	
	pH 4	4.16	
	pH 7	3.82	
	pH 9	2.00	
UV/visible absorption spectrum	Peak maxima at 257 nm		MRID 46246003 / CES

CES (Chemistry Evaluation Section of PMRA).

B. EXPERIMENTAL DESIGN

B.1. Test Site and Crop Information

TABLE B.1.1. Test Site Information.				
Testing Environment	Soil characteristics			
	Type	%OM	pH	CEC (meq/100g)
Plants were grown in 5-gal plastic buckets placed on wooden blocks in a metal stock tub, at Bayer (Stilwell, KS). Plants were started in the greenhouse, then moved outdoors when seedlings were 2" tall.	Sandy loam	2.4	7.4	9.3

Daily greenhouse temperatures ranged from 22-51°C, and relative humidity ranged from 22-64%. Daily outdoor temperatures, precipitation, and relative humidity were reported through harvest. Sugar beet plants received supplemental water and were fertilized, hand weeded, and sprayed

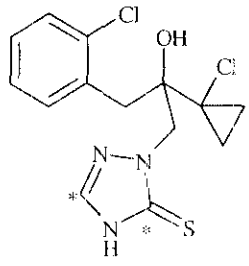
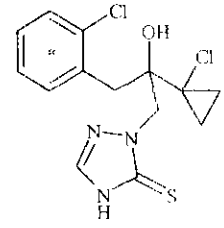
with maintenance chemicals as necessary to maintain healthy plant growth. Accumulated rainfall was drained from the stock tub as needed.

TABLE B.1.2. Crop Information.

Crop; crop group	Variety	Growth stage at application	Growth stage at harvest	Harvested RAC	Harvesting procedure
Sugar beet; Vegetable, root and tuber (Crop Group 1) and Vegetable, leaves of root and tuber (Crop Group 2)	Holly Hybrids	Growth stages were not specified; applications were made 100, 114, 128, and 142 days after planting.	Maturity	Sugar beet roots and tops	Tops were removed from roots at the point of attachment; roots were dug from the soil and rinsed with water to remove dirt.

B.2. Test Materials

TABLE B.2.1. Test Material Characteristics.

Chemical structure		
Radiolabel position	[triazole-UL-^{14}C]-prothioconazole	[phenyl-UL-^{14}C]-prothioconazole
Lot No.	909A and 909B	C-910B and C-910C
Purity	≥98% radiochemical purity (stock solution) ≥97% radiochemical purity (treatment solutions)	≥99% radiochemical purity (stock solution) ≥97% radiochemical purity (treatment solutions)
Specific activity	18.6 mCi/mmol (120,000 dpm/µg)	

B.3. Study Use Pattern

TABLE B.3.1. Use Pattern Information

Chemical name	[triazole-UL-^{14}C]-prothioconazole	[phenyl-UL-^{14}C]-prothioconazole
Application rate	0.232, 0.260, 0.254, and 0.286 lb a.i./A/application (260, 291, 285, and 321 g a.i./ha) for a total application rate of 1.032 lb a.i./A (1157 g a.i./ha).	0.228, 0.269, 0.252, and 0.279 lb a.i./A/application (255, 302, 282, and 313 g a.i./ha) for a total application rate of 1.028 lb a.i./A (1152 g a.i./ha).
Application method	A stock solution of the radiolabeled test substance was evaporated to dryness, mixed with benzene and a seed crystal of nonradiolabeled prothioconazole, evaporated to dryness again, and then formulated as a suspension concentrate with formulation blank. The formulated test substance was diluted with water and added to a spray bottle for application.	
Number of applications	4	
Timing of applications	Applications were made 100, 114, 128, and 142 days after planting at 14-day retreatment intervals.	

TABLE B.3.1. Use Pattern Information	
PHI[1]	7 days

[1] PHI = pre-harvest interval.

B.4. Identification/Characterization of Residues

B.4.1. Sample Handling and Preparation

Sugar beet tops were placed in a freezer (-20 ± 6°C) immediately after harvest; roots were cut into small pieces then placed in the freezer. Tops and roots were processed by homogenizing in the presence of dry ice. Following processing, sugar beet samples were stored frozen at approximately -20°C, and extracts were stored at 0 ± 5°C.

Phenyl-label study:
Tops: Sugar beet tops were extracted 3x with acetonitrile (ACN):water (4:1, v:v) and centrifuged. Cysteine HCl was added to the ACN:water extraction mixture to prevent oxidative decomposition of prothioconazole. The resulting extracts were combined, concentrated to near dryness using rotary evaporation, dissolved in water, then applied to two C-18 SPE cartridges that had been conditioned with ACN and water. Residues were sequentially eluted with water, ACN:water (4:1, v:v), and ACN. The initial column effluent and the eluates were combined, evaporated to dryness under a stream of nitrogen, and redissolved in ACN:0.1% acetic acid (1:9, v:v) and reserved for HPLC analysis. Nonextractable residues were extracted with methanol:water (4:1, v:v) at reflux for 9 hours, then cooled and filtered. The filter cake was rinsed with water, and the rinsate was combined with the methanol/water extract. The remaining non-extractable residues were subjected to accelerated solvent extraction (ASE) with methanol:water (1:1, v:v) at 150°C and 1500 psi. The resulting extract was combined with the methanol/water extract from the previous step, and the combined extracts were evaporated to dryness by rotary evaporation, redissolved in ACN:0.1% acetic acid (1:9, v:v), and reserved for HPLC analysis. Remaining nonextractable residues were subjected to two additional ASE procedures using 0.1% TFA and 1N NaOH.

Root: Subsamples of sugar beet root were extracted with ACN/water, and the extracts were applied to C-18 SPE cartridges as described above for tops. Residues were eluted from the SPE cartridges with water, ACN:water (4:1, v:v), ACN, and ammonium hydroxide:ethanol (1:1, v:v). The initial column effluent and eluates were combined, dissolved in water, and loaded onto three C-18 SPE cartridges. The cartridges were again eluted with water, ACN/water, and ACN; however, eluents were collected separately. The ACN/water eluate was evaporated to dryness under rotary evaporation, dissolved in ACN:0.1% acetic acid (1:9, v:v), and reserved for HPLC analysis. The initial effluent and the water eluate were combined, concentrated to near dryness under nitrogen, and dissolved in water. This sample was further purified using NH_2 SPE cartridges. Residues were sequentially eluted with water, ACN:water (4:1, v:v), ACN, methanol, and ammonium hydroxide:ethanol (1:1, v:v). The initial effluent and the water, ACN/water, and ammonium hydroxide/ethanol eluates were combined and concentrated to near dryness under nitrogen, then redissolved in ACN:0.1% acetic acid (1:9, v:v), and reserved for HPLC analysis.

Non-extractable residues of sugar beet root following the initial ACN/water extraction were extracted with methanol:water (4:1, v:v) at reflux for 8 hours, then cooled and filtered. The filter cake was rinsed with water, and the rinsate was combined with the methanol/water extract. The remaining nonextractable residues were subjected to sequential ASE using methanol:water (1:1, v:v), 0.1% TFA, and 1N NaOH as described above for sugar beet tops, except that the methanol/water extract from ASE was not combined with the initial methanol/water extract.

Triazole-label study:

Tops: Sugar beet tops were extracted three times with acetonitrile (ACN):water (4:1, v:v) and centrifuged. Cysteine HCl was added to the ACN:water extraction mixture to prevent oxidative decomposition of prothioconazole. The resulting extracts were combined, concentrated to near dryness using rotary evaporation, and dissolved in water, then applied to two C-18 SPE cartridges that had been conditioned with ACN and water. Residues were sequentially eluted with water, ACN:water (4:1, v:v), and ACN. The initial column effluent and the eluates were combined, evaporated to dryness under a stream of nitrogen, and redissolved in ACN:0.1% acetic acid (1:9, v:v) and reserved for HPLC analysis. Nonextractable residues were extracted with methanol:water (4:1, v:v) at reflux for 8 hours, then cooled and filtered. The filter cake was rinsed with water, and the rinsate was combined with the methanol/water extract. The remaining nonextractable residues were subjected to accelerated solvent extraction (ASE) with methanol:water (1:1, v:v) at 150°C and 1500 psi. Remaining nonextractable residues were subjected to two additional ASE procedures using 0.1% TFA and 1N NaOH.

Root: Subsamples of sugar beet root were extracted with ACN/water, and the extracts were applied to C-18 SPE cartridges (3 cartridges) as described above for tops. Residues were eluted from the SPE cartridges with water, ACN:water (4:1, v:v), and ACN. The initial effluent and the water eluate were combined, and the ACN/water and ACN eluates were combined. The respective combined eluates were evaporated to dryness under rotary evaporation, dissolved in ACN:0.1% acetic acid (1:9, v:v), and reserved for HPLC analysis. Nonextractable residues of sugar beet root following the initial ACN/water extraction were refluxed with methanol:water (4:1, v:v) for 8 hours, then filtered. The filter cake was rinsed with water, and the rinsate was combined with the methanol/water extract. The remaining nonextractable residues were subjected to sequential ASE using methanol:water (1:1, v:v), 0.1% TFA, and 1N NaOH as described above for sugar beet tops.

The extraction procedures for sugar beet matrices are summarized in FIGURES B.4.1.1 and B.4.1.2.

FIGURE B.4.1.1 Extraction Procedure for Sugar Beet Tops

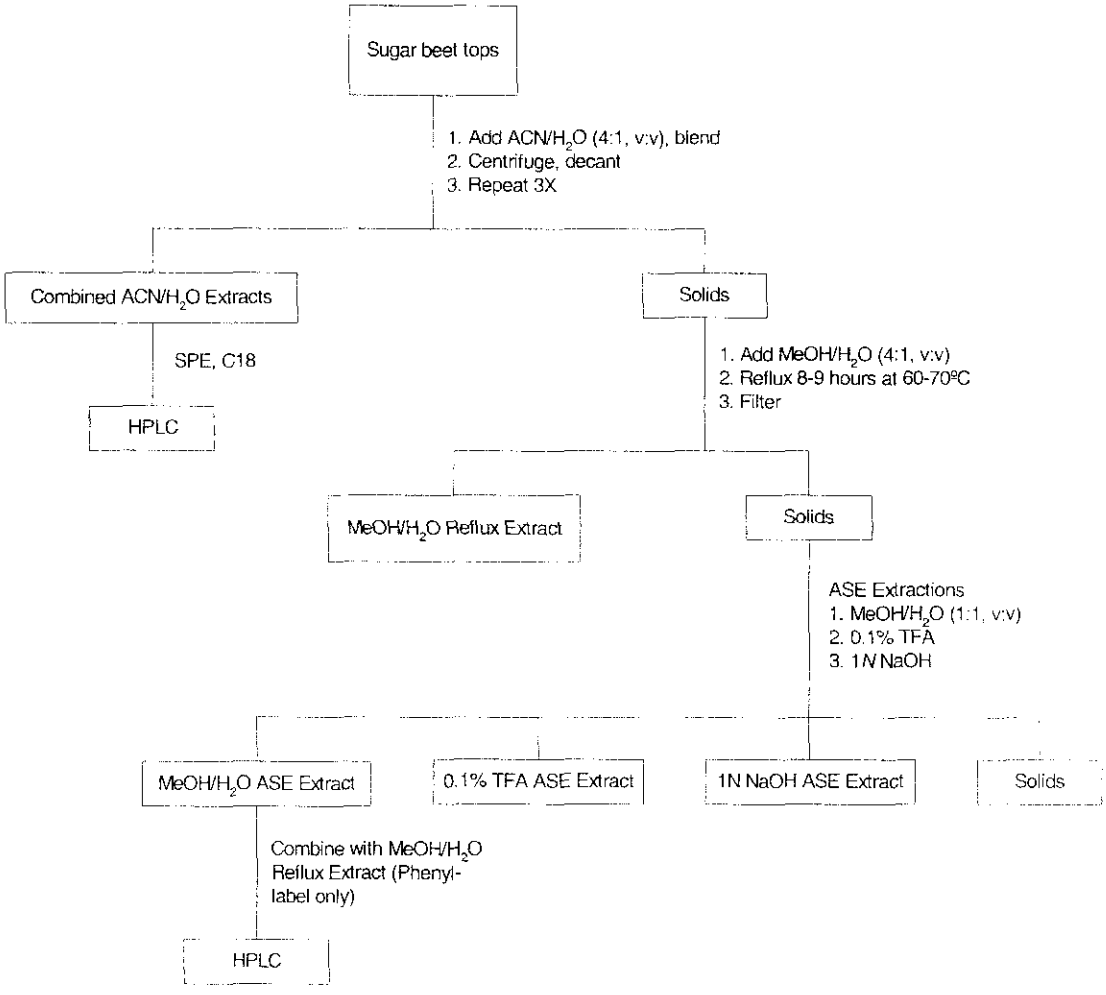
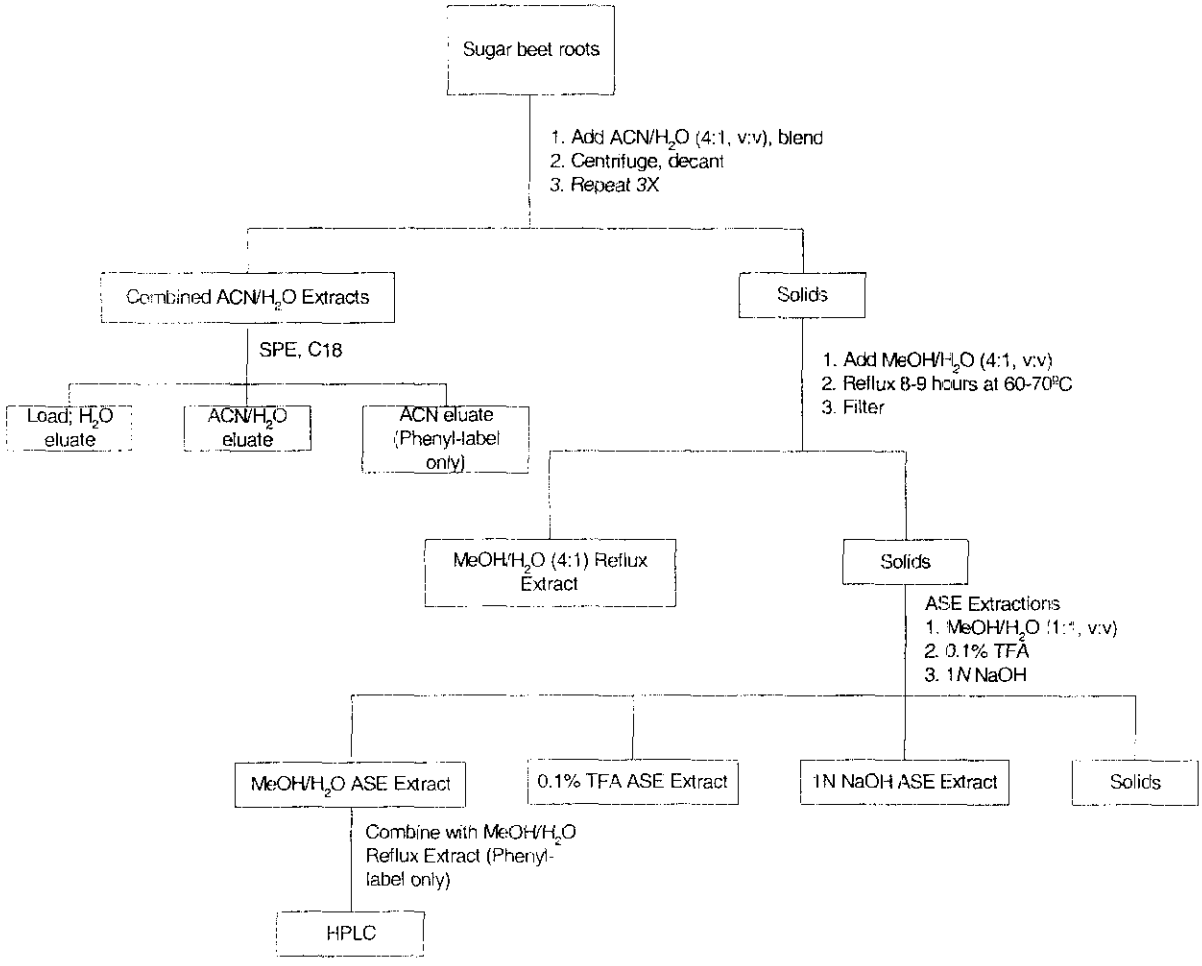


FIGURE B.4.1.2 Extraction Procedure for Sugar Beet Root



B.4.2. Analytical Methodology

Total radioactive residues (TRRs) in sugar beet matrices were determined by combustion/LSC. Extracts and hydrolysates were radioassayed by LSC, and non-extractable residues were radioassayed by combustion/LSC. The reported limits of detection were 0.0004 ppm for liquid samples and 0.0057 ppm for solid samples.

High Performance Liquid Chromatography (HPLC)
Extracts and hydrolysates of sugar beet matrices were subjected to HPLC for identification and quantitation of metabolites, with confirmatory analysis by TLC (phenyl-label study only) and/or LC-MS and LC-MS/MS. HPLC analyses were conducted on systems equipped with a variable wavelength UV detector and a radiodetector. For the triazole-label study, the following column/mobile phase combinations were used: (A) C-18 column with a gradient mobile phase of 0.1% aqueous acetic acid and ACN; (B) C-8 column with a gradient mobile phase of water and ACN; (C) C-18 column with a gradient mobile phase of 0.1% aqueous acetic acid and ACN; (D) C-18 column (new column) with a gradient mobile phase of 0.1% aqueous acetic acid and ACN; (E) C-8 column with a gradient mobile phase of water and ACN; and (F) C-8 column with a gradient mobile phase of water and methanol, each containing 0.005M 1-pentyltriethylammonium phosphate as an ion-pairing agent. The following column/mobile phase combinations were used for the phenyl-label study study: (A) C-18 column with a gradient mobile phase of 0.1% aqueous acetic acid and ACN; (B) C-18 column with a gradient mobile phase of water and ACN; (C) C-8 column with a gradient mobile phase of water and ACN; (D) C-8 column with a gradient mobile phase of water and ACN or methanol; (E) C-18 column with a gradient mobile phase of water and methanol; and (F) C-18 column with a gradient mobile phase of 0.1% aqueous acetic acid and ACN.

System A was used for initial profiling and for preparative HPLC for isolation of metabolites; systems B through F were used for further analysis and for collection and purification of metabolites. Metabolites were identified (TABLE C.3.1) by co-chromatography and/or retention time comparisons with reference standards or by comparison of metabolite patterns between like extracts of different matrices. Chemical names and structures for the reference standards are presented in Appendix I..

Thin Layer Chromatography (TLC)
TLC analyses were conducted using silica gel 60 F_{254} plates and a solvent system of dichloromethane:methanol:ammonium hydroxide (9:1:0.1, v:v:v). Radioactive areas were detected by phosphorimaging.

Mass Spectrometry (MS)
LC-MS and LC-MS/MS analyses were conducted using C8 columns, a gradient mobile phase of 0.1% formic acid and methanol, and MS or MS/MS detection with positive or negative electro-spray ionization.

Derivatization (Triazole-label study only)

Two polar components from the ACN/water extract of sugar beet tops that were isolated by HPLC, and the combined initial effluent/water C-18 SPE eluate of sugar beet root were subjected to purification by SPE and HPLC with ion-pairing prior to derivatization for characterization/identification. The components were combined and loaded onto strong anion exchange (SAX) SPE cartridges that had been conditioned with methanol and water. The initial effluent was collected, the cartridges were rinsed with water, and the initial effluent and water rinse were combined and applied to strong cation exchange (SCX) SPE cartridges that had also been conditioned with methanol and water. Residues were eluted from the SAX cartridges with methanol followed by 5% acetic acid in methanol, and residues were eluted from the SCX cartridges with methanol followed sequentially by 5% and 10% ammonium hydroxide in methanol. The eluates from the SAX and SCX cartridges were separately combined and evaporated to dryness by rotary evaporation. The SCX residue from sugar beet root was dissolved in water and loaded onto two additional SCX cartridges that had been conditioned and rinsed as before; residues were eluted with methanol and 5% ammonium hydroxide in methanol. The resulting residues were evaporated to dryness.

The SAX and SCX residues from sugar beet tops were redissolved in 10% methanol/water containing ion-pairing agent 0.005M 1-pentyltriethylammonium phosphate for HPLC analysis using System F. The SCX residue from sugar beet root was dissolved in ACN:0.1% acetic acid (1:9, v:v) and subjected to preparative HPLC. The resulting polar component was isolated and dissolved in 0.005M 1-pentyltriethylammonium phosphate for HPLC analysis. The isolated components from sugar beet tops and root were then subjected to derivatization with n-butanol by heating with 3N HCl in n-butanol at 110°C for 1 hour. The solvent was removed by evaporation, and the components were further derivatized, if necessary, by heating for 15 minutes at 170°C with heptafluorobutyric anhydride. The resulting product was dissolved in ACN:water (1:4, v:v) for HPLC analysis.

C. RESULTS AND DISCUSSION

Triazole-label study

Total radioactive residues (TRRs) in sugar beet matrices are reported in TABLE C.2.1 and FIGURE C.2.1. TRRs were 5.154 ppm in tops and 0.130 ppm in root.

The distribution of the radioactivity in sugar beet matrices is presented in TABLE C.2.2. Solvent extraction with ACN:water (4:1) released the majority of the radioactivity in tops (90.0% of the TRRs; 4.639 ppm) and root (70.2% of the TRRs; 0.091 ppm). Cysteine HCl was added to the initial extracting solvents to prevent oxidative decomposition of prothioconazole. Additional radioactivity was released from sugar beet matrices by: refluxing with methanol, accelerated solvent extraction (ASE) with methanol/water, and acid and base hydrolysis with 0.1% TFA and 1N NaOH. Non-extractable residues remaining following extraction/hydrolysis accounted for 1.9% of the TRRs (0.099 ppm) in tops, and 6.0% of the TRRs (0.008 ppm) in root. The applicant normalized the extraction results for accountabilites of 100%. However, the reported accountabilities prior to normalization ranged from 85-101%. Residues were identified and quantitated primarily by HPLC co-chromatography with confirmatory analysis and/or structure elucidation by LC-MS, and LC-MS/MS. These methods successfully identified the predominant residues in sugar beet tops and root.

The characterization and identification of residues in sugar beet matrices are summarized in TABLE C.2.4. Approximately 69% of the TRRs (3.536 ppm) were identified in sugar beet tops, and 61% of the TRRs (0.080 ppm) were identified in root. Prothioconazole was identified at 5.1% of the TRRs (0.265 ppm) in tops, but was not identified in root. Metabolite JAU6476-desthio was the major identified residue in tops and was a major residue in root, accounting for 19.2% of the TRRs (0.988 ppm) and 25.5% of the TRRs (0.033 ppm), respectively. Triazolylalanine was the predominant metabolite in sugar beet root at 28.9% of the TRRs (0.038 ppm) and was identified in tops at 1.6% of the TRRs (0.084 ppm). JAU6476-triazolinone was identified in both tops and root, at 1.6-2.0% of the TRRs (0.003-0.105 ppm), as were JAU6476-desthio-hydroxy-dieneyl cysteine isomers, at 9.9% of the TRRs (0.512 ppm) in tops and 5.4% of the TRRs (0.007 ppm) in root. The following additional metabolites were identified in sugar beet tops only: a JAU6476-hydroxy-desthio-glucoside isomer at 6.5% of the TRRs (0.334 ppm); JAU6476-hydroxy-sulfonic acid-glucoside isomers at 6.1% of the TRRs (0.316 ppm); triazolyl-sulfonic acid-ethanol glucoside and triazolyl-ethanol-glucoside together at 5.1% of the TRRs (0.263 ppm); triazolylhydroxypropionic acid (THPA) and triazolyl-ethanol at 3.8-4.0% of the TRRs (0.194-0.207 ppm); JAU6476 sulfonic acid at 4.0% of the TRRs (0.205 ppm); and JAU6476-hydroxy-desthio at 1.2% of the TRRs (0.063 ppm). The remaining radioactivity in sugar beet matrices was characterized as: (1) minor unknowns, accounting for a total of 21.4% of the TRRs (1.102 ppm) in tops (9 components, each present at ≤4.6% of the TRRs, ≤0.236 ppm) and 5.0% of the TRRs (0.007 ppm) in root; (2) methanol-extractable residues (5.3% of the TRRs (0.274 ppm) in tops and 16.1% of the TRRs (0.021 ppm) in root); and (3) acid- and base-hydrolyzable residues (2.7% of the TRRs (0.143 ppm) in tops and 7.7% of the TRRs (0.010 ppm) in root). SAX/SCX SPE eluate/effluents accounted for 3.8% of the TRRs (0.005 ppm) in sugar beet root only.

Following initial HPLC profiling and co-chromatography with reference standards, prothioconazole, JAU6476-desthio, JAU6476-triazolinone, and JAU6476 sulfonic acid were isolated from the ACN/water extract of sugar beet tops by preparative HPLC using System A; identification was confirmed by LC-MS analysis (and LC-MS/MS analysis for JAU6476-triazolinone). JAU6476-hydroxy-desthio was isolated by preparative HPLC (system not specified) and identified by LC-MS and LC-MS/MS. Although the position of the hydroxy substituent was not confirmed by LC-MS, based on HPLC retention time comparisons with reference standards, hydroxylation was at the 3- or 4/5- position. The JAU6476-desthio-hydroxy-dieneyl cysteine isomers were isolated by preparative HPLC of an unknown peak using System A, followed by reinjection on System A for selected peaks and further purification using HPLC System B; the metabolites were positively identified by LC-MS and LC-MS/MS. The JAU6476-hydroxy-desthio-glucoside isomer and the JAU6476-hydroxy-sulfonic acid-glucoside isomers were isolated by preparative HPLC using system A and identified by LC-MS and LC-MS/MS; identification of the sulfonic acid glucoside isomers was confirmed by retention time comparisons using HPLC System C.

Triazolylalanine and THPA were isolated by preparative HPLC using System F, derivatized with acidic n-butanol and heptafluorobutyric anhydride (triazolylalanine only), and identified by LC-MS and LC-MS/MS. Triazolyl-ethanol was isolated by preparative HPLC using systems A and C and identified by LC-MS and LC-MS/MS. Triazolyl-sulfonic acid-ethanol-glucoside and triazolyl-ethanol-glucoside were isolated together by preparative HPLC using System A, subjected to further separation using HPLC system B, and identified by LC-MS and LC-MS/MS.

In sugar beet root, JAU6476-desthio was isolated by preparative HPLC, and identification was confirmed by LC-MS. JAU6476-triazolinone and JAU6476-desthio-hydroxy-dieneyl cysteine isomer were identified by retention time comparisons with the HPLC profile of sugar beet tops. Triazolylalanine was identified by LC-MS and LC-MS/MS following SAX/SCX SPE chromatography, isolation using HPLC System F, and derivatization with acidic n-butanol and heptafluorobutyric anhydride.

Phenyl-label study

Total radioactive residues (TRRs) in sugar beet matrices are reported in TABLE C.2.1 and FIGURE C.2.1. TRRs were 4.333 ppm in tops and 0.119 ppm in root.

The distribution of the radioactivity in sugar beet matrices is presented in TABLE C.2.3. Solvent extraction with ACN:water (4:1) released the majority of the radioactivity in tops (92.9% of the TRRs; 4.025 ppm) and root (69.9% of the TRRs; 0.083 ppm). Cysteine HCl was added to the extracting solvent to prevent oxidative decomposition of prothioconazole. Additional radioactivity was released from sugar beet matrices by: refluxing with methanol, ASE with methanol/water, and acid and base hydrolysis with 0.1% TFA and 1N NaOH. Non-extractable residues remaining following extraction/hydrolysis accounted for 1.3% of the TRRs (0.056 ppm) in tops, and 8.4% of the TRRs (0.010 ppm) in root. The applicant normalized the extraction results for accountabilities of 100%. However, the reported accountability in tops prior to

normalization was 96%. Residues were identified and quantitated primarily by HPLC co-chromatography with confirmatory analysis and/or structure elucidation by TLC, LC-MS, and LC-MS/MS. These methods successfully identified the predominant residues in sugar beet tops and root.

The characterization and identification of residues in sugar beet matrices are summarized in TABLE C.2.4. Approximately 65% of the TRRs (2.837 ppm) were identified in sugar beet tops, and 60% of the TRRs (0.071 ppm) were identified in root. Prothioconazole was identified at 7.4% of the TRRs (0.322 ppm) in tops, but was not identified in root. Metabolite JAU6476-desthio was the major identified residue in both tops and root, accounting for 28.8% of the TRRs (1.248 ppm) and 57.6% of the TRRs (0.068 ppm), respectively. Only one other metabolite, JAU6476-triazolinone, was identified in both sugar beet tops and roots, at 2.0% of the TRRs (0.088 ppm) and 2.4% of the TRRs (0.003 ppm), respectively. The following additional metabolites were identified in sugar beet tops only: JAU6476-desthio-hydroxy-dieneyl-cysteine isomers at 10.5% of the TRRs (0.454 ppm), JAU6476-OH-sulfonic acid glucoside isomers at 8.1% of the TRRs (0.351 ppm), a JAU6476-OH-desthio glucoside isomer at 5.1% of the TRRs (0.222 ppm), and JAU6476-α-OH-desthio and JAU6476-hydroxy-di-sulfonic acid glucoside at ≤1.9% of the TRRs (0.083 ppm) each. Remaining radioactivity in sugar beet matrices was characterized as: (1) multicomponent and minor unknowns, accounting for 31.4% of the TRRs (1.364 ppm) in tops (>13 components, each present at ≤4.3% of the TRRs, ≤0.188 ppm); (2) methanol-extractable residues (10.8% of the TRRs in root; 0.013 ppm); and (3) acid- and base-hydrolyzable residues (1.6% of the TRRs in tops (0.072 ppm) and 11.0% of the TRRs in root (0.013 ppm)). One unknown which accounted for 9.0% of the TRRs (0.011 ppm) in sugar beet root was not conclusively identified but was characterized as having polarity similar to that of JAU6476-desthio-hydroxy-dieneyl-cysteine in sugar beet tops on the basis of HPLC retention time comparison.

Following initial HPLC profiling, identification of prothioconazole and JAU6476-desthio in the ACN:water extract of sugar beet tops was confirmed by LC-MS. Identification of JAU6476-triazolinone and JAU6476-α-OH-desthio was confirmed by LC-MS and TLC analyses. Identification of the JAU6476-hydroxy-sulfonic acid glucoside isomers, JAU6476-desthio-hydroxy-dieneyl cysteine isomers, and the JAU6476-OH-desthio glucoside isomer was confirmed by LC-MS and LC-MS/MS. The JAU6476-hydroxy-di-sulfonic acid glucoside isomer was tentatively identified on the basis of LC-MS and LC-MS/MS analysis. Remaining regions from the initial HPLC profile were subjected to preparative HPLC, using one or more of the systems described above, which indicated that each consisted of multiple minor components. Identification of prothioconazole, JAU6476-desthio, and JAU6476-triazolinone in the methanol/water extracts of sugar beet tops was achieved by retention time comparisons with the metabolites that had been identified in the ACN:water extract. Identification of JAU6476-desthio in the ACN:water SPE eluate of sugar beet root was confirmed by LC-MS, and remaining metabolites in the SPE eluates of sugar beet root were confirmed by retention time comparisons with the identified metabolites in sugar beet tops.

C.1. Storage Stability

Processed sugar beet samples were stored frozen at -20 ± 5°C; extracts were stored at 0 ± 5°C. Dates of initial sample extraction were provided confirming that samples were extracted within 6-17 days of harvest and analyzed by HPLC within 5-28 days of extraction. As sample analysis was completed within 6 months of sample collection, data to demonstrate that the identity of residues did not change during the period between collection and final analysis was not required; refer to OPPTS 860.1300 (d)(7) and PMRA Directive 98-02 (Section 5.5).

TABLE C.1. Summary of Storage Conditions.

Matrix	Storage Temp. (°C)	Actual Storage Duration [1] (Days)		Interval of Demonstrated Storage Stability[2]
		[triazole-UL-^{14}C]-prothioconazole	[phenyl-UL-^{14}C]-prothioconazole	
RAC				
Tops	-20±5	13	6	Not required
Root	-20±5	17	7	
Extract				
Tops	0±5	4	5	Not required
Root	0±5	9 and 10[3]	24 and 28[3]	

1 The storage duration for the RACs is the time from sample harvest to sample extract. The storage duration for the extracts is the time from sample extract to HPLC analysis.

2 Storage stability data are not required for samples analyzed within 6 months of collection, provided that evidence is given that attempts were made to limit degradation of residues by appropriate storage of matrices and extracts during the analytical portion of the study.

3 The extract was divided into two portions based on the elution profile from the C18 cartridges, and both portions were analyzed by HPLC.

C.2. Identification, Characterization, and Distribution of Residues

TABLE C.2.1. Total Radioactive Residues (TRRs) in Sugar Beet Matrices.

Matrix	Timing and Applic. No.	PHI (days)	[triazole-UL-^{14}C]-prothioconazole	[phenyl-UL-^{14}C]-prothioconazole
Tops	4 foliar applications (110, 114, 128, 142 days after planting)	7	5.154 ppm	4.333 ppm
Root			0.130 ppm	0.119 ppm

FIGURE C.2.1 Distribution of the TRRs in sugar beet matrices.

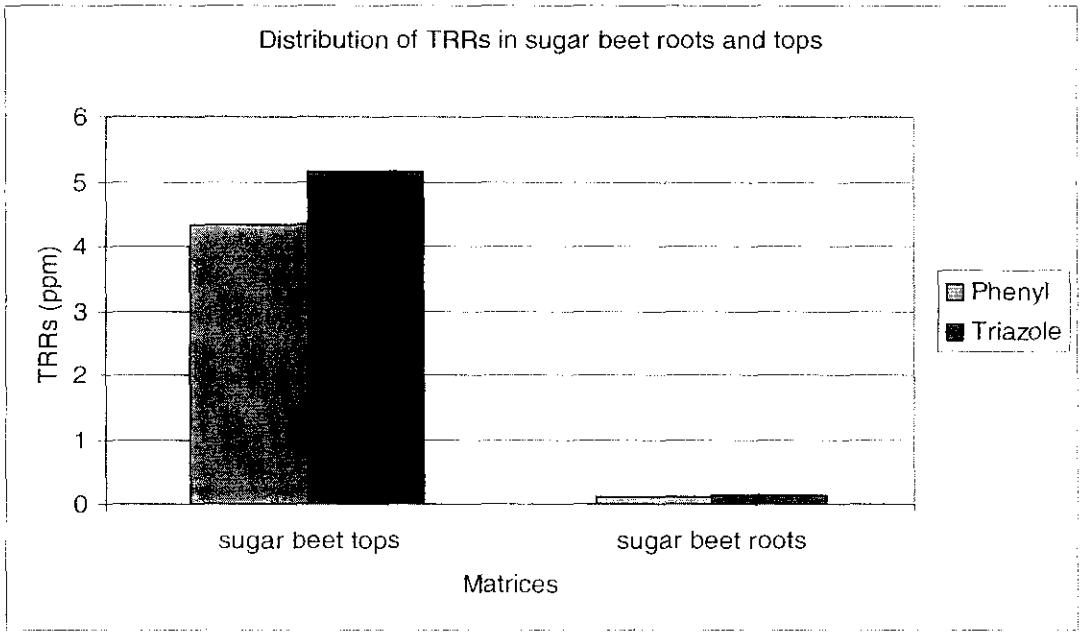


TABLE C.2.2. Distribution of the Parent and the Metabolites in Sugar Beet Matrices Following Foliar Application of [Triazole-UL-^{14}C]-prothioconazole at 1.032 lb a.i./A (1157 g a.i./ha). [1]

Metabolite Fraction	Tops		Root	
	TRR = 5.154 ppm		TRR = 0.130 ppm	
	%TRR	ppm	%TRR	ppm
ACN/water	90.0	4.639	70.2	0.091
Initial SPE effluent + water eluate	NR; combined with other eluates [2]		32.8	0.043
SAX eluate			2.8	0.004
SCX initial effluent			1.0	0.001
SCX eluate			28.9	0.038
Triazolylalanine			28.9	0.038
ACN/water + ACN SPE eluate	NR; combined with other eluates		37.4	0.049
JAU6476-desthio			25.5	0.033
JAU6476-triazolinone			1.6	0.002
JAU6476-desthio-hydroxy-dieneyl-cysteine isomer			5.4	0.007
Unknowns [3]			5.0	0.007
Combined SPE eluates	90.0	4.639		
Prothioconazole	5.2	0.265		
JAU6476-desthio	19.2	0.988		
JAU6476-triazolinone	2.0	0.105		
JAU6476-OH-desthio	1.2	0.063		
JAU6476 sulfonic acid	4.0	0.205		
Triazolylalanine	1.6	0.084		
Triazolylhydroxypropionic acid (THPA)	4.0	0.207		
Triazolyl-ethanol	3.8	0.194		
JAU6476-OH-desthio-glucoside isomer	6.5	0.334		
JAU6476-desthio-hydroxy-dieneyl-cysteine isomers	9.9	0.512		
JAU6476-OH-sulfonic acid-glucoside isomers	6.1	0.316		
Triazolyl-sulfonic acid-ethanol-glucoside	5.1	0.263		
Triazolyl-ethanol-glucoside				
Unknowns [6]	21.4	1.102		
Non-extractable	NR	NR	NR	NR
Methanol/water reflux	3.7	0.189	12.4	0.016
Non-extractable	NR	NR	NR	NR
Methanol/water ASE	1.6	0.085	3.7	0.005
0.1% TFA ASE	1.0	0.054	2.3	0.003
1N NaOH ASE	1.7	0.089	5.4	0.007
Non-extractable	1.9	0.099	6.0	0.008

[1] Shading indicates that the extraction step and/or characterization analysis was not conducted for the matrix in question.
[2] NR = Not reported

[3] Consisting of 4 components, each present at ≤1.7% of the TRRs (≤0.002 ppm).
[4] Two isomers, each present at ≤8.9% of the TRRs (≤0.459 ppm).
[5] Two isomers, each present at ≤3.8% of the TRRs (≤0.196 ppm).
[6] Consisting of 9 components, each present at ≤4.6% of the TRRs (≤0.236 ppm); two components, accounting for ≤1.3% of the TRRs each, had HPLC retention times consistent with JAU6476-hydroxy-desthio isomers.

TABLE C.2.3. *Distribution of the Parent and the Metabolites in Sugar Beet Matrices Following Foliar Application of [Phenyl-UL-^{14}C]-prothioconazole at 1.028 lb a.i./A (1152 g a.i./ha).* [1]

Metabolite Fraction	Tops		Root	
	TRR = 4.333 ppm		TRR = 0.119 ppm	
	%TRR	ppm	%TRR	ppm
ACN/water	92.9	4.025	69.9	0.083
Initial SPE effluent/water eluate	NR; combined with other eluates [2]		26.8	0.032
JAU6476-desthio			17.8	0.021
Unknown [3]			9.0	0.011
ACN/water SPE eluate	NR; combined with other eluates		42.2	0.050
JAU6476-desthio			39.8	0.047
JAU6476-triazolinone			2.4	0.003
ACN eluate	NR; combined with other eluates		0.8	0.001
Combined SPE eluates	92.9	4.025		
Prothioconazole	6.3	0.274		
JAU6476-desthio	26.3	1.141		
JAU6476-triazolinone	1.5	0.066		
JAU6476-α-OH-desthio	1.6	0.069		
JAU6476-OH-sulfonic acid glucoside isomers	8.1	0.351		
JAU6476-OH-desthio glucoside isomer	5.1	0.222		
JAU6476-desthio-hydroxy-dieneyl-cysteine isomers	10.5	0.454		
JAU6476-hydroxy-di-sulfonic acid glucoside [4]	1.9	0.083		
Multicomponent unknown	4.9	0.214		
Unknowns [5]	26.5	1.150		
Non-extractable	NR	NR	NR	NR
Methanol reflux	2.6	0.110	7.8	0.009
Non-extractable	NR	NR	NR	NR
Methanol/water ASE	1.6	0.068	3.0	0.004
Combined methanol reflux + methanol ASE	4.1	0.178		
Prothioconazole	1.1	0.048		
JAU6476-desthio	2.5	0.107		
JAU6476-triazolinone	0.5	0.022		
Non-extractable	NR	NR	NR	NR
0.1% TFA ASE	0.5	0.023	2.6	0.003
Non-extractable	NR	NR	NR	NR
1N NaOH ASE	1.1	0.049	8.4	0.010
Non-extractable	1.3	0.056	8.4	0.010

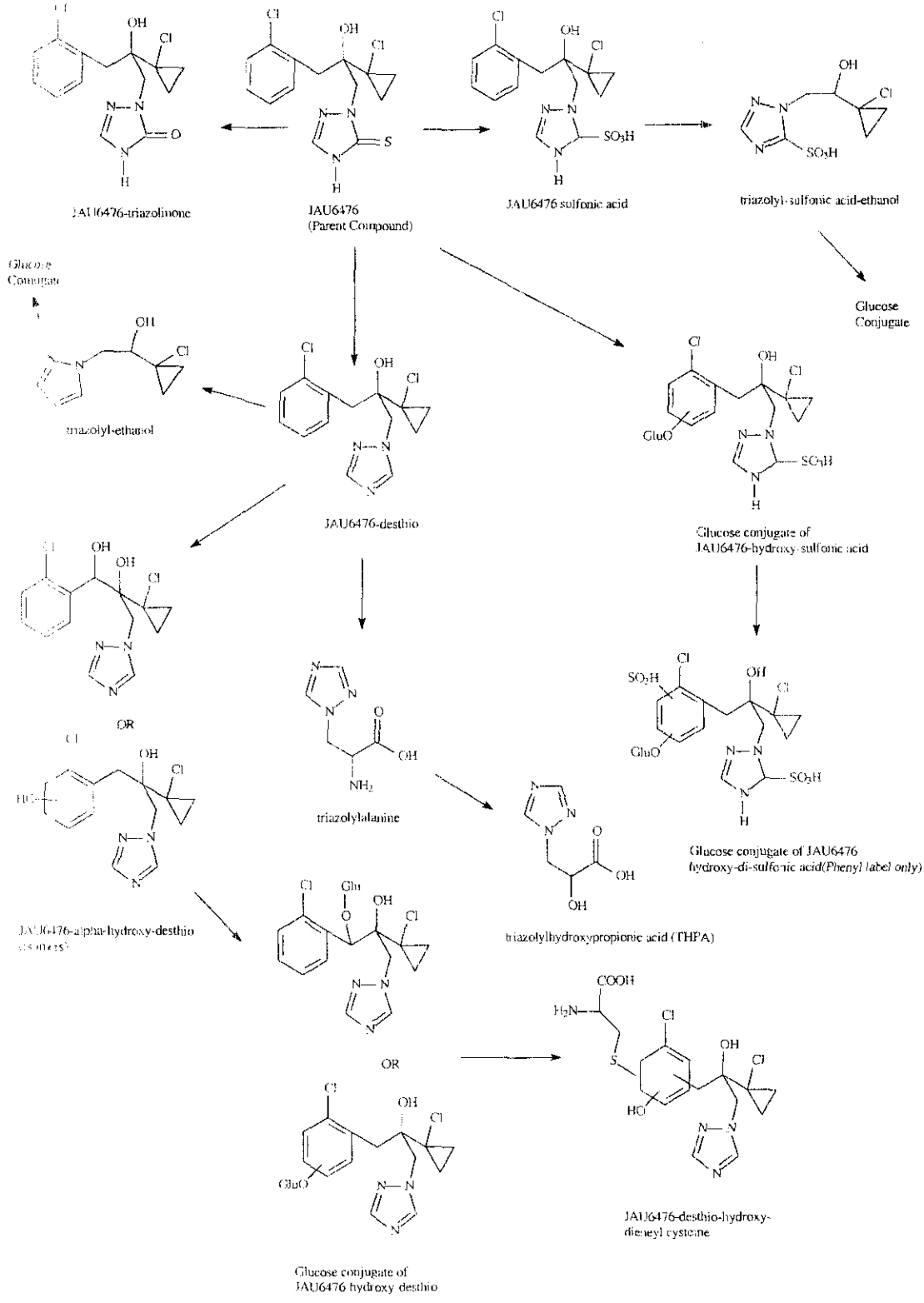
[1] Shading indicates that the extraction step and/or characterization analysis was not conducted for the matrix in question.
[2] NR = Not reported.
[3] The applicant noted that the polarity of this component was similar to that of JAU6476-desthio-hydroxy-dieneyl-cysteine in sugar beet tops.
[4] Tentative identification.
[5] Consisting of 13 components, each present at ≤4.3% of the TRRs (≤0.188 ppm).

TABLE C.2.4. Summary of Characterization and Identification of Radioactive Residues in Sugar Beet Matrices Following Foliar Application of [Triazole-UL-^{14}C]-prothioconazole at 1.032 lb a.i./A (1157 g a.i./ha) and [Phenyl-UL-^{14}C]-prothioconazole at 1.028 lb a.i./A (1152 g a.i./ha).

Compound	[Triazole-UL-^{14}C]-prothioconazole				[Phenyl-UL-^{14}C]-prothioconazole			
	Tops		Root		Tops		Root	
	TRR = 5.154 ppm		TRR = 0.130 ppm		TRR = 4.333 ppm		TRR = 0.119 ppm	
	%TRR	ppm	%TRR	ppm	%TRR	ppm	%TRR	ppm
Identified								
Prothioconazole	5.1	0.265	--	--	7.4	0.322	--	--
JAU6476-desthio	19.2	0.988	25.5	0.033	28.8	1.248	57.6	0.068
JAU6476-triazolinone	2.0	0.105	1.6	0.003	2.0	0.088	2.4	0.003
JAU6476-α-OH-desthio	--	--	--	--	1.6	0.069	--	--
JAU6476-OH-desthio	1.2	0.063	--	--				
JAU6476 sulfonic acid	4.0	0.205	--	--				
JAU6476-OH-sulfonic acid glucoside isomers	6.1	0.316	--	--	8.1	0.351	--	--
JAU6476-OH-desthio glucoside isomer	6.5	0.334	--	--	5.1	0.222	--	--
JAU6476-desthio-hydroxy-dieneyl-cysteine isomers	9.9	0.512	5.4	0.007	10.5	0.454	--	--
JAU6476-OH-di-sulfonic acid glucoside	--	--	--	--	1.9	0.083	--	--
Triazolylalanine	1.6	0.084	28.9	0.038	--	--	--	--
Triazolylhydroxypropionic acid (THPA)	4.0	0.207	--	--	--	--	--	--
Triazolyl-ethanol	3.8	0.194	--	--	--	--	--	--
Triazolyl-sulfonic acid-ethanol-glucoside	5.1	0.263	--	--	--	--	--	--
Triazolyl-ethanol-glucoside			--	--	--	--	--	--
Characterized								
SAX eluate/SCX effluent	--	--	3.8	0.005	--	--	--	--
Multicomponent unknown	--	--	--	--	4.9	0.214	--	--
Unknowns[1]	21.4	1.102	5.0	0.007	26.5	1.150	9.0	0.011
ACN SPE eluate	--	--	--	--	--	--	0.8	0.001
Methanol/water extractable (reflux + ASE)	5.3	0.274	16.1	0.021	--	--	10.8	0.013
0.1% TFA hydrolysate	1.0	0.054	2.3	0.003	0.5	0.023	2.6	0.003
1N NaOH hydrolysate	1.7	0.089	5.4	0.007	1.1	0.049	8.4	0.010
Total identified	68.5	3.536	61.4	0.080	65.4	2.837	60.0	0.071
Total characterized	29.4	1.519	32.6	0.043	33.0	1.436	31.6	0.038
Total extractable	98.0	5.056	94.0	0.122	98.4	4.273	91.7	0.109
Unextractable (PES)[2]	1.9	0.099	6.0	0.008	1.3	0.056	8.4	0.010
Accountability[3]	100.0		100.0		100.0		100.0	

[1] For triazole-label study, see TABLE C.2.2 for distribution. For phenyl-label study, unknowns consist of 13 components in tops and one component in root.

FIGURE C.3.1. Proposed Metabolic Profile of Prothioconazole in Sugar Beet



[2] Residues remaining after exhaustive extractions.
[3] Accountability = (Total extractable + Total unextractable)/(absolute values of the TRRs (ppm)) * 100.

C.3. Proposed Metabolic Profile

Based on the results of the triazole-label and phenyl-label prothioconazole sugar beet metabolism studies, it was concluded that prothioconazole was extensively metabolized in sugar beet via: (1) oxidation of the sulfur of the triazolinethione ring to the corresponding sulfonic acid and subsequent elimination of the sulfonic acid group to form JAU6476-desthio; (2) hydroxylation of the phenyl ring or the benzyl carbon to form multiple isomers, with subsequent conjugation with glucose or further reaction to produce JAU6476-desthio-hydroxy-dieneyl-cysteine; (3) release of the triazole moiety to form triazolylalanine and triazolylhydroxypropionic acid (THPA); and (4) elimination of the phenyl ring. Free triazole (1*H*-1,2,4-triazole) was not identified in any of the sugar beet matrices.

TABLE C.3.1. Identification of Compounds from the Metabolism Studies.

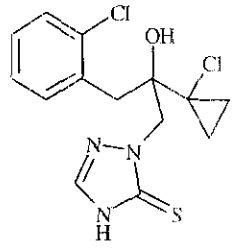
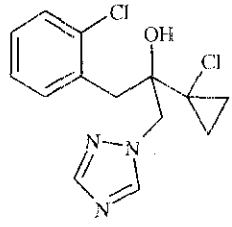
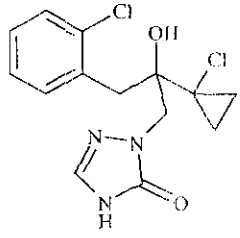
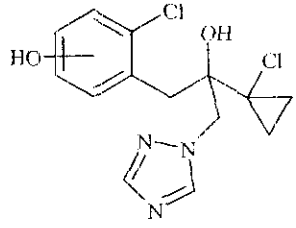
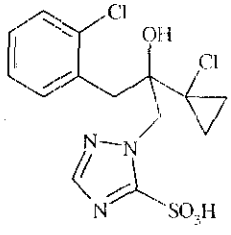
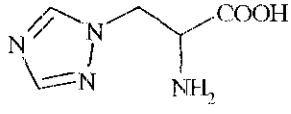
Common name/code FIGURE C.3.1 ID No.	Chemical name	Chemical structure
Prothioconazole JAU6476	2-[2-(1-chlorocyclopropyl)-3-(2-chlorophenyl)-2-hydroxypropyl]-1,2-dihydro-3*H*-1,2,4-triazole-3-*thione*	
JAU6476-desthio	α-(1-chlorocyclopropyl)-α-[(2-chlorophenyl)methyl]-1*H*-1,2,4-triazole-1-ethanol	
JAU6476-*triazolinone*	2-[2-(1-chlorocyclopropyl)-3-(2-chlorophenyl)-2-hydroxypropyl]-2,4-dihydro-3*H*-1,2,4-triazole-3-one	
JAU6476-OH desthio		
JAU6476 sulfonic acid	1-[2-(1-chlorocyclopropyl)-3-(2-chlorophenyl)-2-*hydroxypropyl*]-1*H*-1,2,4-triazole-5-sulfonic acid	
Triazolylalanine (TA)	α-amino-1*H*-1,2,4-triazole-1-propanoic acid	

TABLE C.3.1. Identification of Compounds from the Metabolism Studies.

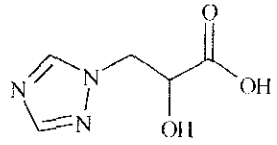
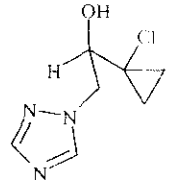
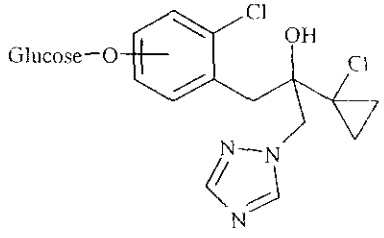
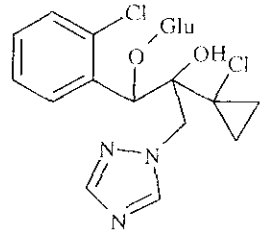
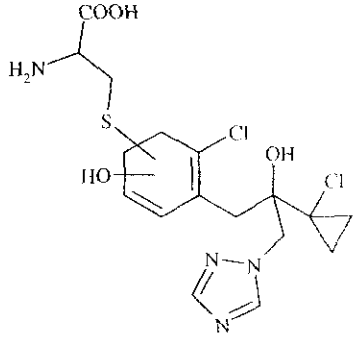
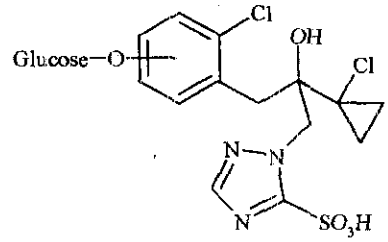
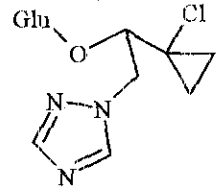
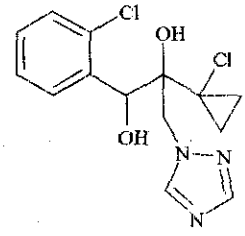
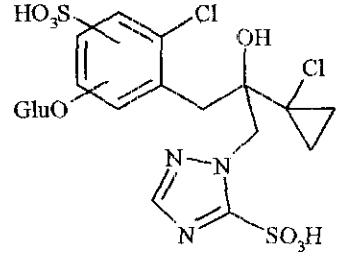
Common name/code FIGURE C.3.1 ID No.	Chemical name	Chemical structure
Triazolylhydroxypropionic acid (THPA)	α-hydroxy-1*H*-1,2,4-triazole-1-propanoic acid	
Triazolyl-ethanol	1-(1-chlorocyclopropyl)-2-(1*H*-1,2,4-triazol-1-yl)ethanol	
JAU6476-OH-desthio glucoside isomer		 OR 
JAU6476-desthio-hydroxy-dieneyl-cysteine isomers		

TABLE C.3.1. Identification of Compounds from the Metabolism Studies.

Common name/code FIGURE C.3.1 ID No.	Chemical name	Chemical structure
JAU6476-OH-sulfonic acid-glucoside isomers		
Triazolyl-ethanol-glucoside		
JAU6476-α-OH-desthio	2-(1-chlorocyclopropyl)-1-(2-chlorophenyl)-3-(1*H*-1,2,4-triazol-1-yl)-1,2-propanediol	
JAU6476-hydroxy-di-sulfonic acid glucoside		

D. CONCLUSION

For both radiolabels, total radioactive residues (TRRs) were 4.333-5.154 ppm in sugar beet tops and 0.119-0.130 ppm in sugar beet root harvested 7 days following the last application. The parent, prothioconazole, was identified in tops only and constituted 5.1-7.4% of the TRRs (0.27-0.30 ppm). Major metabolites accounting for ≥10% of the TRRs in tops were JAU6476-desthio and JAU6476-desthio-hydroxy-dieneyl-cysteine isomers while major metabolites identified in roots were JAU6476-desthio and triazolylalanine (triazole-label study only). JAU6476-triazolinone and JAU6476-desthio-hydroxy-dieneyl-cysteine isomers (triazole-label study only) were the only other metabolites identified in roots, each accounting for less than 5.4% of the TRRs (≤0.007 ppm). Several minor metabolites accounting for ≤10% of the TRRs were identified in tops including: JAU6476-triazolinone, JAU6476-α-OH-desthio (phenyl-label study

only), JAU6476-OH-sulfonic acid glucoside isomers, a JAU6476-OH-desthio glucoside isomer, JAU6476-hydroxy-di-sulfonic acid glucoside (phenyl-label study only), JAU6476-OH-desthio (triazole-label study only) and JAU6476 sulfonic acid (triazole-label study only). The following minor triazole-specific metabolites were also identified in the tops: triazolylalanine (TA), triazolylhydroxypropionic acid (THPA), triazolyl-ethanol, triazolyl-sulfonic acid-ethanol-glucoside and triazolyl-ethanol-glucoside. The presence of these triazole-specific metabolites was evidence of the release of the triazole moiety from the molecule. Remaining radioactivity in sugar beet matrices was characterized as multicomponent and minor unknowns.

Prothioconazole was extensively metabolized in sugar beet with the initial formation of JAU6476-desthio with further hydroxylation of the phenyl ring or the benzyl carbon to form multiple isomers. Subsequent conjugation with glucose or further reactions took place to produce JAU6476-desthio-hydroxy-dieneyl-cysteine. Release of the triazole moiety formed triazolylalanine and triazolylhydroxypropionic acid (THPA). Free triazole (1*H*-1,2,4-triazole) was not identified in any of the sugar beet matrices.

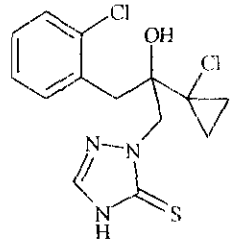
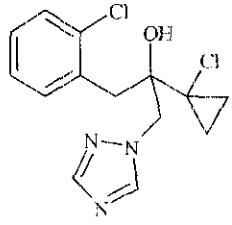
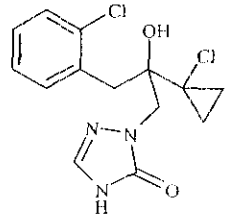
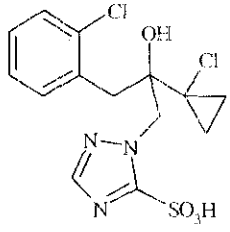
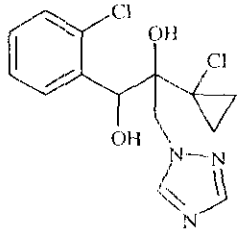
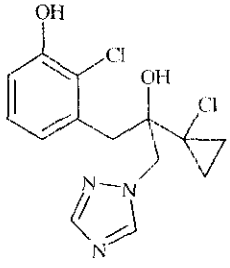
E. REFERENCES

None.

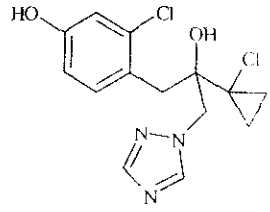
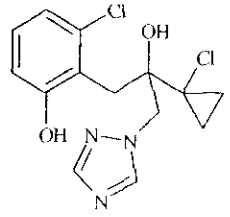
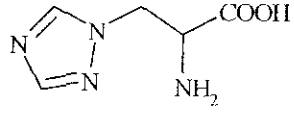
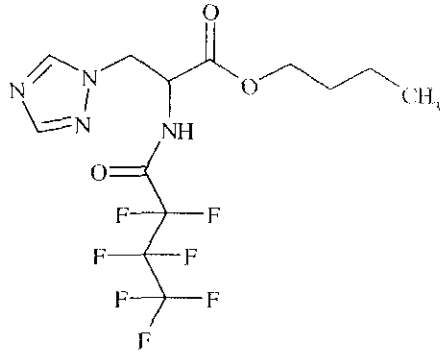
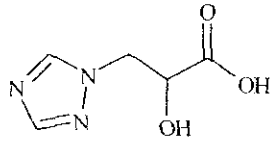
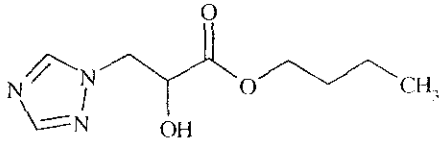
F. DOCUMENT TRACKING

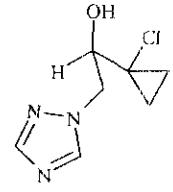
RDI: Louise Croteau, Suzan Mathew (PMRA): 23/01/2006; Henri Bietlot (PMRA) 24/01/2006; Stephen Funk (IO)13/03/2006; Leung Cheng (RAB3)
Petition Number: PP#4F6830
DP Barcode: D303508
PC Code: 113961

APPENDIX 1. Chemical Names and Structures of Reference Standards Used in the Sugar Beet Metabolism Studies.

Common name: Company code	Chemical name	Chemical structure
Prothioconazole: JAU6476	2-[2-(1-chlorocyclopropyl)-3-(2-chlorophenyl)-2-hydroxypropyl]-1,2-dihydro-3*H*-1,2,4-triazole-3-thione	
JAU6476-desthio	α-(1-chlorocyclopropyl)-α-[(2-chlorophenyl)methyl]-1*H*-1,2,4-triazole-1-ethanol	
JAU6746 triazolinone	2-[2-(1-chlorocyclopropyl)-3-(2-chlorophenyl)-2-hydroxypropyl]-2,4-dihydro-3*H*-1,2,4-triazole-3-one	
JAU6476 sulfonic acid	1-[2-(1-chlorocyclopropyl)-3-(2-chlorophenyl)-2-hydroxypropyl]-1*H*-1,2,4-triazole-5-sulfonic acid	
JAU6476-α-OH-desthio	2-(1-chlorocyclopropyl)-1-(2-chlorophenyl)-3-(1*H*-1,2,4-triazol-1-yl)-1,2-propanediol	
JAU6476-3-OH-desthio	α-(1-chlorocyclopropyl)-α-[(2-chloro-3-hydroxyphenyl)methyl]-1*H*-1,2,4-triazole-1-ethanol	

APPENDIX I. Chemical Names and Structures of Reference Standards Used in the Sugar Beet Metabolism Studies.

Common name; Company code	Chemical name	Chemical structure
JAU6476-4/5-hydroxy-desthio (structure and chemical name correspond to JAU6476-4-hydroxy-desthio)	α-(1-chlorocyclopropyl)-α-[(2-chloro-4-hydroxyphenyl)methyl]-1*H*-1,2,4-triazole-1-ethanol	
JAU6476-6-hydroxy-desthio	α-(1-chlorocyclopropyl)-α-[(2-chloro-6-hydroxyphenyl)methyl]-1*H*-1,2,4-triazole-1-ethanol	
Triazolylalanine (TA)	α-amino-1*H*-1,2,4-triazole-1-propanoic acid	
Triazolylalanine derivative	butyl α-[(2,2,3,4,4,4,4-heptafluoro-1-oxobutyl)amino]-1*H*-1,2,4-triazole-1-propanoate	
Triazolylhydroxypropionic acid (THPA)	α-hydroxy-1*H*-1,2,4-triazole-1-propanoic acid	
Triazolylhydroxypropionic acid derivative (THPA n-butyl ester)	butyl α-hydroxy-1*H*-1,2,4-triazole-1-propanoate	

APPENDIX I. Chemical Names and Structures of Reference Standards Used in the Sugar Beet Metabolism Studies.		
Common name; Company code	Chemical name	Chemical structure
Triazolyl-ethanol	1-(1-chlorocyclopropyl)-2-(1*H*-1,2,4-triazol-1-yl)ethanol	

Primary Evaluators	Stephen Funk, Ph.D., Senior Chemist Immediate Office	Date: Mar. 13 2006
	Louise G. Croteau, Senior Evaluation Officer FREAS, HED	Date: 23/01/06
	Suzan Mathew, Evaluation Officer FREAS, HED	Date: January 23/06
Approved by	Leung Cheng, Ph. D. Team Leader HED/RAB3	Date:
	Henri P. Bietlot, Acting Section Head FREAS, HED	Date: Jan 24/06

In the absence of signatures, this document is considered to be a draft with deliberative material for internal use only.

This DER was originally prepared under contract by Dynamac Corporation (20440 Century Boulevard, Suite 100; Germantown, MD 20874; submitted 05/03/2005). The DER has been reviewed by the Health Effects Division (HED) and Health Canada's Pest Management Regulatory Agency (PMRA) and revised to reflect current Office of Pesticide Programs (OPP) policies, and PMRA Directive 98-02.

STUDY REPORT:

46246142 Haas, M. (2001) Metabolism of JAU 6476 in Spring Wheat after Seed Dressing. Project Number: M/1730885/2, 110881, MR/467/99. Unpublished study prepared by Bayer Ag Institut fuer Ruckstands-Analytik. 84 p.

EXECUTIVE SUMMARY:

Bayer CropScience has submitted a study investigating the metabolism of [phenyl-UL-^{14}C]-prothioconazole (specific activity 2.97 MBq/mg) in wheat as a seed treatment. The radiolabeled test substance was applied at 7.3 µg a.i./seed (equivalent to 18.4 g a.i./kg seed; low-rate) and 37 µg a.i./seed (equivalent to 93.3 g a.i./kg seed; high-rate). Wheat plants were grown from the treated seed in the greenhouse. Forage was harvested at BBCH 41, hay was harvested BBCH 83,

and grain and straw were harvested at maturity (57, 110, and 153 days, respectively, after planting).

Total radioactive residues (TRRs) in forage, hay, and straw were determined by combustion followed by liquid scintillation counting (LSC). In wheat matrices harvested following the low-rate seed treatment, TRRs were 0.020 ppm in forage and hay, 0.030 ppm in straw, and 0.008 ppm in grain. In wheat matrices harvested following high-rate seed treatment, TRRs were 0.07 ppm in forage, 0.09 ppm in hay, 0.28 ppm in straw, and 0.01 ppm in grain. Only forage, hay, and straw were subjected to further analysis.

Solvent extraction with acetonitrile/water released the majority of the TRRs (~71.2-85.2%) in wheat matrices from both treatment rates. We note that cysteine HCl was added to extracting solvents and to extracts during concentration procedures to prevent oxidative decomposition of prothioconazole. Hydrolysis with dioxane/HCl solubilized an additional 7.8% of the TRRs in straw (high-rate treatment only). Non-extractable residues remaining following extraction/hydrolysis accounted for 17.1-28.8% of the TRRs (0.003-0.006 ppm) in wheat matrices from the low-rate treatment, and for 7.7-25.7% of the TRRs (0.01-0.02 ppm) in wheat matrices from the high-rate treatment. Because TRRs were determined by summing extractable and non-extractable radioactivity, accountabilities ranged from 100-119%. Residues were identified primarily by TLC co-chromatography with some confirmatory analysis by HPLC. These methods successfully identified the predominant residues in wheat forage, hay, straw, and grain following seed treatment. Only extracts and hydrolysates from the high-rate treatment were subjected to analysis for characterization/identification of residues. Extraction and analysis of all samples were conducted within 30 days of harvest.

Approximately 18-33% of the TRRs (0.018-0.092 ppm) were identified in wheat forage, hay, and straw. Prothioconazole was identified at <1% of the TRRs in all matrices (≤0.002 ppm). Metabolite JAU6476-desthio was the major identified residue, accounting for 10.9% of the TRRs (0.01 ppm) in forage and 6.4-6.6% of the TRRs (0.005-0.019 ppm) in hay and straw. Metabolites JAU6476-3-OH-desthio and JAU6476-4-OH-desthio together accounted for 3.8-12.0% of the TRRs (≤0.017 ppm). In addition, JAU6476-OH-glucosides were tentatively identified at 10.6% of the TRRs (0.030 ppm) in straw and were tentatively identified but not quantitated in wheat forage and hay. Remaining identified metabolites, including JAU6476-α-OH-desthio, JAU6476-6-OH-desthio, JAU6476-triazolinone, JAU6476 sulfonic acid, JAU6476-α-acetoxy-desthio, benzylpropyldiol glucoside, and JAU6476-disulfide were present at ≤3.3% of the TRRs (≤0.008 ppm) each.

Based on the results of the phenyl-label seed treatment wheat metabolism study, prothioconazole was extensively metabolized in wheat via: (1) oxidation and loss of sulfur to form JAU6476-desthio; and (2) hydroxylation of the chlorobenzyl methylene C-atom to form JAU6476-α-hydroxy-desthio and hydroxylation of the chlorobenzyl ring at positions 3, 4, and 6 of JAU6476-desthio to form the hydroxy desthio metabolites. Exchange of oxygen for sulfur, the elimination of the triazole moiety and conjugation of the benzylpropyldiol portion of the remaining molecule,

and the formation of glucosides of the monohydroxylated JAU6476-desthio isomers were proposed as minor metabolic reactions.

STUDY/WAIVER ACCEPTABILITY/DEFICIENCIES/CLARIFICATIONS:

Under the conditions and parameters used in the study, the wheat metabolism data are classified as scientifically acceptable.

The acceptability of this study for regulatory purposes is addressed in the forthcoming U.S. EPA Residue Chemistry Summary Document, DP Barcode D303508, PP#4F6830, and in Canada's Regulatory Decision Document.

COMPLIANCE:

Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided. No deviations from regulatory requirements were reported which would have an impact on the validity of the study.

A. BACKGROUND INFORMATION

Prothioconazole is a broad-spectrum systemic fungicide intended for the control of many diseases in different crops such as cereals, oilseed rape or peanuts. The biochemical mode of action is the inhibition of the demethylation of lanosterol or 24-methylene-dihydrolanosterol, which are precursors of sterols in fungi. The chemical has been submitted for joint EPA and PMRA review by Bayer CropScience. The crops proposed for joint review include barley, the dried shell and bean subgroup, the oilseed crop group, and wheat. Uses on peanuts and rice are being proposed for the U.S. only.

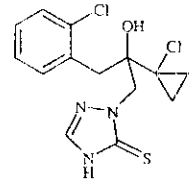
TABLE A.1. Test Compound Nomenclature.	
Chemical structure	
Common name	Prothioconazole (ISO accepted)
Company experimental name	JAU6476
IUPAC name	2-[(2*RS*)-2-(1-chlorocyclopropyl)-3-(2-chlorophenyl)-2-hydroxypropyl]-2*H*-1,2,4-triazole-3(4*H*)-thione
CAS name	2-[2-(1-chlorocyclopropyl)-3-(2-chlorophenyl)-2-hydroxypropyl]-1,2-dihydro-3*H*-1,2,4-triazole-3-thione
CAS registry number	178928-70-6
PC Code	113961
End-use product (EP)	Proline® 480 SC (4 lb/gal suspension concentrate), EPA File Symbol 264-IEL

TABLE A.2. Physicochemical Properties of the Technical Grade Test Compound.

Parameter	Value	References
Melting range	139.1 to 144.5°C	MRID 46246003/CES
pH	5.8 (1% solution)	MRID 46246003/CES
Density at 20°C	1.36 g/mL	MRID 46246003/CES
Water solubility at 20°C	pH: mg/L 4: 5 8: 300 9: 2000	MRID 46246003/CES
Solvent solubility at 20°C	Solvent: g/L Acetone: >250 Acetonitrile: 69 Dichloromethane: 88 Dimethysulfoxide: 126 Ethyl acetate: >250 n-Heptane: <0.1 1-Octanol: 58 Polyethylene glycol: >250 2-Propanol: 87 Xylene: 8	MRID 46246003/CES
Vapor pressure at 20 or 25°C	$<4 \times 10^{-7}$ Pa (calculated from determinations at 70°C)	MRID 46246003/CES
Dissociation constant, pK_a	6.9 (calculated from K_{OW})	MRID 46246003/CES
Octanol/water partition coefficient, at 20°C	pH: $Log(K_{OW})$ unbuffered water: 4.05 4: 4.16 7: 3.82 9: 2.00	MRID 46246003/CES
UV/visible absorption spectrum	Peak maxima at 257 nm. No absorption at > 300 nm.	MRID 46246003/CES

CES - Chemistry Evaluation Section of PMRA

B. EXPERIMENTAL DESIGN

B.1. Test Site and Crop Information

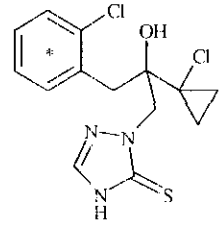
TABLE B.1.1. Test Site Information.

Testing Environment	Soil characteristics			
	Type	%OM	pH	CEC
Plants were grown in 0.5-m^2 planting containers in a climate-controlled chamber in the greenhouse (Leverkusen, Germany).	Sandy loam	1.98	6.5	10.0 meq/100g

The growth chamber was operated on a 14-hour day/10-hour night schedule at 65% relative humidity. Daylight temperatures were 19-20°C, and nighttime temperatures were 13-14°C. Wheat plants were watered as necessary and were fertilized 9 days after emergence. The applicant reported that plants remained healthy over the growth period.

TABLE B.1.2. Crop Information.					
Crop; crop group	Variety	Growth stage at application	Growth stage at harvest	Harvested RAC	Harvesting procedure
Wheat; Grain, cereal, group 15 and Grain, cereal, forage, fodder and straw, group 16	Spring wheat, var. Kadett	Seed	BBCH 41	Forage	Plants were removed by cutting at the soil surface; hay was dried for 4 days in a fumehood.
			BBCH 83 (late milk, early dough)	Hay	
			Mature	Grain and straw	Wheat ears were removed by cutting from the stalks; straw was then cut at the soil surface.

B.2. Test Materials

TABLE B.2.1. Test Material Characteristics.	
Chemical structure	
Radiolabel position	[phenyl-UL-^{14}C]-Prothioconazole
Lot No.	11403/1
Purity	>99% radiochemical purity; >99% chemical purity
Specific activity (Bq) [1]	2.97 MBq/mg (80.3 µCi/mg)

[1] Bq = disintegrations per second

B.3. Study Use Pattern

TABLE B.3.1. Use Pattern Information.	
Chemical name	[phenyl-UL-^{14}C]-Prothioconazole
Application method	The radiolabeled test substance was dissolved in acetonitrile (ACN) and combined with seed in a beaker. The beaker was gently shaken until the ACN dissolved. The seeds were planted on the day of treatment.
Application rate	Low-rate: 7.3 µg/seed, equivalent to 18.4 g a.i./100 kg seed High-rate: 37 µg/seed, equivalent to 93 g a.i./100 kg seed
Number of applications	1
Timing of applications	Seed treatment
PHI[1]	57 days (forage), 110 days (hay), and 153 days (straw and grain) after seed treatment/planting

[1] PHI = pre-harvest interval.

B.4. Identification/Characterization of Residues

B.4.1. Sample Handling and Preparation

Wheat grain was separated from the chaff by hand, then frozen in liquid nitrogen and homogenized. Samples of forage, hay, and straw (including chaff) were chopped into 1-cm pieces, then frozen in liquid nitrogen and homogenized. Samples were stored at 4°C during processing, after which they were stored at ~-20°C.

Because the total radioactive residues (TRRs) in grain from both treatment rates were <0.01 ppm, no further attempts were made to extract or characterize residues in grain.

General extraction procedures: Cysteine HCl was added to all extracting solvents and to extracts during concentration procedures to prevent oxidative decomposition of prothioconazole. Extracts were centrifuged to remove precipitated material including cysteine HCl. Following partitioning with dichloromethane (DCM), a small volume of acetonitrile (ACN) was added to each DCM phase prior to concentration.

Forage and hay: Subsamples were extracted 3x with ACN:water (80:20, v:v), then vacuum filtered. The filtrates were combined and concentrated, then partitioned with n-hexane. The resulting aqueous phase (Aqueous 1) from the high-rate treatment for each matrix was partitioned with DCM (3x), and the DCM phases were combined and concentrated. The aqueous phases following DCM partitioning (Aqueous 2) were subjected to acid hydrolysis with 6N HCl at 100°C for 16 hours; the hydrolysate was neutralized with 6N NaOH. The resulting hydrolysates were partitioned with DCM, and the DCM phases were concentrated and reserved for TLC analysis.

Straw: Subsamples were soaked in water overnight (at 4°C) then extracted three times with ACN:water and vacuum filtered. The filtrates were combined and concentrated, then partitioned three times with DCM (both treatment rates), and the DCM phases were combined and

concentrated. The aqueous phase from the high-rate treatment following DCM partitioning was subjected to acid hydrolysis as described above for forage and hay.

The remaining solids of straw from the high-rate treatment were subjected refluxed in dioxane:2N HCl (9:1, v:v). The hydrolysate was partitioned with DCM and the DCM phase was reserved for TLC analysis.

The extraction procedures for wheat forage, hay, and straw are summarized in the flow charts (FIGURES B.4.1 to B.4.1.3), which were copied without alteration from MRID 46246142.

FIGURE B.4.1 Extraction Procedure for Forage.

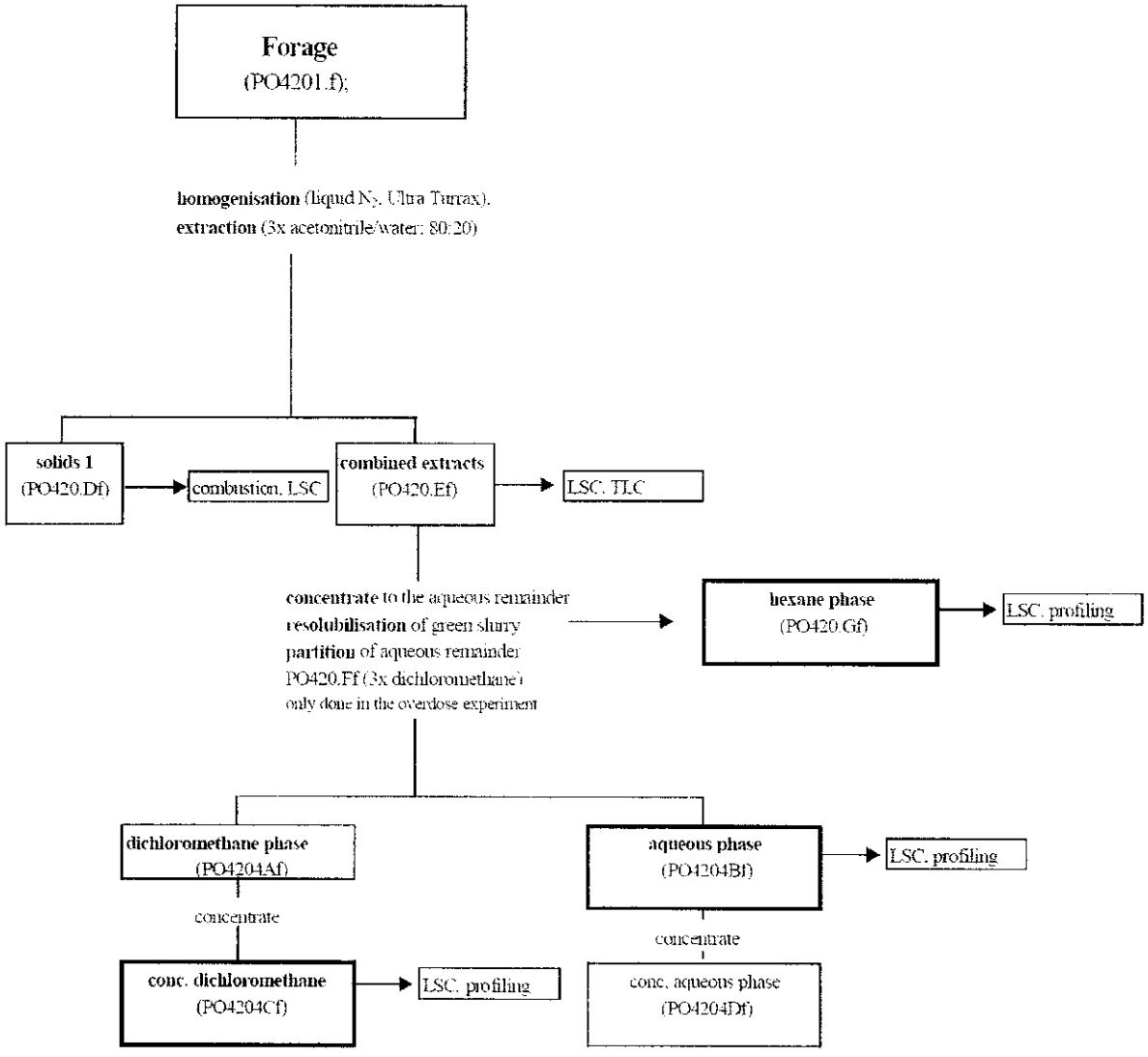


FIGURE B.4.1.2 Extraction Procedure for Hay.

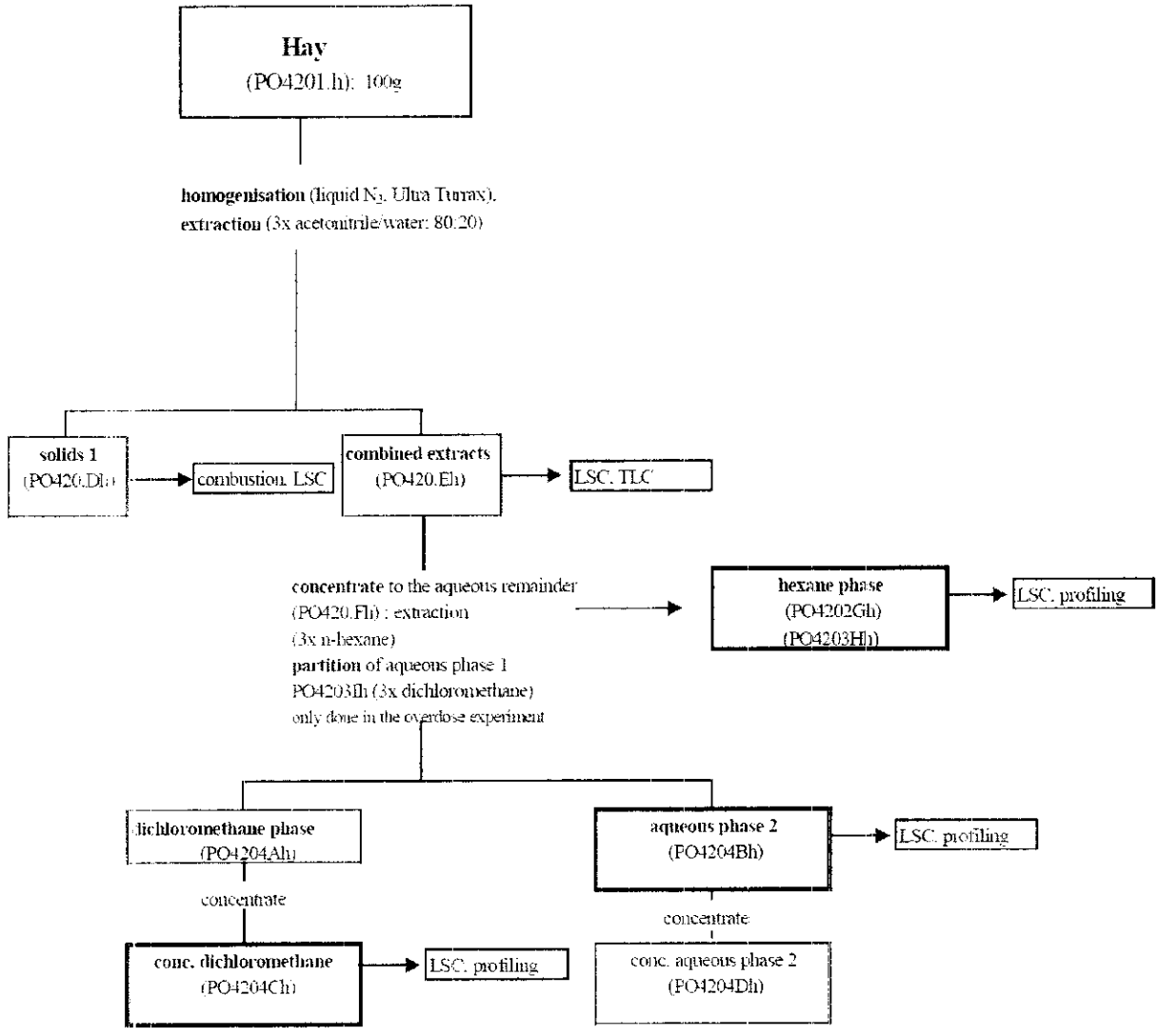
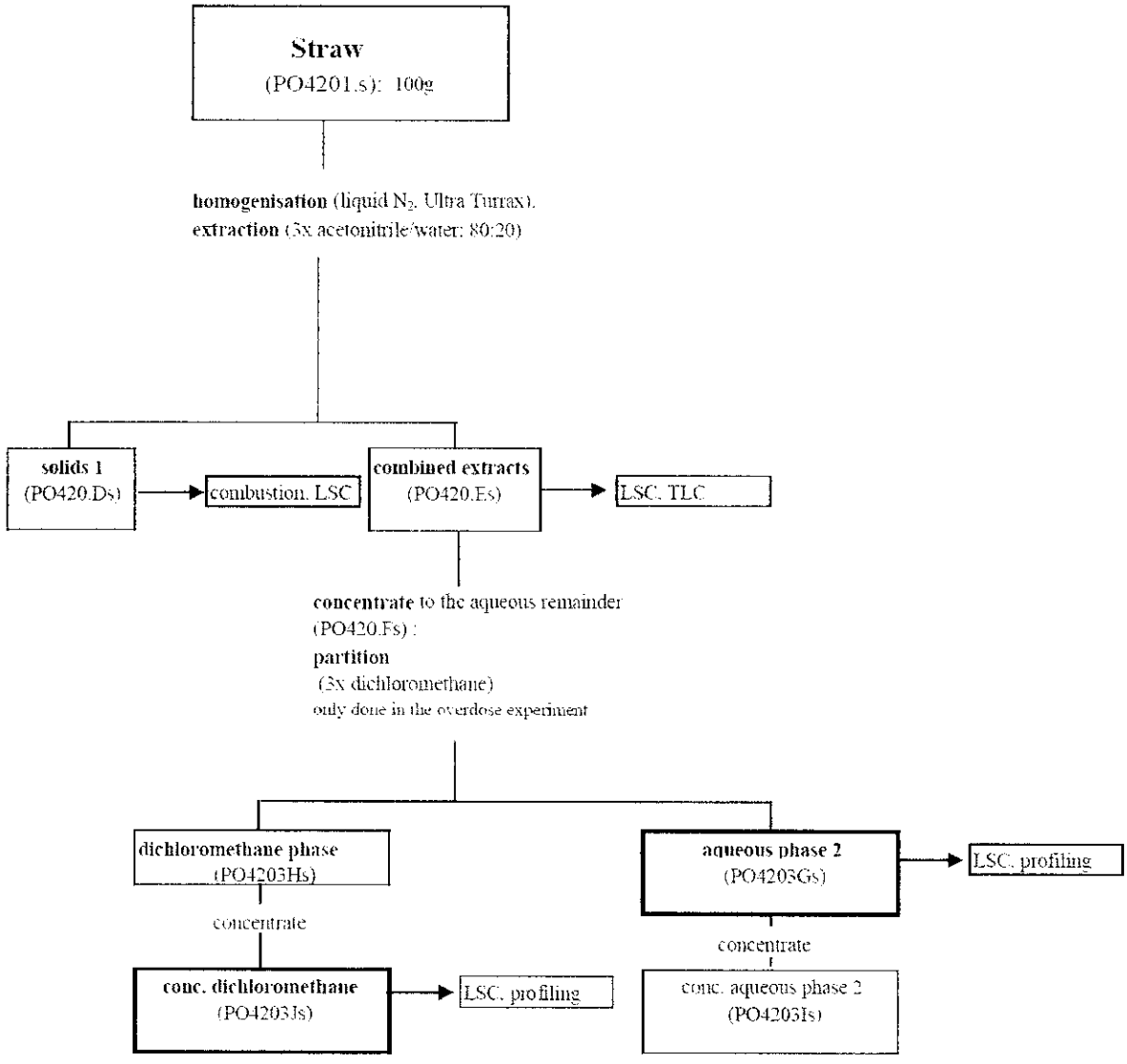


FIGURE B.4.1.3 Extraction Procedure for Straw.



B.4.2. Analytical Methodology

Total radioactive residues (TRRs) in wheat forage, hay, and straw were determined by summing radioactivity in extractable and non-extractable residues following extraction with ACN/water. TRRs in grain were determined by combustion and liquid scintillation counting (LSC). Extracts and hydrolysates were radioassayed by LSC, and non-extractable residues were radioassayed by combustion/LSC. The limit of detection was reported as twice the background.

Extracts and hydrolysates of wheat matrices from the high-rate treatment were subjected to TLC analyses using silica gel Si60 F_{254} plates and RP-18 plates and three solvent systems: DCM:methanol:25% ammonia (90:10:1, v:v:v; silica gel plates); n-butanol:water:acetic acid (4:1:1, v:v:v; silica gel plates); and ACN:water:methanol:acetic acid (70:25:5:1, v:v:v; RP-18 plates). The applicant noted that prothioconazole reference standard and cysteine HCl were spiked to each TLC origin before applying sample solutions to minimize decomposition of prothioconazole. Radioactive areas were detected and quantitatively evaluated using bioimaging, and non-radioactive reference standards were visualized under UV light. Metabolites were identified by cochromatography or comparison of R_f values with those of reference standards. The chemical names and structures for the reference standards are presented in Appendix I. The applicant indicated that isolated components from the wheat metabolism study reflecting foliar application of [phenyl-UL-^{14}C]-prothioconazole were also used for co-chromatography.

Confirmatory analysis was conducted using reverse phase HPLC on a system equipped with a C18 column and a UV detector, and using a gradient mobile phase of water containing 0.1% acetic acid and ACN. The applicant stated that the extracts did not contain sufficient radioactivity for analysis via a flow-through radio-detector; therefore, a fraction collector was used, and individual components were applied to deep well scintillator-coated microplates and analyzed with a microplate scintillation and luminescence counter for potential comparison of HPLC retention times with those of the components from the foliar application study. The applicant noted that these data were not obtained under GLP.

C. RESULTS AND DISCUSSION

The in-life and analytical phases of the study were conducted by Bayer CropScience (Leverkusen, Germany). The storage conditions for wheat samples are presented in TABLE C.1. Extraction and analysis of all samples were conducted within 30 days of harvest.

Total radioactive residues (TRRs) in wheat matrices are reported in TABLE C.2.1. TRR in forage, hay, and straw were determined by summing radioactivity in extractable and non-extractable residues; TRR in grain were determined by combustion/LSC. In wheat matrices harvested following seed treatment at 7.3 µg a.i./seed (equivalent to 18.4 g a.i./kg seed), TRRs were 0.020 ppm in forage and hay, 0.030 ppm in straw, and 0.008 ppm in grain. In wheat matrices harvested following seed treatment at an exaggerated rate of 37 µg a.i./seed (equivalent to 93.3 g a.i./kg seed), TRRs were 0.07 ppm (forage), 0.09 ppm (hay), 0.28 ppm (straw), and 0.01 ppm (grain). Only forage, hay, and straw were subjected to further analysis.

The distribution of the radioactivity in wheat matrices is presented in TABLES C.2.2.1 (low-rate treatment) and C.2.2.2 (high-rate treatment). Solvent extraction with ACN/water released the majority of the TRRs (~71.2-85.2%) in wheat matrices from both treatment rates. Hydrolysis with dioxane/HCl solubilized an additional 7.8% of the TRRs in straw (high-rate treatment only). Non-extractable residues remaining following extraction/hydrolysis accounted for 17.1-28.8% of the TRRs (0.003-0.006 ppm) in wheat matrices from the low-rate treatment, and for 7.7-25.7% of the TRRs (0.01-0.02 ppm) in wheat matrices from the high-rate treatment. Because TRRs were determined by summing extractable and non-extractable radioactivity (ppm), accountabilities ranged from 100-119%. Residues were identified primarily by TLC co-chromatography with some confirmatory analysis by HPLC. These methods successfully identified the predominant residues in wheat forage, hay, straw, and grain following seed treatment. Only extracts and hydrolysates from the high-rate treatment were subjected to analysis for characterization/identification of residues.

The characterization and identification of residues in wheat matrices from the high-rate treatment are summarized in TABLE C.2.3. Approximately 18-33% of the TRRs (0.018-0.092 ppm) were identified in wheat forage, hay, and straw. Prothioconazole was identified at <1% of the TRRs in all matrices (≤0.002 ppm). Metabolite JAU6476-desthio was the major identified residue, accounting for 10.9% of the TRRs (0.01 ppm) in forage and 6.4-6.6% of the TRRs (0.005-0.019 ppm) in hay and straw. Metabolites JAU6476-3-OH-desthio and JAU6476-4-OH-desthio together accounted for 3.8-12.0% of the TRRs (≤0.017 ppm). In addition, JAU6476-OH-glucosides were tentatively identified at 10.6% of the TRRs (0.030 ppm) in straw and were tentatively identified but not quantitated in wheat forage and hay. Remaining identified metabolites, including JAU6476-α-OH-desthio, JAU6476-6-OH-desthio, JAU6476-triazolinone, JAU6476 sulfonic acid, JAU6476-α-acetoxy-desthio, benzylpropyldiol glucoside, and JAU6476-disulfide were present at ≤3.3% of the TRRs (≤0.008 ppm) each.

C.1. Storage Stability

Processed wheat samples were stored frozen at approximately -20°C. Extraction and analysis of all samples was conducted within 30 days of harvest.

TABLE C.1. Summary of Storage Conditions.

Matrix	Storage Temp.	Actual Storage Duration	Interval of Demonstrated Storage Stability
Forage	~-20°C	≤30 days.	Not required.
Hay			
Straw			
Grain			

C.2. Identification, Characterization, and Distribution of Residues

TABLE C.2.1. Total Radioactive Residues (TRRs) in Wheat Matrices.

Matrix	Timing and Applic. No.	PHI (days)	[^{14}C]-Prothioconazole, ppm	
			Low-rate	High-rate
Forage	Seed treatment	57	0.020	0.07
Hay		110	0.020	0.09
Straw		153	0.030	0.28
Grain		153	0.008	0.012

TABLE C.2.2.1. Distribution of the Parent and the Metabolites in Wheat Matrices Following Seed Treatment with [Phenyl-UL-^{14}C]-Prothioconazole at 7.3 µg/seed.

Metabolite Fraction	Forage		Hay		Straw	
	TRR = 0.020 ppm		TRR = 0.020		TRR = 0.030 ppm	
	%TRR	ppm	%TRR	ppm	%TRR	ppm
ACN/water	71.2	0.014	71.2	0.014	82.9	0.027
Hexane (forage/hay) or DCM (straw)	5.1	0.001	5.1	0.001	33.3	0.011
Aqueous	64.4	0.013	66.1	0.013	49.5	0.016
Non-extractable	28.8	0.006	28.8	0.006	17.1	0.003

TABLE C.2.2.2. Distribution of the Parent and the Metabolites in Wheat Matrices Following Seed Treatment with [Phenyl-UL-^{14}C]-Prothioconazole at 37 µg/seed. [1]

Metabolite Fraction	Forage		Hay		Straw	
	TRR = 0.07 ppm		TRR = 0.09		TRR = 0.28 ppm	
	%TRR	ppm	%TRR	ppm	%TRR	ppm
ACN/water	85.2	0.06	74.3	0.07	84.4	0.24
Hexane	18.0	0.01	13.4	0.01		
JAU6476-desthio	6.8	0.004	6.0	0.004		
JAU6476-α-OH-desthio	--	--	0.9	0.001		
JAU6476-3- or 4-OH-desthio	3.9	0.002	2.1	0.002		
JAU6476-6-OH-desthio	0.5	<0.001	1.8	0.001		
JAU6476-triazolinone	0.7	<0.001	--	--		
JAU6476-disulfide	2.1	0.001	1.5	0.001		
TLC origin	2.0	0.001	1.2	0.001		
Unassigned	2.1 [2]	0.002	--	--		
Aqueous I	67.2	0.05	61.0	0.06		
DCM	21.3	0.02	7.1	0.01	23.2	0.06
Prothioconazole	0.4	<0.001	0.8	0.001	0.6	0.001
JAU6476-desthio	4.1	0.004	0.4	0.001	5.4	0.014
JAU6476-α-OH-desthio	1.5	0.001	1.6	0.002	3.3	0.007
JAU6476-3-OH-desthio	8.1	0.008	1.7	0.002	3.3	0.009

TABLE C.2.2.2. Distribution of the Parent and the Metabolites in Wheat Matrices Following Seed Treatment with [Phenyl-UL-^{14}C]-Prothioconazole at 37 µg/seed. [1]

Metabolite Fraction	Forage		Hay		Straw	
	TRR = 0.07 ppm		TRR = 0.09		TRR = 0.28 ppm	
	%TRR	ppm	%TRR	ppm	%TRR	ppm
JAU6476-4-OH-desthio					2.1	0.005
JAU6476-6-OH-desthio	1.0	0.001	--	--	2.9	0.007
JAU6476-triazolinone	0.6	0.001	--	--	--	--
JAU6476 sulfonic acid	0.6	0.001	0.2	<0.001	0.4	0.001
JAU6476-α-acetoxy-desthio	--	--	0.2	<0.001	0.8	0.002
Benzylpropyldiol glucoside	--	--	0.8	0.001	1.4	0.004
Polar metabolites	1.9	0.002	--	--	--	--
TLC origin	3.1	0.003	0.2	<0.001	1.8	0.005
Unassigned [3]	--	--	1.4	0.003	1.2	0.003
Aqueous 2 [4]	47.5 [5]	0.04	54.3 [5]	0.05	60.9	0.17
JAU6476-OH-desthio glucosides					10.6	0.030
Unknown ST11					5.7	0.016
Unknown ST12					5.1	0.014
Unknown STSD1					11.8	0.033
Unknown STSD2					8.9	0.025
Unknown STSD3					10.5	0.029
TLC origin					8.4	0.023
Non-extractable	14.8	0.01	25.7	0.02	15.6	0.04
Dioxane/HCl					7.8	0.02
DCM					2.4	0.04
JAU6476-desthio					1.2	0.005
JAU6476-3-OH-desthio					0.5	0.002
JAU6476-4-OH-desthio					0.3	0.001
Unknown STSD6					0.3	0.001
TLC origin					0.1	<0.001
Aqueous					1.0	<0.01
Precipitated fines					4.5	0.01
Non-extractable					7.7	0.02

[1] Shading indicates that the extraction step and/or characterization analysis was not conducted for the matrix in question.
[2] Consisting of 2 components in forage, each ≤1.2% of the TRRs.
[3] Consisting of 3 components in hay, each ≤0.5% of the TRRs; and 1 component in straw.
[4] This fraction was additionally subjected to acid hydrolysis with 6N HCl followed by partitioning with DCM; quantitative results were not provided.
[5] Consisting of at least 5 metabolites; acid hydrolysis of this fraction yielded monohydroxylated desthio metabolites, indicating the presence of JAU6476-OH-glucosides in the unhydrolyzed fraction.

TABLE C.2.3. Summary of Characterization and Identification of Radioactive Residues in Wheat Matrices Following Seed Treatment with [Phenyl-UL-^{14}C]-Prothioconazole at 37 µg/seed.

Compound	Forage		Hay		Straw	
	TRR = 0.07 ppm		TRR = 0.09 ppm		TRR = 0.28 ppm	
	% of the TRRs	ppm	% of the TRRs	ppm	% of the TRRs	ppm
Identified						
Prothioconazole	0.4	<0.001*	0.8	0.001	0.6	0.002
JAU6476-desthio	10.9	0.008	6.4	0.005	6.6	0.019
JAU6476-α-OH-desthio	1.5	0.001	2.5	0.003	3.3	0.007
JAU6476-3-OH-desthio	12.0	0.010	3.8	0.004	3.8	0.011
JAU6476-4-OH-desthio					2.4	0.006
JAU6476-6-OH-desthio	1.5	0.002	1.8	0.001	2.9	0.008
JAU6476-triazolinone	1.3	0.002	–	–	–	–
JAU6476 sulfonic acid	0.6	0.001	0.2	<0.001	0.4	0.001
JAU6476-α-acetoxy-desthio	--	--	0.2	<0.001	0.8	0.002
Benzylpropyldiol glucoside	--	--	0.8	0.001	1.4	0.004
Tentatively identified						
JAU6476-disulfide	2.1	0.001	1.5	0.001	--	--
JAU6476-OH-desthio glucosides [1]	--	--	--	--	10.6	0.030
Characterized						
Polar metabolites (2)	1.9	0.002	--	--	--	--
Straw unknowns (6) [2]	--	--	--	--	42.3	0.118
Aqueous phase (>5 metabolites)	47.5	0.04	54.3	0.05	1.0	0.01
Precipitated fines after dioxane/HCl	--	--	--	--	4.5	0.01
TLC origins	5.1	0.004	1.4	0.002	10.2	0.03
Unassigned	2.1	0.002	1.4	0.001	1.2	<0.001
Total identified	30.3	0.024	18.0	0.018	32.8	0.090
Total characterized	56.6	0.048	57.1	0.053	59.2	0.169
Total extractable	86.9	0.072	75.1	0.071	92.2	0.26
Unextractable (PES)[3]	14.8	0.01	25.7	0.02	7.7	0.02
Accountability[4]	119		101		100	

[1] JAU6476-OH-desthio-glucosides were tentatively identified but not quantitated in wheat forage and hay.

[2] See TABLE C.2.2 for distribution.

[3] Residues remaining after exhaustive extractions.

[4] Accountability = (Total extractable + Total unextractable)/(absolute values of the TRRs (ppm)) * 100.

* Values <0.001 ppm were included in the calculations for total identified and/or total characterized as 0.001 ppm. This resulted in high accountability values (>100%).

C.3. Proposed Metabolic Profile

The following metabolic reactions were involved: (1) oxidation and loss of sulphur, resulting in JAU6474-desthio; (2) hydroxylation of the chlorobenzyl methylene carbon and hydroxylation of the chlorobenzyl ring at positions 3, 4 and 6 of the JAU6476-desthio; (3) exchange of sulphur against oxygen; (4) cleavage of the triazole moiety and conjugation of the benzylpropyldiol.

FIGURE C.3.1. Proposed Metabolic Profile of Prothioconazole in Wheat Following Seed Treatment. (This FIGURE was copied without alteration from MRID 46246142).

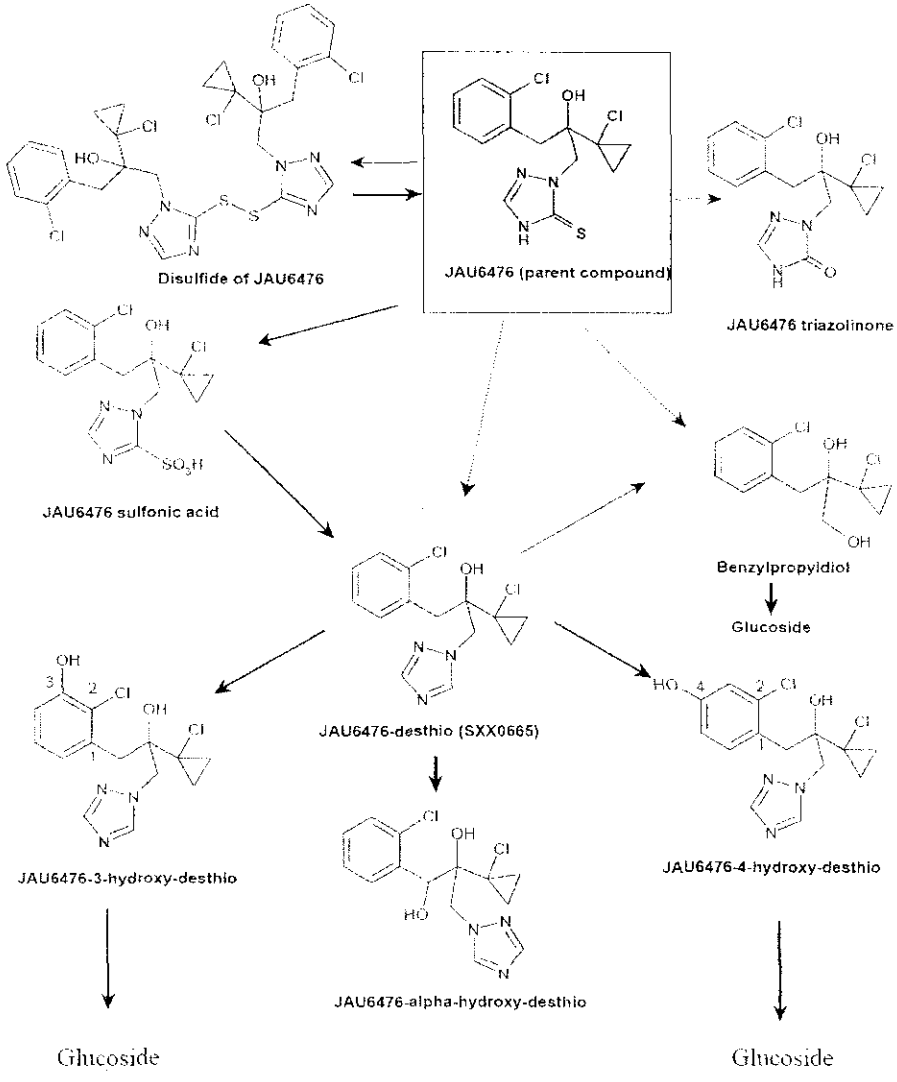


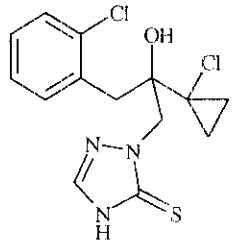
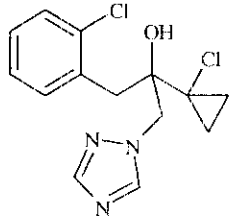
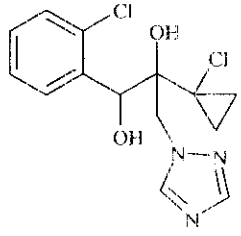
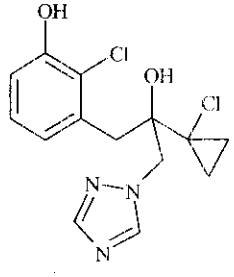
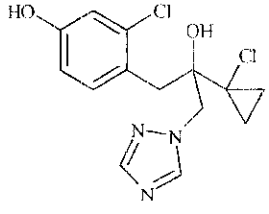
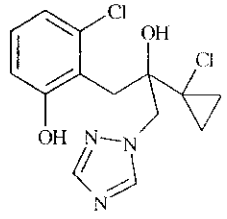
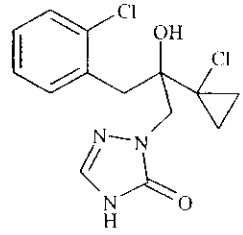
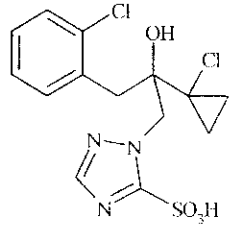
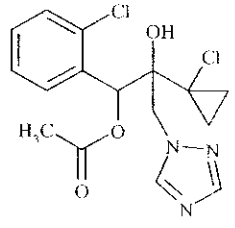
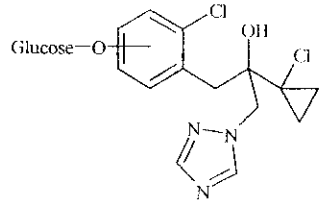
TABLE C.3.1. Identification of Compounds from Metabolism Study.		
Common name/Code FIGURE C.3.1 ID No.	Chemical name	Chemical structure
Prothioconazole JAU6476	2-[2-(1-chlorocyclopropyl)-3-(2-chlorophenyl)-2-hydroxypropyl}-1,2-dihydro-3*H*-1,2,4-triazole-3-thione	
JAU6476-desthio	α-(1-chlorocyclopropyl)-α-[(2-chlorophenyl)methyl]-1*H*-1,2,4-triazole-1-ethanol	
JAU6476-α-OH-desthio	2-(1-chlorocyclopropyl)-1-(2-chlorophenyl)-3-(1*H*-1,2,4-triazol-1-yl)-1,2-propanediol	
JAU6476-3-OH-desthio	α-(1-chlorocyclopropyl)-α-[(2-chloro-3-hydroxyphenyl)methyl]-1*H*-1,2,4-triazole-1-ethanol	
JAU6476-4-OH-desthio	α-(1-chlorocyclopropyl)-α-[(2-chloro-4-hydroxyphenyl)methyl]-1*H*-1,2,4-triazole-1-ethanol	

TABLE C.3.1. Identification of Compounds from Metabolism Study.		
Common name/code FIGURE C.3.1 ID No.	Chemical name	Chemical structure
JAU6476-6-OH-desthio	α-(1-chlorocyclopropyl)-α-[(2-chloro-6-hydroxyphenyl)methyl]-1*H*-1,2,4-triazole-1-ethanol	
JAU6476-triazolinone	2-[2-(1-chlorocyclopropyl)-3-(2-chlorophenyl)-2-hydroxypropyl]-2,4-dihydro-3*H*-1,2,4-triazole-3-one	
JAU6476 sulfonic acid	1-[2-(1-chlorocyclopropyl)-3-(2-chlorophenyl)-2-hydroxypropyl]-1*H*-1,2,4-triazole-5-sulfonic acid	
JAU6476-α-acetoxy-desthio	2-(1-chlorocyclopropyl)-1-(2-chlorophenyl)-2-hydroxy-3-(1*H*-1,2,4-triazol-1-yl)propyl acetate	
JAU6476-OH-desthio glucoside isomers		

D. CONCLUSION

The TRRs found in wheat matrices from the low-rate treatment (18.4 g a.i./kg seed) were low; 0.020 ppm in forage and hay, 0.030 ppm in straw, and 0.008 ppm in grain. Only extracts and hydrolysates of wheat forage, hay and straw from the high-rate treatment (93.3 g a.i./kg seed) were subjected to analysis for characterization and identification of residues. The TRRs in grain were less than 0.01 ppm from both treatment rates and no further analyses were attempted. The majority of the residues were characterized or unassigned, with only 18 to 33% of the TRRs identified. The major components of the TRRs in wheat forage were JAU6476-desthio (10.9%; 0.008 ppm) and JAU6476-3-OH-desthio/JAU6476-4-OH-desthio (<12%; <0.02 ppm) when treated at the high-rate. JAU6476-OH-desthio glucoside was the major residue identified (10.6%; 0.03 ppm) in wheat straw. Prothioconazole was less than 1% of the TRRs (≤0.002 ppm) in all wheat matrices. Other minor components (<10% of the TRRs) identified in the wheat matrices were JAU6476-α-OH-desthio, JAU6476-6-OH-desthio, JAU6476-triazolinone, JAU6476 sulfonic acid, JAU6476-α-acetoxy-desthio, benzylpropyldiol glucoside and JAU6476-disulfide.

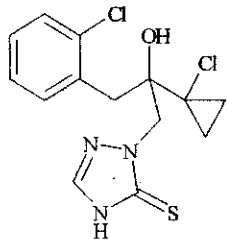
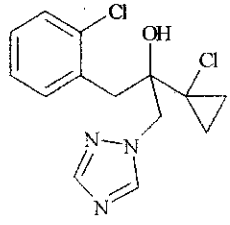
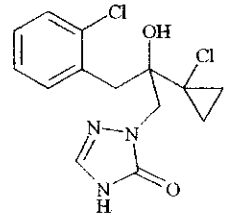
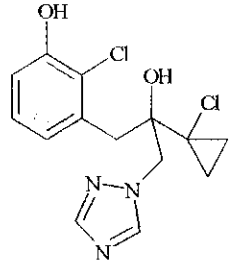
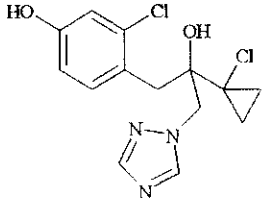
The major metabolic process is the hydroxylation of prothioconazole (JAU6476) to form JAU6476-desthio. Since JAU6476 has multiple positions that could potentially undergo hydroxylation, the majority of the remaining metabolites are multiple isomers of monohydroxylated JAU6476-desthio and their corresponding glucosides. Exchange of the sulfur for oxygen resulted in JAU6476 triazolinone. Cleavage of the triazole moiety occurred resulting in the formation of JAU6476-benzylpropyldiol and its glucoside.

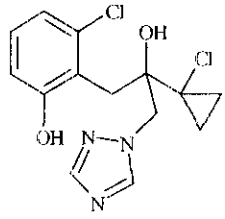
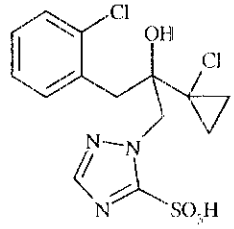
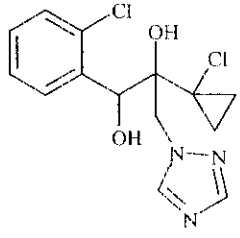
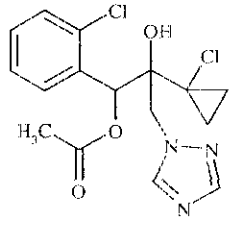
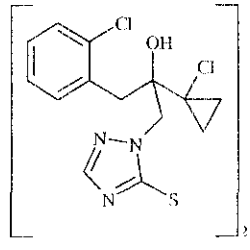
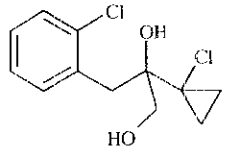
E. REFERENCES

None.

F. DOCUMENT TRACKING

RDI: Louise Croteau, Suzan Mathew (PMRA) 23/01/2006; Henri Bietlot (PMRA) 24/01/2006; Stephen Funk (IO)13/03/2006; Leung Cheng (RAB3).
Petition Number: PP#4F6830
DP Barcode: D303508
PC Code: 113961

APPENDIX I. Chemical Names and Structures of Reference Standards Used in the Wheat Metabolism Study.		
Common name; Company code	Chemical name	Chemical structure
Prothioconazole; JAU6476	2-[2-(1-chlorocyclopropyl)-3-(2-chlorophenyl)-2-hydroxypropyl]-1,2-dihydro-3*H*-1,2,4-triazole-3-thione	
JAU6476-desthio (a phenyl-labeled standard also used)	α-(1-Chlorocyclopropyl)-α-[(2-chlorophenyl)methyl]-1*H*-1,2,4-triazole-1-ethanol	
JAU6746 triazolinone	2-[2-(1-chlorocyclopropyl)-3-(2-chlorophenyl)-2-hydroxypropyl]-2,4-dihydro-3*H*-1,2,4-triazole-3-one	
JAU6476-3-hydroxy-desthio	α-(1-chlorocyclopropyl)-α-[(2-chloro-3-hydroxyphenyl)methyl]-1*H*-1,2,4-triazole-1-ethanol	
JAU6476-4-hydroxy-desthio	α-(1-chlorocyclopropyl)-α-[(2-chloro-4-hydroxyphenyl)methyl]-1*H*-1,2,4-triazole-1-ethanol	

APPENDIX I. Chemical Names and Structures of Reference Standards Used in the Wheat Metabolism Study.		
Common name; Company code	Chemical name	Chemical structure
JAU6476-6-hydroxy-desthio	α-(1-chlorocyclopropyl)-α-[(2-chloro-6-hydroxyphenyl)methyl]-1*H*-1,2,4-triazole-1-ethanol	
JAU6476 sulfonic acid	1-[2-(1-chlorocyclopropyl)-3-(2-chlorophenyl)-2-hydroxypropyl]-1*H*-1,2,4-triazole-5-sulfonic acid	
JAU6476-α-OH-desthio	2-(1-chlorocyclopropyl)-1-(2-chlorophenyl)-3-(1*H*-1,2,4-triazol-1-yl)-1,2-propanediol	
JAU6476-α-acetoxy-desthio	2-(1-chlorocyclopropyl)-1-(2-chlorophenyl)-2-hydroxy-3-(1*H*-1,2,4-triazol-1-yl)propyl acetate	
JAU6476-disulfide; dimer of prothioconazole		
Benzylpropyldiol	2-(1-chlorocyclopropyl)-3-(2-chlorophenyl)propane-1,2-diol [1]	

[1] *Chemical name generated using ACD chemical naming software.*

Primary Evaluators	Stephen Funk, Ph.D., Senior Chemist Immediate Office	Date: May 13 2006
	Suzan Mathew, Evaluation Officer FREAS, HED	Date: January 23/06
Approved by	Leung Cheng, Ph. D., Team Leader HED/RAB3	Date:
	Henri P Bietlot, Acting Section Head FREAS, HED	Date: Jan 27/06

In the absence of signatures, this document is considered to be a draft with deliberative material for internal use only.

This DER was originally prepared under contract by Dynamac Corporation (20440 Century Boulevard, Suite 100; Germantown, MD 20874; submitted 04/28/2005). The DER has been reviewed by the Health Effects Division (HED) and Health Canada's Pest Management Regulatory Agency (PMRA). The DER has been revised to reflect current Office of Pesticide Programs (OPP) policies and Directive 98-02.

STUDY REPORT:

46246210 Rodgers, C. (2003) Testing of JAU 6476 Plus Five Metabolites Through the FDA Multiresidue Methods as Described in the FDA Pesticide Analytical Manual (PAM) I, Appendix II, Updated /94. Project Numbers: 200480, J6162302 and 47947. Unpublished study prepared by Analytical Bio-Chemistry Laboratories, Inc. 133 p.

EXECUTIVE SUMMARY:

Bayer CropScience has submitted multiresidue method data for prothioconazole, the metabolites JAU6476-desthio and JAU6476-4-hydroxy, and the triazole-related compounds triazole, triazolylalanine, and triazolylacetic acid. The test substances were analyzed according to the FDA Multi-Residue Method Test guidelines in PAM Vol. I (dated 1/94). Prothioconazole, JAU6476-desthio, JAU6476-4-hydroxy, triazole and triazolylacetic acid were tested through Protocols A and C. As a result of Protocol C testing, prothioconazole, JAU6476-desthio, and JAU6476-4-hydroxy were tested through Protocol F. JAU6476-4-hydroxy and triazolylacetic acid were tested through Protocol B. Based on the results of the Protocol F testing, testing under

Protocols D and E was not required for prothioconazole, and testing under Protocol E was not required for JAU6476-4-hydroxy. Because the test substances are not substituted ureas, no testing under Protocol G was required. A suitable solvent for triazolylalanine could not be found; therefore, testing of this compound could not be conducted.

Sensitivity for triazolylacetic acid was poor using Protocol A, and no response was obtained for the other test compounds. Protocol C testing indicated that further testing using Protocols D, E, and F was not required for triazolylacetic acid and triazole. Triazolylacetic acid and JAU6476-4-hydroxy could not be adequately recovered under Protocol B. Prothioconazole and JAU6476-4-hydroxy were not adequately recovered using the Florisil column cleanup steps of Protocol F, and JAU6476-4-hydroxy did not yield adequate chromatography using Protocol D; thus, no further testing of these compounds was conducted. JAU6476-desthio could not be adequately recovered under Protocols D or E, using wheat hay. Recovery of JAU6476-desthio was variable (66-100%) under Protocol F, using ground beef.

STUDY/WAIVER ACCEPTABILITY/DEFICIENCIES/CLARIFICATIONS:

Under the conditions and parameters used in the study, the multiresidue method residue data are classified as scientifically acceptable. These data will be forwarded to the U.S. FDA for further evaluation.

The acceptability of this study for regulatory purposes is addressed in the forthcoming U.S. EPA Residue Chemistry Summary Document, [DP Barcode D303508], and in Canada's Regulatory Decision Document.

COMPLIANCE:

Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided. No deviations from regulatory requirements were reported.

A. BACKGROUND INFORMATION

Prothioconazole is a broad-spectrum systemic fungicide intended for the control of many diseases in different crops such as cereals, oilseed rape or peanuts. The biochemical mode of action is the inhibition of the demethylation of lanosterol or 24-methylene-dihydrolanosterol, which are precursors of sterols in fungi. The chemical has been submitted for joint EPA and PMRA review by Bayer CropScience. The crops proposed for joint review include barley, the dried shell and bean subgroup, the oilseed crop group, and wheat. Uses on peanuts and rice are being proposed for the U.S. only.

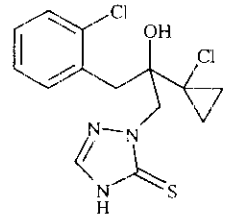
TABLE A.1. Test Compound Nomenclature.	
Chemical structure	
Common name	Prothioconazole (ISO accepted)
Company experimental name	JAU6476
IUPAC name	2-[(2*RS*)-2-(1-chlorocyclopropyl)-3-(2-chlorophenyl)-2-hydroxypropyl]-2*H*-1,2,4-triazole-3(4*H*)-thione
CAS name	2-[2-(1-chlorocyclopropyl)-3-(2-chlorophenyl)-2-hydroxypropyl]-1,2-dihydro-3*H*-1,2,4-triazole-3-thione
CAS registry number	178928-70-6
PC Code	113961
End-use product (EP)	Proline® 480 SC (4 lb/gal suspension concentrate), EPA File Symbol 264-IEL

TABLE A.2. Physicochemical Properties of the Technical Grade Test Compound.		
Parameter	Value	References
Melting range	139.1 to 144.5°C	MRID 46246003/CES
pH	5.8 (1% solution)	MRID 46246003/CES
Density at 20°C	1.36 g/mL	MRID 46246003/CES
Water solubility at 20°C	pH mg/L 4 5 8 300 9 2000	MRID 46246003/CES
Solvent solubility at 20°C	Solvent g/L Acetone >250 Acetonitrile 69 Dichloromethane 88 Dimethysulfoxide 126 Ethyl acetate >250 n-Heptane <0.1 1-Octanol 58 Polyethylene glycol >250 2-Propanol 87 Xylene 8	MRID 46246003/CES
Vapor pressure at 20 or 25°C	$<4 \times 10^{-7}$ Pa (calculated from determinations at 70°C)	MRID 46246003/CES
Dissociation constant, pK_a	6.9 (calculated from K_{OW})	MRID 46246003/CES
Octanol/water partition coefficient, at 20°C	pH $Log(K_{OW})$ unbuffered water 4.05 4 4.16 7 3.82 9 2.00	MRID 46246003/CES
UV/visible absorption spectrum	Peak maxima at 257 nm. No absorption at > 300 nm.	MRID 46246003/CES

CES - Chemistry Evaluation Section of PMRA

B. MATERIALS AND METHODS

The test substances were analyzed according to the FDA Multi-Residue Method Test guidelines in PAM Vol. I (dated 1/94).

Prothioconazole, JAU6476-desthio, JAU6476-4-hydroxy, triazole and triazolylacetic acid were tested through Protocols A and C. Protocol B involved the testing of JAU6476-4-hydroxy and triazolylacetic acid. Prothioconazole, JAU6476-desthio and JAU6476-4-hydroxy were tested through Protocol F due to the results of protocol C testing. Testing of prothioconazole (through protocols D and E) and JAU6476-4-hydroxy (through Protocol E) was not required due the results of Protocol F. Since none of the test substances were substituted ureas, no testing under Protocol G was required. The compound triazolylalanine was also tested. However, because no solvent was found to dissolve the compound, testing through protocols A, B, or C could not be conducted.

C. RESULTS AND DISCUSSION

TABLE C.1. Results of Multiresidue Methods Testing with Prothioconazole, JAU6476-Desthio, JAU6476-4-Hydroxy, Triazolylacetic Acid, and Triazole.

PAM I Protocol	Results	Comments
A	Prothioconazole, JAU6476-desthio, JAU6476-4-hydroxy, triazole, and triazolylacetic acid were tested for natural fluorescence. Triazolylacetic acid exhibited natural fluorescence (272-398 nm); however, the fluorescence response was too low for adequate sensitivity. The other compounds did not naturally fluoresce.	No further testing was conducted.
B	JAU6476-4-hydroxy and triazolylacetic acid were tested. Because methylated JAU6476-4-hydroxy gave acceptable chromatography under Protocol C conditions, further testing was conducted. Methylated JAU6476-4-hydroxy was adequately recovered (90%) from the Florisil cleanup system (section 402 C1c) and ELCD. The methylated compound was then tested for recovery via GPC cleanup (section 402 C1a); the compound was not recovered (0.4% recovery) so no further testing was conducted. Methylated triazolylacetic acid did not yield adequate chromatography when tested under Protocol C conditions, so no further testing was conducted.	Prothioconazole, JAU6476-desthio, and triazole are not acids or phenols; therefore, testing of these compounds was not required.
C	Prothioconazole: Peaks with acceptable relative retention time and sensitivity were observed using DB1 column with ECD; all other column/detector combinations [DB1 or DB17 column with electrolytic conductivity detection (ELCD) or nitrogen-phosphorus (NP) detection; DB17 with ECD or flame-photometric detection (FPD); DB225 with ECD] yielded peaks with inadequate sensitivity, peaks with unacceptable relative retention time, or no observable peaks. JAU6476-desthio: Peaks with acceptable relative retention time and sensitivity were observed using DB1 and DB17 columns with ECD, ELCD, and NPD; and DB225 column with ECD. JAU6476-4-hydroxy: Peaks with acceptable relative retention time and sensitivity were observed using DB1 column with ELCD; the applicant noted that the response did not seem to be linear. Triazole: No peaks observed using DB1, DB17, or DB225 columns with ECD. Triazolylacetic acid: No peaks observed using DB225 column and ECD.	Based on results, prothioconazole, JAU6476-desthio, and JAU6476-4-hydroxy were tested through Protocol F, and JAU6476-desthio was tested through Protocols D, E, and F; no testing of triazole or triazolylacetic acid through Protocols D, E, or F was required.

TABLE C.1. Results of Multiresidue Methods Testing with Prothioconazole, JAU6476-Desthio, JAU6476-4-Hydroxy, Triazolylacetic Acid, and Triazole.		
PAM I Protocol	Results	Comments
D	Prothioconazole was not tested because of low recovery from Florisil cleanup under Protocol F; the Florisil cleanup systems of Protocol D are similar to those of Protocol F [Florisil cleanup step would be required for prothioconazole because it was not found to have sufficient sensitivity to any of the selective detectors]. JAU6476-desthio was tested under Protocol D using a representative non-fatty food commodity (wheat hay) without Florisil cleanup using extraction method 302 E4, at spiking levels of 0.10 and 0.50 ppm. A significant peak at the retention time of JAU6476-desthio was observed in the reagent blank and in the control sample, and an apparent matrix effect degraded the chromatographic response. Further testing was not conducted. JAU6476-4-hydroxy was tested under Protocol D without Florisil cleanup; however, chromatographic results were erratic and further testing could not be conducted.	
E	Prothioconazole and JAU6476-4-hydroxy were not tested because of low recovery from Florisil cleanup under Protocol F; the Florisil cleanup systems of Protocol E are identical to those of Protocol F. JAU6476-desthio was tested through Protocol E (method 303), using extraction E3 with Florisil cleanup C1, by spiking wheat hay at 0.05 ppm and 0.5 ppm. Recoveries were 14% and 14% at 0.05 ppm, and 23% and 33% at 0.5 ppm.	
F	Prothioconazole, JAU6476-desthio, and JAU6476-4-hydroxy were tested for recovery via Florisil cleanup (304 C1 and C2). Prothioconazole was not recovered through either system. JAU6476-desthio was recovered at a total of 53% using 304 C1 and at 5% using 304 C2. JAU6476-4-hydroxy was recovered at a total of 30% using 304 C1, and was not recovered using 304 C2. JAU6476-desthio was tested through Protocol F (method 304), using extraction E1 with Florisil cleanup C1, by spiking ground beef (representative fatty food) at 0.05 ppm and 0.5 ppm. Recoveries were 66% and 100% at 0.05 ppm, and 81% and 85% at 0.5 ppm.	Testing of prothioconazole and JAU6476-4-hydroxy was terminated because of low recovery from Florisil cleanup.
G	Not tested because none of the compounds are substituted ureas.	

D. CONCLUSION

Prothioconazole, the metabolites JAU6476-desthio and JAU6476-4-hydroxy, and the triazole-related compounds triazole, triazolylalanine, and triazolylacetic acid were adequately evaluated for their recovery through FDA multiresidue methods. Because the test substances are not substituted ureas, testing under Protocol G was not conducted. A suitable solvent for triazolylalanine could not be found; therefore, testing of this compound could not be conducted. Sensitivity for triazolylacetic acid was poor using Protocol A; and the other test compounds were not naturally fluorescent. Protocol C testing indicated that further testing using Protocols D, E, and F was not required for triazolylacetic acid and triazole. Triazolylacetic acid and JAU6476-4-

hydroxy could not be adequately recovered under Protocol B. Prothioconazole and JAU6476-4-hydroxy were not adequately recovered using the Florisil column cleanup steps of Protocol F, and JAU6476-4-hydroxy did not yield adequate chromatography using Protocol D; therefore, no further testing of these compounds was conducted. In wheat hay, JAU6476-desthio could not be adequately recovered under Protocols D or E. In ground beef, the recovery of JAU6476-desthio was variable (66-100%) under Protocol F. The submitted data will be forwarded to the U.S. FDA for further evaluation.

E. REFERENCES

None.

F. DOCUMENT TRACKING

RDI: Suzan Mathew (PMRA) 23/01/2006; Henri Bietlot (PMRA) 24/01/2006; Stephen Funk (IO) 13/03/2006; Leung Cheng (RAB3) .
Petition Number: PP#4F6830
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PC Code: 113961

Template Version September 2003

Primary Evaluators	Stephen Funk, Ph.D., Senior Chemist Immediate Office	Date: Mar 13 2006
	Louise G. Croteau, Senior Evaluation Officer FREAS, HED	Date: 23/01/06
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Approved by	Leung Cheng, Ph.D., Team Leader HED/RAB3	Date:
	Henri P. Bietlot, Acting Section Head FREAS, HED	Date: Jan 24/06

In the absence of signatures, this document is considered to be a draft with deliberative material for internal use only.

This DER was originally prepared under contract by Dynamac Corporation (20440 Century Boulevard, Suite 100; Germantown, MD 20874; submitted 05/05/2005). The DER has been reviewed by the Health Effects Division (HED) and Health Canada's Pest Management Regulatory Agency (PMRA). The DER has been revised to reflect current Office of Pesticide Programs (OPP) policies, and PMRA Directive 98-02.

STUDY REPORT:

46246145 Haas, M. (2001) Metabolism of [Phenyl-UL-(Carbon 14)]JAU6476 in Peanuts. Project Number: M1730984-2, MR-193/01. Unpublished study prepared by Bayer Ag Institut fuer Ruckstands-Analytik. 130 p.

46246146 Haas, M. (2003) Metabolism of [Triazole-UL-(Carbon 14)]JAU6476 in Peanuts. Project Number: M1731145/2, MR/194/02. Unpublished study prepared by Bayer Ag, Institute of Product h fo. 145 p.

EXECUTIVE SUMMARY:

Phenyl-label study

Bayer CropScience has submitted studies investigating the metabolism of [phenyl-UL-^{14}C]-prothioconazole (specific activity 2.77 MBq/mg) in peanut plants grown in a greenhouse. The radiolabeled test substances were formulated as an emulsifiable concentrate (EC) formulation. [Phenyl-UL-^{14}C]-JAU6476 were applied to peanut plants 'Georgia Green' as three foliar spray applications with 20 to 22-days interval at growth stages beginning at pod development (BBCH codes 66, 71 and 75). Each treatment was performed at a rate of approximately 0.267 lb a.i./A (299 g a.i./ha) for a maximum seasonal rate of 0.800 lb a.i./A (897 g a.i./ha). A 5-fold rate exaggeration study was also performed to allow for metabolite identification. Peanut plants were harvested at maturity (BBCH growth stages 89-91) at a pre-harvest interval of 21 days. Nuts were removed and cleaned from adhering soil. The plants (hay and nuts with shells) were allowed to dry for 4-5 days. The hay and nutmeat samples were individually homogenized with liquid nitrogen. All samples were stored at -18°C or below. The experimental work from extraction to first analysis (TLC-profiling) was completed within 17-91 days (peanut hay) and 51-99 days (nutmeat). Nutmeat extracted 355 days after harvest using MSPD, indicated similar metabolic distribution

The overall distribution of TRRs was achieved by combustion and radioassay by liquid scintillation counting (LSC). Identification and characterization of metabolites were performed by HPLC with photodiode array or variable wavelength UV detector and a flow-through radiodetector. Confirmation of residues was by radio-TLC co-chromatography with authentic reference standards or by mass spectrometry (MS) and when possible ^{1}H-NMR. Homogenized peanut hay was extracted with acetonitrile (ACN)/water, with added cysteine hydrochloride to prevent oxidative decomposition of the parent during extraction. Further extraction was achieved using an accelerated solvent extractor (ASE). The homogenized nutmeat samples were extracted by two separate methods; refluxing with hexane and matrix solid phase dispersion (MSPD)/microwave extractions.

The TRRs found in peanut hay and nutmeat were 107.51 ppm and 0.29 ppm, respectively. Solvent extraction with acetonitrile/water released 77.5% of the TRRs in peanut hay. Hexane reflux and/or MSPD extraction with a series of solvents released approximately 67-74% of the TRRs in nutmeat. Accelarated solvent extraction (ASE) and microwave extraction was useful to release additional radioactivity from the peanut matrices. Non-extractable residues remaining following extraction/hydrolysis accounted for <7% of the TRRs (6.73 ppm) in hay and <13% of the TRRs (<0.05 ppm) in nutmeat. Accountabilities ranged from 100-124%.

Approximately 65 to 74% of the TRRs were identified in peanut matrices for the phenyl-label study. Prothioconazole was identified at about 2% of the TRRs (2.0 ppm) in hay. Metabolite JAU6476-desthio was the major identified residue in hay, accounting for 28.2% of the TRRs (30.37 ppm), and one additional metabolite, JAU6476-desthio-dihydroxyolefin glucosides, was identified at 14.1% of the TRRs (15.09 ppm). All remaining metabolites were identified at <10% of the TRRs and included JAU6476-3-OH-desthio, JAU6476-4-OH-desthio, JAU6476-

dihydroxydiene sulfonic, JAU6476-dihydroxyolefin sulfonic acid, glucoside conjugates of the JAU6476-OH-desthio isomers, JAU6476-desthio-hydroxydienyl-cysteine, JAU6476-triazolinone, JAU6476 sulfonic acid, and JAU6476-disulfide. Neither prothioconazole nor JAU6476-desthio were identified in nutmeat. The majority of the TRRs in nutmeat (42.6-47.8%; 0.13-0.14 ppm) were associated with peanut oil and determined as fatty acids, indicating that prothioconazole may be completely metabolized to CO_2 in plants. Identified metabolites (each found at <10% of the TRRs) included JAU6476-desthio-dihydroxyolefin glucosides, JAU6476-desthio-hydroxydienyl-cysteine, JAU6476-OH-desthio glucosides, and JAU6476 sulfonic acid.

Triazole-label Study

Bayer CropScience has submitted studies investigating the metabolism of [triazole-UL-^{14}C]-prothioconazole (specific activity 2.11 MBq/mg) in peanut plants grown in a greenhouse. The radiolabeled test substances were formulated as an emulsifiable concentrate (EC) formulation. [Triazole-UL-^{14}C]-JAU6476 was applied to peanut plants 'Georgia Green' as three foliar spray applications with 20 to 22-days interval at growth stages beginning at pod development (BBCH code 66, 71 and 75). Each application was between 0.365-0.267 lb a.i./A (297-299 g a.i./ha) for a maximum seasonal rate of 0.799 lb a.i./ha (895 g a.i./ha). Peanut plants were harvested at maturity (BBCH growth stages 89-91) at a pre-harvest interval of 14 days (triazole-label study). Nuts were removed and cleaned from adhering soil. The plants (hay and nuts with shells) were allowed to dry for 4-5 days. The hay and nutmeat samples were individually homogenized with liquid nitrogen. All samples were stored at -18°C or below. The experimental work from extraction to first analysis (TLC-profiling) was completed within 17-91 days (peanut hay) and 51-99 days (nutmeat). The aqueous phase of peanut hay and polar fractions of nutmeat were monitored for stability using different HPLC systems throughout the study.

The overall distribution of TRRs was achieved by combustion and radioassay by liquid scintillation counting (LSC). Identification and characterization of metabolites were performed by HPLC with photodiode array or variable wavelength UV detector and a flow-through radiodetector. Confirmation of residues was by radio-TLC co-chromatography with authentic reference standards or by mass spectrometry (MS) and when possible ^{1}H-NMR. Isolation and characterization of triazole metabolites was conducted by incubating heterotrophic plant cell suspension cultures prepared from apples with [^{14}C]-triazole for 7 days. Homogenized peanut hay was extracted with acetonitrile (ACN)/water, with added cysteine hydrochloride to prevent oxidative decomposition of the parent during extraction. Further extraction was achieved using an accelerated solvent extractor (ASE). The homogenized nutmeat samples were extracted by two separate methods; refluxing with hexane and matrix solid phase dispersion (MSPD)/microwave extractions.

The TRRs found in peanut hay and nutmeat were 47.38 ppm and 1.4 ppm, respectively. Solvent extraction with acetonitrile/water released 85% of the TRRs in peanut hay. Hexane reflux and/or MSPD extraction with a series of solvents released approximately 77% of the TRRs in nutmeat. Accelarated solvent extraction (ASE) and microwave extraction was useful to release additional radioactivity from the peanut matrices. Non-extractable residues remaining following

extraction/hydrolysis accounted for 5.4% of the TRRs (2.55 ppm) in hay and 1.9% of the TRRs (0.03 ppm) in nutmeat. Accountabilities ranged from 100-102%.

Approximately 80-85% of the TRRs were identified in peanut matrices for the triazole-label study. Prothioconazole was identified at 6.6% of the TRRs (3.11 ppm) in hay. Metabolite JAU6476-desthio was the major identified residue in hay, accounting for 23.6% of the TRRs (11.15 ppm). All remaining metabolites were identified at <10% of the TRRs and included JAU6476-3-OH-desthio; JAU6476-4-OH-desthio; JAU6476-triazolinone; JAU6476 sulfonic acid; JAU6476-desthio-phenyl-cysteine; JAU6476-disulfide; glucoside conjugates of JAU6476-3-OH-desthio, JAU6476-4-OH-desthio, unspecified JAU6476-OH-desthio isomers, JAU6476-desthio-dihydroxyolefin, and JAU6476-desthio-dihydroxydiene; JAU6476-malonyl glucoside isomers; JAU6476-dihydroxydiene sulfonic acid; and JAU6476-dihydroxyolefin sulfonic acid. Triazolyl metabolites, including triazolylalanine (TA), triazole acetic acid (TAA), triazolylhydroxy-propionic acid (THPA), JAU6476-triazolyl-ethanol, and JAU6476-triazolyl-ethanol-glucoside were minor components in peanut hay, each accounting for ≤ 1.5% of the TRRs (≤0.71 ppm). Prothioconazole was not identified in nutmeat, and JAU6476-desthio was identified at 6.2% of the TRRs (0.09 ppm). Triazolyl metabolites were the major identified residues in nutmeat, with TA accounting for 49.8% of the TRRs (0.70 ppm), and THPA accounting for 24.7% of the TRRs (0.35 ppm). TAA was identified at 1.2% of the TRRs (0.02 ppm) and triazolyl unknowns accounted for 4.3% of the TRRs (0.07 ppm). Radioactivity determined as fatty acids in peanut oil accounted for 3.0% of the TRRs (0.05 ppm) in nutmeat.

JAU6476 was extensively metabolized in peanut by: (1) oxidation and loss of sulfur to form JAU6476-desthio; (2) hydroxylation of the chlorobenzyl ring of JAU6476-desthio at positions 3 and 4 to form the hydroxy desthio metabolites; (3) conjugation of the hydroxylated metabolites; (4) exchange of oxygen for sulfur; and (5) release of the triazole moiety to form triazolylalanine (TA) and triazolylhydroxypropionic acid (THPA). Free triazole (1*H*-1,2,4-triazole) was not detected in any peanut matrix.

STUDY/WAIVER ACCEPTABILITY/DEFICIENCIES/CLARIFICATIONS:

Under the conditions and parameters used in the study, the peanut metabolism data are classified as scientifically acceptable. The acceptability of this study for regulatory purposes is addressed in the forthcoming U.S. EPA Residue Chemistry Summary Document, DP Barcode D303508, PP#4F6830, and in Canada's Regulatory Decision Document.

COMPLIANCE:

Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided. No deviations from regulatory requirements were reported which would have an impact on the validity of the study.

A. BACKGROUND INFORMATION

Prothioconazole is a broad-spectrum systemic fungicide intended for the control of many diseases in different crops such as cereals, oilseed rape or peanuts. The biochemical mode of action is the inhibition of the demethylation of lanosterol or 24-methylene-dihydrolanosterol, which are precursors of sterols in fungi. The chemical has been submitted for joint EPA and PMRA review by Bayer CropScience. The crops proposed for joint review include barley, the dried shell and bean subgroup, the oilseed crop group, and wheat. Uses on peanuts and rice are being proposed for the U.S. only.

TABLE A.1. Test Compound Nomenclature.

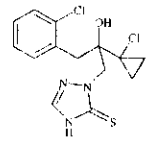
Chemical structure	
Common name	Prothioconazole (ISO accepted)
Company experimental name	JAU6476
IUPAC name	2-[(2*RS*)-2-(1-chlorocyclopropyl)-3-(2-chlorophenyl)-2-hydroxypropyl]-2*H*-1,2,4-triazole-3(4*H*)-thione
CAS name	2-[2-(1-chlorocyclopropyl)-3-(2-chlorophenyl)-2-hydroxypropyl]-1,2-dihydro-3*H*-1,2,4-triazole-3-thione
CAS registry number	178928-70-6
PC Code	113961
End-use product (EP)	Proline® 480 SC (4 lb/gal suspension concentrate), EPA File Symbol 264-IEL

TABLE A.2. Physicochemical Properties of the Prothioconazole

Parameter	Value	References
Melting range	139.1 to 144.5°C	MRID 46246003/CES
pH	5.8 (1% solution)	MRID 46246003/CES
Density at 20°C	1.36 g/mL	MRID 46246003/CES
Water solubility at 20°C	pH: mg/L 4: 5 8: 300 9: 2000	MRID 46246003/CES
Solvent solubility at 20°C	Solvent: g/L Acetone: >250 Acetonitrile: 69 Dichloromethane: 88 Dimethysulfoxide: 126 Ethyl acetate: >250 n-Heptane: <0.1 1-Octanol: 58 Polyethylene glycol: >250 2-Propanol: 87 Xylene: 8	MRID 46246003/CES

TABLE A.2. Physicochemical Properties of the Prothioconazole		
Parameter	Value	References
Vapor pressure at 20 or 25°C	<4 x 10^{-7} Pa (calculated from determinations at 70°C)	MRID 46246003/CES
Dissociation constant, pK_a	6.9 (calculated from K_{OW})	MRID 46246003/CES
Octanol/water partition coefficient, at 20°C	pH — Log(K_{OW}) unbuffered water — 4.05 4 — 4.16 7 — 3.82 9 — 2.00	MRID 46246003/CES
UV/visible absorption spectrum	Peak maxima at 257 nm. No absorption at > 300 nm	MRID 46246003/CES

CES - Chemistry Evaluation Section of PMRA

B. EXPERIMENTAL DESIGN

B.1. Test Site and Crop Information

TABLE B.1.1. Test Site Information.				
Testing Environment	Soil characteristics			
	Type	%OM	pH	CEC
Phenyl-label study				
Plants were grown in standard lysimeter planting containers with a 1.0-m^2 surface area in a climate-controlled chamber in a greenhouse (Leverkusen, Germany).	Monheim 3 Sandy loam soil	1.98	pH ($CaCl_2$) 6.3 pH (water) 6.5	10

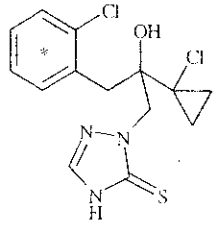
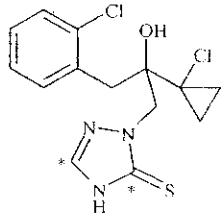
TABLE B.1.1. Test Site Information.				
Testing Environment	Soil characteristics			
	Type	%OM	pH	CEC
Triazole-label study				
Plants were grown in standard lysimeter planting containers with a 1.0- m^2 surface area in a climate-controlled chamber in a greenhouse (Monheim, Germany).	Monheim 3 Sandy loam soil	1.98	pH ($CaCl_2$) 6.3 pH (water) 6.5	10

The growth chamber was operated on a 14-hour day/10-hour night schedule (light 35 Klux) at 60% relative humidity. Daylight temperatures were 23-24°C (phenyl-label study) and 20-24°C (triazole-label study), and night time temperatures were 16-17°C. Peanut plants were watered as necessary and received maintenance pesticides as needed. The applicant reported that plants remained healthy over the growth period.

To facilitate isolation and identification of triazole metabolites, the applicant also conducted a cell culture experiment in which heterotrophic plant cell suspension cultures prepared from apples were incubated with uniformly ring labeled [^{14}C]-triazole for 7 days.

TABLE B.1.2. Crop Information.					
Crop; crop group	Variety	Growth stage at application	Growth stage at harvest	Harvested RAC	Harvesting procedure
Peanut; Miscellaneous commodities	Georgia Green	1: BBCH 66 (beginning of pegging) 2: BBCH 71 (first pod development) 3: BBCH 75 (pod development)	BBCH 89-91	Peanuts and hay	Plants were removed from the soil using a spade. Peanuts were removed from foliage, and peanuts and foliage were allowed to dry for 4 days (triazole-label study) and 5 days (phenyl-label study).

B.2. Test Materials

TABLE B.2.1. Test Material Characteristics		
Chemical structure		
Radiolabel position	[phenyl-UL-^{14}C]-prothioconazole	[triazole-UL-^{14}C]-prothioconazole
Lot No.	1811/1822/1826	SYP11997, SYP11999, SYP12103
Purity	>97% radiochemical purity by radio-HPLC; >99% chemical purity by HPLC-UV	>98% radiochemical purity; >99% chemical purity
Specific activity (Bq) [1]	2.77 MBq/mg (74.9 µCi/mg)	2.11 MBq/mg (56.9 µCi/mg)

[1] Bq = disintegrations per second

B.3. Study Use Pattern

TABLE B.3.1. Use Pattern Information		
Chemical name	[phenyl-UL-^{14}C]-prothioconazole	[triazole-UL-^{14}C]-prothioconazole
Application method	The radiolabeled test substance was formulated as an EC with formulation blank, then diluted with water and applied using a computer-controlled track sprayer.	The radiolabeled test substance was formulated as an EC with formulation blank, then diluted with water and applied using a computer-controlled track sprayer.
Application rate	Three applications at 0.266 lb a.i./A (297 g a.i./ha); 0.267 lb a.i./A (299 g a.i./ha); 265 lb a.i./A (297.3 g a.i./ha) for a total application rate of 0.799 lb a.i./A (895 g a.i./ha).	Three applications at approximately 0.267 lb a.i./A (299 g a.i./ha) for a total application rate of 0.800 lb a.i./A (897 g a.i./ha).
Number of applications	3	3
Timing of applications	Applications were made at 94, 114, and 136 days after planting at BBCH 66, 71, and 75; 20- to 22-day retreatment intervals.	Applications were made at 93, 113, and 134 days after planting at BBCH 66, 71, and 75; 20- to 21-day retreatment intervals.
PHI[1]	21 days	14 days

[1] PHI = pre-harvest interval.

The applicant also conducted a "5-fold overdose" experiment for the phenyl-label study study only, in which applications were made to plants grown in 10-L buckets using a hand sprayer; application rates in terms of g a.i./ha were not provided and could not be determined from the available information.

B.4. Identification/Characterization of Residues

B.4.1. Sample Handling and Preparation

Peanut hay was cut into small pieces and peanut nutmeats were separated from shells; hay and nutmeat samples were then frozen in liquid nitrogen and homogenized. Following processing, peanut samples were stored frozen at ≤-18°C. Extracts were stored either refrigerated (4°C) or frozen (-20°C) for longer periods. Nutmeat samples from the 5-fold overdose treatment (phenyl-label study only) were subjected to extraction but were not further analyzed.

Cells from the cell culture experiment were separated from the nutrient media by filtration (triazole-label study only).

General extraction procedures: Cysteine HCl was added to all extracting solvents and to extracts during concentration procedures to prevent oxidative decomposition of prothioconazole. Extracts were centrifuged to remove precipitated material including cysteine HCl. Following partitioning with dichloromethane (DCM), a small volume of acetonitrile (ACN) was added to each DCM phase prior to concentration.

Phenyl-label study:

Hay: A subsample of hay from the low-rate treatment was soaked in water for 3 hours, after which ACN was added to the water, and the subsample was homogenized and vacuum filtered. Extraction with ACN:water (80:20, v:v) was repeated twice followed by vacuum filtration, and the filtrates were combined and concentrated. Non-extractable residues following ACN:water extraction were subjected to accelerated solvent extraction (ASE) with ACN:water (80:20, v:v) at 50 and 100°C (two extractions each temperature). The ACN:water extracts from the conventional and ASE procedures were combined, concentrated to aqueous, and partitioned with DCM (three times). The resulting DCM phases were combined and concentrated. Non-extractable residues following ASE were subjected to acid hydrolysis using dioxane:2N HCl (9:1, v:v) at reflux for 2 hours. The hydrolysate was separated by filtration and concentrated, and the remaining non-extractable residues were washed with water (yielding an aqueous fraction) and then lyophilized prior to combustion and liquid scintillation counting (LSC). Peanut hay from the high-rate treatment was not subjected to extraction procedures.

Nutmeat:
Hexane extraction: A subsample of nutmeat was refluxed with n-hexane for 16 hours. The n-hexane phase was partitioned three times with ACN, and the ACN phases were combined and reserved for TLC analysis. The resulting n-hexane phase (Hexane 1) was concentrated to an oily liquid and then subjected to alkaline saponification with 10% KOH in ethanol for 16 hours. The hydrolysate was cooled and acidified with concentrated HCl. The phases were separated, yielding a hexane phase (Hexane 2) and an aqueous phase (Aqueous 1); Aqueous 1 was partitioned twice with n-hexane, and the combined hexane phases (Hexane 3) were combined with the hexane phase (Hexane 2) from saponification. Both the aqueous (Aqueous 2) and the

hexane phases were concentrated, and the concentrated hexane phase was subjected to methylation with diazomethane. The methylated residues were reserved for TLC analysis.

The non-extractable residues following the initial n-hexane extraction were extracted three times with ACN:water (80:20, v:v), filtered, and the filtrates were combined, concentrated to aqueous, and reserved for TLC analysis. The combined filtrates were then partitioned three times with DCM, and the resulting DCM and aqueous phases were concentrated [the applicant indicated that TLC analyses were conducted on these fractions; however, no TLC results for these fractions were presented]. The non-extractable residues following extraction with ACN:water were subjected to enzyme hydrolysis with diastase (α-amylase) in citrate/NaOH buffer, pH 6, containing NaN_3, at room temperature; the mixture was filtered on days 5 and 11, and fresh enzyme solution was added to the remaining solids. The applicant reported that the enzyme hydrolysate was partitioned three times with ethyl acetate and the resulting organic and aqueous phases were subjected to TLC analyses; however, no results for the partitioning or TLC analyses were reported. The non-extractable residues remaining following enzyme hydrolysis were subjected to acid hydrolysis with 5N HCl at reflux for 4 hours. The hydrolysate was partitioned twice with ethyl acetate (the hydrolysate was adjusted to pH 7 after the first partition), and the ethyl acetate phases were combined, concentrated to dryness, and redissolved in ACN.

MSPD extraction: A second subsample of peanut nutmeat was subjected to extraction using Matrix Solid Phase Dispersion (MSPD) by mixing the subsample with MSPD Isolute® material and packing the mixture into a column reservoir. Residues were sequentially eluted from the mixture with n-heptane, n-hexane, DCM, ACN, methanol:water (1:1, v:v; twice), and water:tetrahydrofuran (8:2, v:v). The n-heptane phase was subjected to saponification as described above. Non-extractable residues (including MSPD material) were subjected to microwave extraction twice with ACN:water (80:20, v:v), at 140°C for 10 minutes each. The applicant noted that although both extraction procedures for nutmeat yielded similar quantitative results, this procedure yielded more highly purified extracts.

Nutmeat from the high-rate treatment was subjected to extraction procedures from a minimum amount of plant matrix to allow the identification and characterization of metabolites. This was done to avoid extracting large amounts of plant matrix (minimize plant co-extractives and to obtain sufficient radioactivity for identification work. Since the high-rate treatment samples were only used in the qualitative process of identification and characterization, quantitative data was provided in the study report.

Triazole-label study:

Hay: A subsample of hay was soaked in water overnight, after which ACN was added to the water, and the subsample was homogenized and vacuum filtered. Extraction with ACN:water (80:20, v:v) was repeated twice followed by vacuum filtration, and the filtrates were combined and concentrated. Non-extractable residues following ACN:water extraction were subjected to accelerated solvent extraction (ASE) with ACN:water (80:20, v:v) at 50 and 100°C (two extractions at each temperature). The ACN:water extracts from the conventional and ASE

procedures were separately partitioned with DCM (three times). Resulting like phases were combined and concentrated. Non-extractable residues following ASE were subjected to acid hydrolysis using dioxane:2N HCl (9:1, v:v) at reflux for 2 hours. The hydrolysate was separated by filtration, concentrated, and reserved for TLC analysis.

An aliquot of the concentrated aqueous phase following DCM partitioning was subjected to enzyme hydrolysis with cellulase (in 0.05M acetate buffer, pH 5, at ~40°C for 24 hours).

Nutmeat: A subsample of peanut nutmeat was subjected to extraction using Matrix Solid Phase Dispersion (MSPD) by mixing the subsample with MSPD Isolute® material and packing the mixture into a column reservoir. Residues were sequentially eluted with n-heptane, n-hexane, DCM, ACN, methanol:water (1:1, v:v; twice), and water:tetrahydrofuran (8:2, v:v). The heptane phase was concentrated and subjected to alkaline saponification with 10% KOH in ethanol for 16 hours. The hydrolysate was cooled and acidified with concentrated HCl. The resulting phases were separated, the aqueous phase was partitioned twice with n-hexane, and the hexane phases were combined and subjected to methylation with diazomethane under a stream of nitrogen. Non-extractable residues following MSPD extraction (including MSPD material) were subjected to microwave extraction twice with ACN:0.1 N HCl (80:20, v:v), at 140°C for 10 minutes each. The resulting suspension was filtered.

Cell culture: Cells were extracted three times with ACN/water (80:20, v:v), then filtered through a glass filter. The resulting ACN/water extracts were combined and concentrated, then partitioned three times with ethyl acetate. The original combined nutrient media was also partitioned three times with ethyl acetate. Non-extractable residues were allowed to dry for 7 days. Samples with sufficient radioactivity were analyzed by HPLC and TLC, then subjected to semi-preparative HPLC for isolation and purification of metabolites.

The extraction procedures for peanut matrices are summarized in FIGURES B.4.1 to B.4.1.2.

FIGURE B.4.1. Extraction Procedure for Peanut Hay for Both Radiolabels

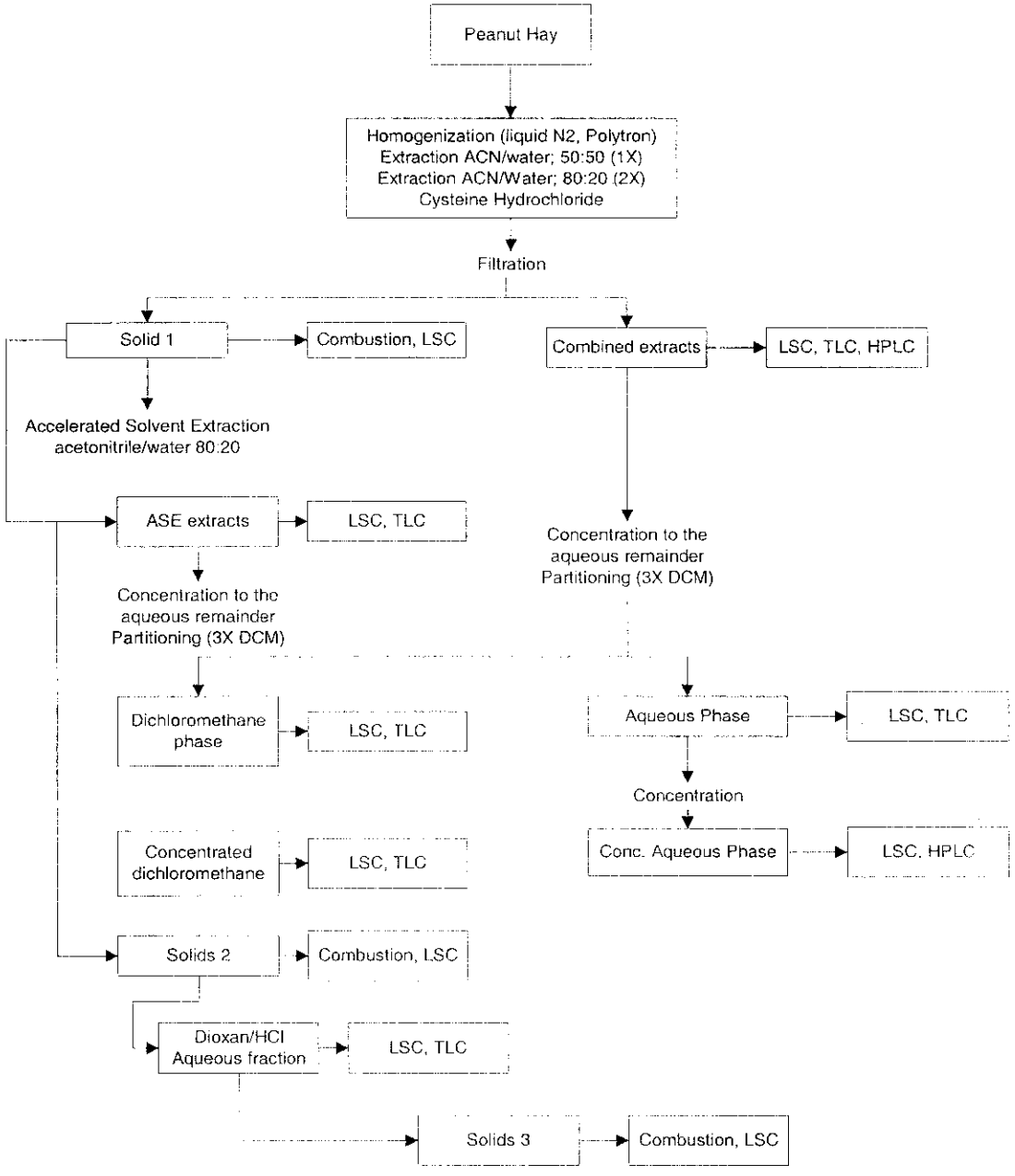
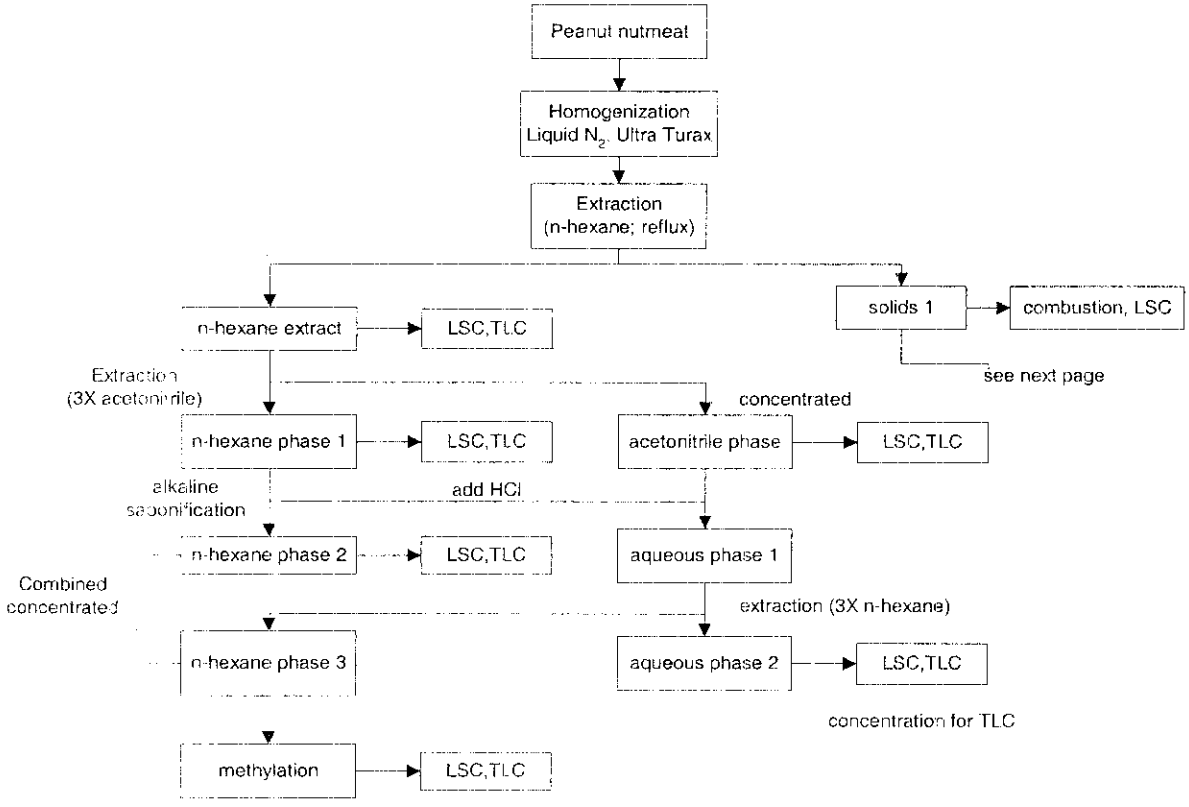


FIGURE B.4.1.1 Extraction Procedure for Nutmeat (Hexane Extraction; Phenyl-label study)



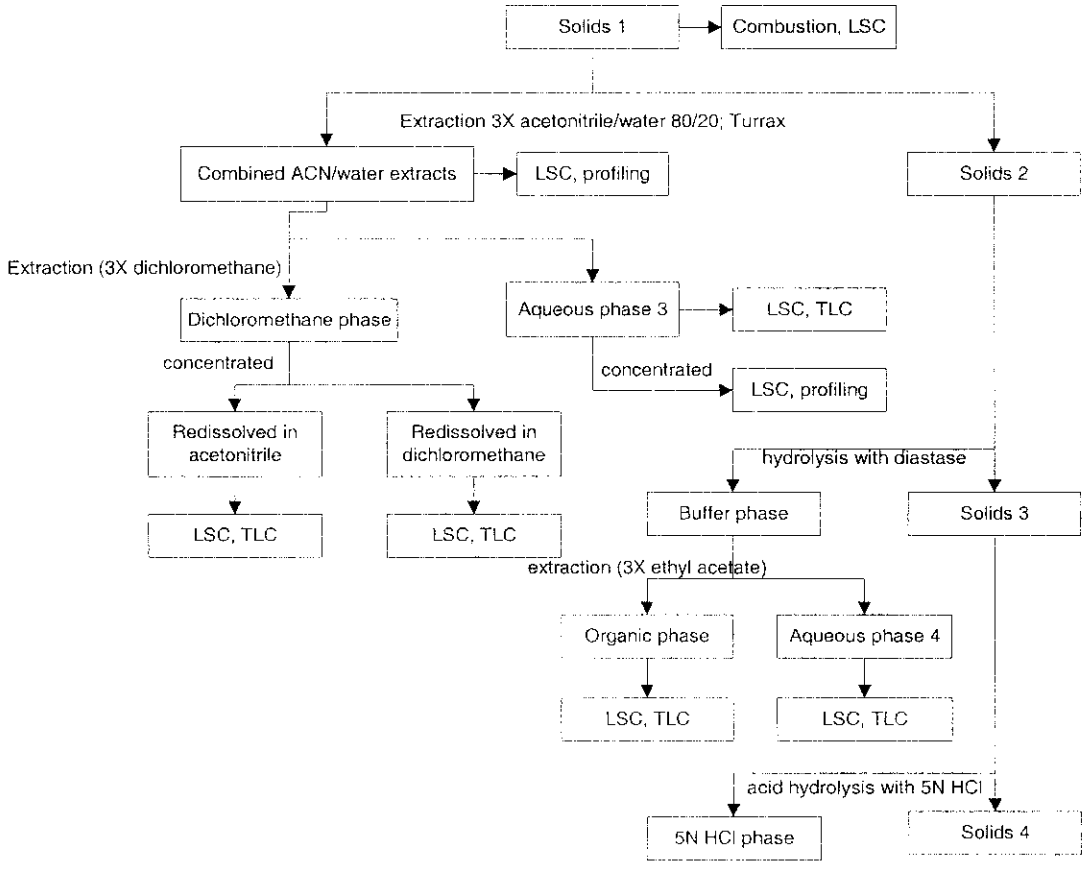
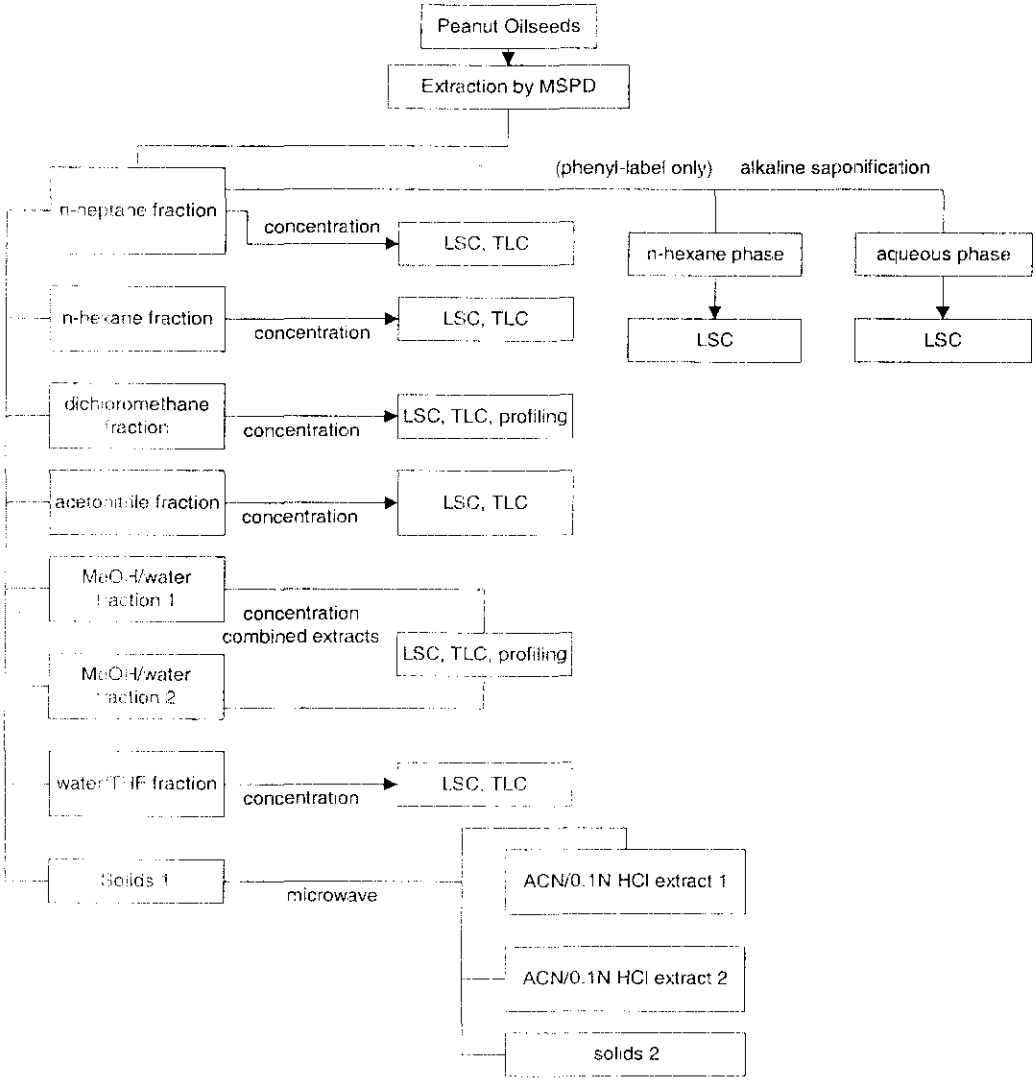

Solids 1
Combustion, LSC
Extraction 3X acetonitrile/water 80/20; Turrax
Combined ACN/water extracts
LSC, profiling
Solids 2
Extraction (3X dichloromethane)
Dichloromethane phase
Aqueous phase 3
LSC, TLC
concentrated
concentrated
LSC, profiling
Redissolved in acetonitrile
Redissolved in dichloromethane
hydrolysis with diastase
Buffer phase
Solids 3
LSC, TLC
LSC, TLC
extraction (3X ethyl acetate)
Organic phase
Aqueous phase 4
LSC, TLC
LSC, TLC
acid hydrolysis with 5N HCl
5N HCl phase
Solids 4

FIGURE B.4.1.2 Extraction Procedure for Nutmeat (MSPD extraction)



B.4.2. Analytical Methodology

Total radioactive residues (TRRs) in peanut matrices were determined following extraction with ACN/water for hay or hexane for nutmeat following conventional extraction; for nutmeat subjected to MSPD, TRRs were determined by summing radioactivity in the organic eluates and the non-extractable residues. TRRs in samples from the overdose experiment were determined by combustion/LSC. Extracts and hydrolysates were radioassayed by LSC, and non-extractable residues were radioassayed by combustion/LSC. The limit of detection was reported as twice the background.

Extracts and hydrolysates of peanut matrices were subjected to TLC analysis and/or reverse or normal phase HPLC for identification and quantitation of metabolites. The applicant noted that prothioconazole reference standard and cysteine HCl were spiked to each TLC origin before applying sample solutions to minimize decomposition of prothioconazole. Radioactive areas were detected and quantitatively evaluated using bioimaging (radioluminography), and non-radioactive reference standards were visualized under UV light. Because a relatively high percentage of the TRRs in hay remained at the TLC origin following initial analysis using SS1, the radioactive zone was isolated by preparative TLC and re-chromatographed using SS2. In addition to conventional TLC analysis, peanut oil fractions from the MSPD extraction (n-heptane, n-hexane, DCM, and the hexane extract of n-heptane) were analyzed on RP-18 plates using a solvent system of acetone:ACN:chloroform (5:4:2; v:v:v); residues were detected by spraying plates with a methanol solution of molybdophosphoric acid and heating to 110°C for 15 minutes.

Phenyl-label study:

For the phenyl-label sutdy, TLC analyses were conducted using silica gel Si60 F_{254} plates and three solvent systems: DCM:methanol:25% ammonia (90:10:1; v:v:v; SS1); n-butanol:water:acetic acid (4:1:1; v:v:v; SS2); and hexane:diisopropylether:acetic acid (50:50:2; v:v:v; SS3). HPLC analyses were conducted on systems equipped with a UV detector and a flow-through radiodetector. The following column/mobile phase combinations were used: (1) C18 column (Lichrospher 100 RP 18e) with a gradient mobile phase of water and ACN, each containing 0.1% acetic acid; (2) silica column (Lichrospher 100 Diol) with a gradient mobile phase of n-hexane and aqueous ethanol containing ammonia; and (3) C18 column (Hypersil RP-18) with a gradient mobile phase of 0.2 M ammonia acetate buffer and ACN containing 0.5% acetic acid (used for isolation and purification of metabolites from the aqueous phases).

Metabolites were identified (TABLE C.3.1) by co-chromatography and/or retention time comparisons with reference standards or by comparison of metabolite patterns between like extracts of different matrices. Chemical names and structures for the reference standards are presented in Appendix I. The applicant additionally generated methylated reference standards of [^{14}C]-oleic acid and [^{14}C]-linoleic acid by subjecting the compounds to methylation with diazomethane as described above for the saponified hexane extract of nutmeat.

Individual metabolites were isolated from the organic and aqueous phases of peanut hay using solid phase extraction (SPE) columns and/or micropreparative HPLC using the systems described above. Metabolites were isolated from the DCM phase of peanut hay by micropreparative HPLC using systems 1 and 2 described above. Metabolites in the aqueous phase of peanut hay were isolated by applying to a C18 SPE column; nine separate fractions were sequentially eluted with a stepwise gradient of ACN and water, each containing 0.1% acetic acid. Fractions containing radioactivity were subjected to micropreparative HPLC which resulted in the isolation of four major peaks. These were subjected to HPLC chromatography using system 3 described above, and three of the purified metabolites were reserved for structure elucidation. Isolated metabolites from the DCM phase of peanut hay were analyzed by LC-MS, LC-MS/MS, and/or ^{1}H NMR for structure elucidation/identification.

LC-MS, LC-MS/MS, and in some cases ^{1}H NMR analysis were used for metabolite confirmation or for identification of metabolites which could not be identified by TLC and HPLC. LC-MS and LC-MS/MS analyses were conducted using a reverse-phase C8 column, gradient mobile phases of water and ACN, each containing 0.1% formic acid or water containing 1% acetic acid and ACN, and MS or MS/MS detection with positive or negative electro-spray ionization.

Selected isolates/extracts were subjected to acid and/or alkaline hydrolysis with 5N HCl at pH 1 or 1N NaOH at 100°C for 16 hours. The resulting hydrolysates were partitioned with DCM and the DCM phases were analyzed by HPLC or TLC. To further characterize residues in the aqueous phase of hay, one fraction resulting from the initial SPE purification was further purified by partitioning three times with butanol, then subjected to acid hydrolysis.

Triazole-label study:

For the triazole-label sutdy, TLC analyses were conducted using silica gel Si60 F_{254} plates and three solvent systems: DCM:methanol:25% ammonia (90:10:1; v:v:v; SS1); n-butanol:water:acetic acid (4:1:1; v:v:v; SS2); and 2-propanol:25% ammonia:water (70:25:5, v:v:v SS3). HPLC analyses were conducted on systems equipped with a photodiode array or variable wavelength UV detector (210 and/or 254 nm) and a flow-through radiodetector. The following column/mobile phase combinations were used: (1) C18 column (Lichrospher 100 RP 18e) with a gradient mobile phase of water and ACN, each containing 0.1% acetic acid (used for metabolite profile in organic phases); (2) C18 column (Phenomenex Aqua C18) with a gradient mobile phase of water and ACN, each containing 2.5% formic acid (used for isolation of metabolites from the aqueous phase of hay and for co-chromatography); (3) silica column (Lichrospher 100 Diol) with a gradient mobile phase of n-hexane and aqueous ethanol containing ammonia (used for further purification of metabolites from the aqueous phase of hay and the cell culture experiment); (4) graphite column (Hypercarb) with a gradient mobile phase of water/ammonia acetate buffer and ACN, each containing up to 0.2% formic acid (used for isolation of metabolites derived from 1*H*-1,2,4-triazole in the cell culture experiment and for co-chromatography of the aqueous phases of hay and extracts of nutmeat with cell culture metabolites); and (5) C8 column (Alltech Econosil C8) with a gradient mobile phase of water and methanol, each containing 0.005 M tetra-n-butyl ammonium hydrogen sulfate (used for

further purification of triazolyl metabolites and for co-chromatography of the aqueous phases of hay and the extracts of nutmeat).

Metabolites were identified by co-chromatography and/or retention time comparisons with reference standards or by comparison of metabolite patterns between like extracts of different matrices. Chemical names and structures for the reference standards are presented in Appendix I.B. The petitioner additionally generated methylated reference standards of [^{14}C]-oleic acid and [^{14}C]-linoleic acid by subjecting the compounds to methylation with diazomethane as described above for the saponified n-heptane phase of nutmeat.

LC-MS, LC-MS/MS, and in some cases ^{1}H NMR analyses were used for metabolite confirmation or for identification of metabolites which could not be identified by TLC and HPLC. LC-MS and LC-MS/MS analyses were conducted using a reverse-phase C8 or a C18 column, gradient mobile phases of water and ACN, each containing 0.1% formic acid, and MS or MS/MS detection with positive or negative electro-spray ionization.

Metabolites were isolated and purified from the cell culture experiment by semi-preparative HPLC using systems 3 and 4 described above and were identified by HPLC-MS/MS. Metabolites from the aqueous phase of hay were isolated by micropreparative HPLC using system 2 described above. Each HPLC run was fractionated into 24 regions, and corresponding regions were combined to yield eight major fractions which were analyzed using HPLC system 3. This procedure yielded 16 purified metabolite fractions, 14 of which were concentrated and used for structure elucidation by LC-MS and LC-MS/MS. The cellulase hydrolysate from the aqueous phase of hay was analyzed by HPLC using system 1, then fractionated using system 2. The resulting regions were incubated with cellulase (in acetate buffer, pH 5, at 40°C for 24 hours), and enzyme hydrolysis was repeated for three of the resulting metabolites. These hydrolysates were analyzed using HPLC system 2.

C. RESULTS AND DISCUSSION

Phenyl-label study:

Total radioactive residues (TRRs) in peanut matrices are reported in TABLE C.2.1 and FIGURE C.2.1. TRRs were determined by summing extractable and non-extractable radioactivity. TRRs were 107.51 ppm in hay and 0.30 ppm in nutmeat.

The distribution of the radioactivity in peanut matrices is presented in TABLES C.2.2.1 (hay) and C.2.2.2 (nutmeat). Solvent extraction with acetonitrile/water released the majority of the TRRs (77.5%) in hay. Peanut nutmeat was subjected to two separate extraction procedures. Reflux with n-hexane followed by extraction with acetonitrile/water released 66.7% of the TRRs from one subsample of nutmeat, and MSPD extraction with n-heptane, n-hexane, dichloromethane, acetonitrile, methanol/water and water/tetrahydrofuran sequentially released a total of 74.3% of the TRRs in a second subsample. Cysteine HCl was added to all extracting solvents and to extracts during concentration procedures to prevent oxidative decomposition of

prothioconazole. Additional radioactivity was released from peanut matrices by: (1) accelerated solvent extraction and acid hydrolysis with dioxane/HCl for hay; (2) enzyme hydrolysis with diastase and acid hydrolysis with 5N HCl for nutmeat following n-hexane reflux; and (3) microwave extraction with acetonitrile/water for nutmeat following MSPD extraction. Residues were identified and quantified primarily by TLC and/or HPLC co-chromatography with confirmatory analysis and structure elucidation by LC-MS, LC-MS/MS, and/or ^{1}H NMR spectroscopy. These methods successfully identified the predominant residues in peanut hay and nutmeat.

The characterization and identification of residues in peanut matrices are summarized in TABLE C.2.3. Approximately 74% of the TRRs were identified in peanut hay, and 65% of the TRRs were identified in nutmeat. The metabolite profiles differed significantly between peanut hay and nutmeat. Prothioconazole was identified at 1.8% of the TRRs (1.98 ppm) in hay. Metabolite JAU6476-desthio was the major identified residue in hay, accounting for 28.2% of the TRRs (30.37 ppm). Glucosides of JAU6476-hydroxy-desthio isomers accounted for 14.1% of the TRRs (15.09 ppm) in hay. All remaining metabolites were identified at <10% of the TRRs. JAU6476-3-OH-desthio and JAU6476-4-OH-desthio together accounted for 9.6% of the TRRs (10.31 ppm). Two sulfonic acid metabolites, JAU6476-dihydroxydiene sulfonic acid and JAU6476-dihydroxyolefin sulfonic acid, accounted for 7.4% of the TRRs (7.89 ppm). Glucoside conjugates of the JAU6476-OH-desthio isomers were tentatively identified at 0.9% of the TRRs (0.97 ppm). JAU6476-desthio-hydroxydienyl-cysteine was identified at 5.2% of the TRRs (5.59 ppm), JAU6476-triazolinone and JAU6476 sulfonic acid were identified at ≤2.1% of the TRRs (<2.3 ppm) each, and JAU6476-disulfide was tentatively identified at 3.2% of the TRRs (3.48 ppm).

Neither prothioconazole nor JAU6476-desthio were identified in nutmeat. The majority of the TRRs (42.6-47.8% of the TRRs; 0.13-0.14 ppm) was found to be associated with peanut oil and determined to be fatty acids, suggesting that prothioconazole may be completely metabolized to CO_2 in plants. Identified metabolites in nutmeat included glucosides of JAU6476-hydroxy-desthio isomers at up to 12.2% of the TRRs (0.04 ppm), JAU6476-desthio-hydroxydienyl-cysteine at up to 9.0% of the TRRs (0.04 ppm), JAU6476-OH-desthio glucosides at up to 3.4% of the TRRs (0.01 ppm), and JAU6476 sulfonic acid at 1.5% of the TRRs (<0.01 ppm).

Remaining radioactivity in peanut matrices was characterized as: (1) radioactivity remaining at the TLC origins (1.4-8.3% of the TRRs; 0.02-8.87 ppm); (2) unassigned or diffuse radioactivity (4.3% of the TRRs; 4.64 ppm, hay only); and (3) discrete unknowns (1.7-6.8% of the TRRs; <0.01-5.15 ppm). In nutmeat, 17% of the TRRs (0.05 ppm) was characterized based on diastase hydrolysis, and 8% of the TRRs (0.02 ppm) was characterized based on acid hydrolysis (5N HCl). Non-extractable residues remaining following extraction/hydrolysis accounted for 6.3% of the TRRs (6.73 ppm) of the TRRs in hay and 8.3-12.8% of the TRRs (0.03-0.04 ppm) in nutmeat. Accountabilities ranged from 100-124%.

Identification of prothioconazole in peanut hay was confirmed by TLC and HPLC co-chromatography. Identification of metabolites JAU6476-desthio, JAU6476-4-OH-desthio,

JAU6476-dihydroxydiene sulfonic acid, JAU6476-dihydroxy-olefin sulfonic acid, and JAU6476-desthio-OH-dienyl-cysteine was confirmed by LC-MS and LC-MS/MS. Identification of JAU6476-triazolinone, JAU6476 sulfonic acid, JAU6476-3-OH-desthio, and JAU6476-desthio-dihydroxy-olefin glucosides was confirmed by LC-MS, LC-MS/MS, and ^{1}H NMR. Metabolite JAU6476-disulfide was tentatively identified by HPLC retention time comparisons with the reference standard and by comparison of TLC results with those of the phenyl wheat metabolism study, and the JAU6476-OH-desthio glucoside metabolites were tentatively identified by comparison of TLC results with those of the wheat metabolism study.

Acid hydrolysis of metabolites isolated from the aqueous phase of hay by SPE released six aglycones corresponding to JAU6476-desthio, JAU6476-3-OH- and JAU6476-4-OH-desthio isomers, as well as JAU6476-α-OH-desthio and traces of benzylpropyldiol. These results indicate the presence of conjugates of these compounds in the aqueous phase of hay. Acidic hydrolysis of the methanol/water fractions from the MSPD extraction of nutmeat released the same six aglycones.

Residues in the n-hexane reflux extract and the n-heptane, hexane, and DCM phases from the MSPD extraction of nutmeat were characterized as fatty acids based on the results of alkaline saponification and subsequent methylation with diazomethane and co-chromatography with methylated oleic and linoleic acid reference standards.

Triazole-label study:

Total radioactive residues (TRRs) in peanut matrices are reported in TABLE C.2.1 and FIGURE C.2.1. TRRs were 47.38 ppm in hay and 1.40 ppm in nutmeat. The distribution of the radioactivity in peanut matrices is presented in TABLES C.2.2.3 (nutmeat) and C.2.2.4 (hay). Combined acetonitrile/water and ASE released a total of 89.6% of the TRRs (42.33 ppm) in peanut hay. In nutmeat, approximately 77% of the TRRs (1.08 ppm) were extracted with acetonitrile, methanol/water, and water/tetrahydrofuran. Cysteine HCl was added to all extracting solvents and to extracts during concentration procedures to prevent oxidative decomposition of prothioconazole. Additional radioactivity was released from peanut matrices by ASE with ACN/water and acid hydrolysis with dioxane/HCl for hay, and by microwave extraction with ACN/0.1N HCl for nutmeat. Non-extractable residues remaining following extraction/hydrolysis accounted for 5.4% of the TRRs (2.55 ppm) in hay and 1.9% of the TRRs (0.03 ppm) in nutmeat. Residues were identified and quantitated primarily by TLC and/or HPLC co-chromatography with confirmatory analysis and structure elucidation by LC-MS, LC-MS/MS, and/or ^{1}H NMR spectroscopy.

The characterization and identification of residues in peanut matrices are summarized in TABLE C.2.3.1. Approximately 80-85% of the TRRs were identified in peanut matrices. The metabolite profiles differed significantly between peanut hay and nutmeat. Prothioconazole was identified at 6.6% of the TRRs (3.11 ppm) in hay. Metabolite JAU6476-desthio was the major identified residue in hay, accounting for 23.6% of the TRRs (11.15 ppm). All remaining metabolites were identified at <10% of the TRRs (≤3.13 ppm), and included JAU6476-3-OH-desthio; JAU6476-4-

OH-desthio; JAU6476-triazolinone; JAU6476 sulfonic acid; JAU6476-desthio-phenyl-cysteine; JAU6476-disulfide (tentatively identified); glucoside conjugates of JAU6476-3-OH-desthio, JAU6476-4-OH-desthio, and unresolved JAU6476-OH-desthio isomers; JAU6476-malonyl glucoside isomers; JAU6476-desthio-dihydroxyolefin-glucoside; JAU6476-desthio-dihydroxydiene-glucoside; JAU6476-dihydroxydiene sulfonic acid; and JAU6476-dihydroxyolefin sulfonic acid. Triazolyl metabolites, including triazolylalanine (TA), triazolylacetic acid (TAA), triazolylhydroxypropionic acid (THPA), JAU6476-triazolyl-ethanol, and JAU6476-triazolyl-ethanol-glucoside were minor components in peanut hay, each accounting for ≤ 1.5% of the TRRs (≤0.71 ppm).

Prothioconazole was not identified in nutmeat, however JAU6476-desthio was identified at 6.2% of the TRRs (0.09 ppm). Triazolyl metabolites were the major identified residues in nutmeat, with TA accounting for 49.8% of the TRRs (0.70 ppm) and THPA accounting for 24.7% of the TRRs (0.35 ppm). TAA was identified at 1.2% of the TRRs (0.02 ppm). Radioactivity determined as fatty acids in peanut oil accounted for 3.0% of the TRRs (0.05 ppm) in nutmeat.

Remaining radioactivity in peanut matrices was characterized as radioactivity remaining at the TLC origins (<5% of the TRRs (≤2.2 ppm) in hay; and 0.4% of the TRRs (0.01 ppm) in nutmeat). In nutmeat, 3.1% of the TRRs (0.04 ppm) was characterized based on microwave extraction with acetonitrile/0.1N HCl. Multicomponents of unknowns were characterized at a maximum of 10.4% of the TRRs (4.92 ppm) in peanut hay and 9.59% of the TRRs (0.14 ppm) in peanut nutmeat. Accountabilites ranged from 100-102%.

Prothioconazole, JAU6476-desthio, JAU6476-3-OH-desthio, JAU6476-4-OH-desthio, JAU6476-triazolinone, and JAU6476 sulfonic acid were identified by TLC and HPLC co-chromatography with reference standards. JAU6476-disulfide was tentatively identified in the DCM phase of hay by TLC co-chromatography. JAU6476-dihydroxyolefin sulfonic acid and JAU6476-dihydroxydiene sulfonic acid were identified by TLC R_f value comparison with metabolites isolated from the phenyl-label study peanut study; identification of these metabolites had been confirmed in the previous study by LC-MS and LC-MS/MS. The JAU6476-desthio-dihydroxy-diene and JAU6476-desthio-dihydroxyolefin glucoside metabolites and the malonic acid glucosides of JAU6476-OH-desthio isomers were tentatively identified by LC-MS and LC-MS/MS; the position of the hydroxy groups could not be determined. LC-MS and LC-MS/MS analysis also indicated the presence of a metabolite identified as either glucosylated JAU6476-desthio-dihydroxy or an S-glucoside of JAU6476-desthio that eluted in the same HPLC region as JAU6476-desthio-dihydroxy-diene glucoside; this metabolite was not quantitated. JAU6476-desthio-phenyl-cysteine was identified in the aqueous phase of hay by LC-MS and LC-MS/MS. The applicant noted that a similar metabolite, JAU6476-desthio-hydroxy-dienyl-cysteine, was identified in the phenyl-label study peanut study. Glucosides of JAU6476-OH-desthio were tentatively identified by LC-MS and LC-MS/MS; although the position of hydroxylation could not be determined, for at least one of the metabolites, hydroxylation at the 3- and 6-positions was excluded by ^{1}H NMR analysis. These metabolites were further characterized on the basis of TLC co-chromatography with a JAU6476-OH-desthio glucoside isolated from the phenyl-label study wheat metabolism study.

TA and THPA were isolated and purified from the cell culture experiment and further identified by HPLC-MS/MS and confirmed by ^{1}H NMR spectroscopy. These purified metabolites were used for identification of the metabolites in nutmeat and hay (THPA only) via HPLC co-chromatography on two systems. Identification of TAA in hay was confirmed by co-chromatography with the radiolabeled standard. JAU6476-triazolyl-ethanol was identified in the aqueous phase of hay by co-chromatography with the purified metabolite which had been isolated and identified from a hen metabolism study. The JAU6476-triazolyl-ethanol-glucoside metabolite was identified by LC-MS and LC-MS/MS.

Enzyme hydrolysis with cellulase released at least nine aglycones in the aqueous phase of peanut hay, the majority of which were characterized as JAU6476-OH-desthio isomers based on co-chromatography; JAU6476-triazolyl-ethanol and JAU6476-desthio were also identified in the hydrolysate.

Residues in the n-heptane, n-hexane, and DCM phases of nutmeat were characterized as fatty acids based on the results of alkaline saponification and subsequent methylation with diazomethane and co-chromatography with methylated oleic and linoleic acid.

Results for peanut hay in the triazole-label study were similar to the results in the corresponding phenyl-label peanut study, with the same major metabolites identified and comprising about the same relative percentages of TRRs. Detection of malonyl glucoside metabolites in the triazole-label study was attributed to refinements in the analytical methods and processing procedures. For nutmeat, triazolyl metabolites were major components of the residue, whereas distribution into peanut oil represented the major allocation of the residues in the phenyl-label study study.

C.1. Storage Stability

The storage intervals for peanut samples are presented in TABLE C.1. Processed peanut samples for both sutdies were stored frozen at ≤-18°C. Extracts were stored either refrigerated (4°C) or frozen (-20°C) for longer periods. Dates of sample extraction and analysis were not provided, however, the applicant stated that initial extracts were profiled by TLC within 17 days (phenyl) and 91 days (triazole) of collection for peanut hay and 51 days (phenyl-label study) and 99 days (triazole-label study) for nutmeat. For the phenyl-label study, the applicant also noted that MSPD extraction of nutmeat was conducted 355 days after harvest and yielded a metabolite profile similar to the initial n-hexane reflux extraction of nutmeat. In the case of the triazole-label study, the applicant also stated that HPLC analyses of the aqueous phase of peanut hay and the polar fractions of nutmeat were repeated over the course of the experimental phase of the study, with final HPLC analysis conducted 475 days (15.6 months after harvest), and that no significant changes in the metabolite profile were observed. Representative chromatograms of the aqueous phase of peanut hay reflecting analysis at 131 and 344 days (4.3 and 11.3 months) after harvest confirmed that the metabolite profile was essentially unchanged during this interval. No additional data was considered necessary to support the sample storage conditions and intervals of the submitted studies.

TABLE C.1. Summary of Storage Conditions.				
Matrix	Storage Temp.	Phenyl-label Actual Storage Duration [1] (Days)	Triazole-label Actual Storage Duration [1] (Days)	Interval of Demonstrated Storage Stability (Days)
Hay	≤-18°C	17	91	up to 344 days for aqueous phase extract
Nutmeat		51 355 (MSPD)	99	355

[1] Interval from extraction to initial profiling.

C.2. Identification, Characterization, and Distribution of Residues

TABLE C.2.1. Total Radioactive Residues (TRRs) in Peanut Matrices.					
Matrices	Timing and application	PHI (days)	[Phenyl-^{14}C]-Prothioconazole, ppm [1]		[Triazole-^{14}C]-Prothioconazole, ppm [1]
			Normal rate	5-fold rate	Normal rate
Hay	3 foliar applications at 20-22 days interval	21 (Phenyl) 14 (Triazole)	107.51	296.37	47.38
Nutmeat - hexane reflux			0.3	0.39	1.4
Nutmeat - MSPD extraction			0.29		

[1] TRRs in normal rate samples were determined by summing extractable and non-extractable radioactivity; TRRs in high-rate samples were determined by combustion.

FIGURE C.2.1. Distribution of TRRs in peanut hay and nutmeat.

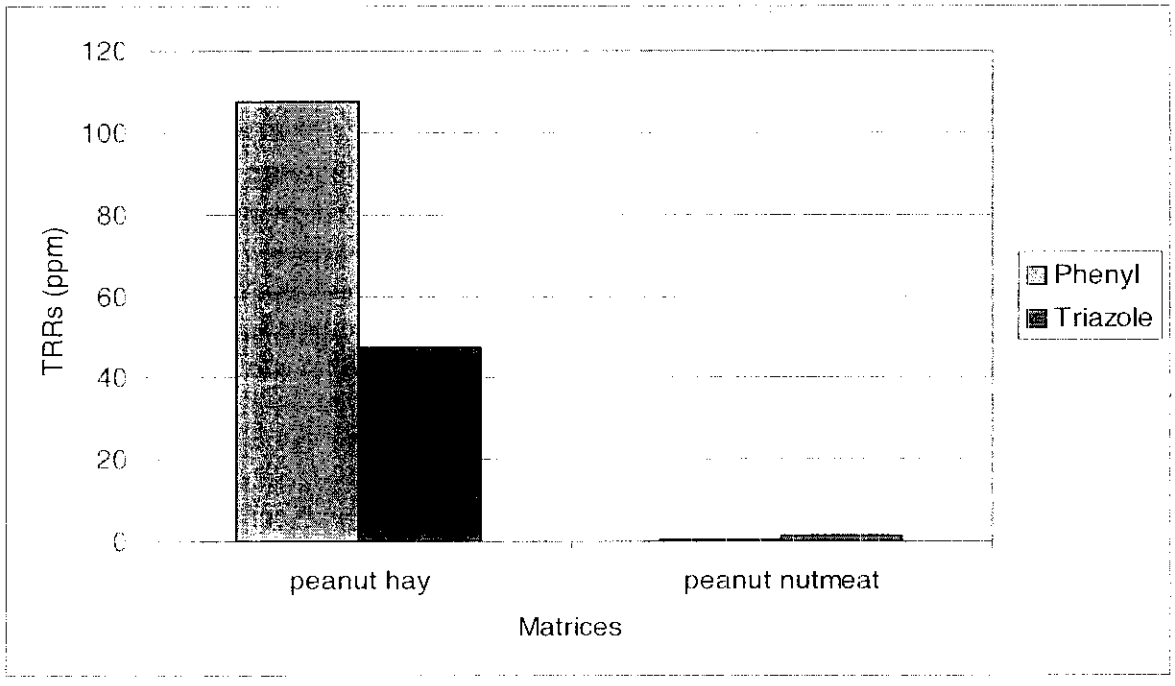


TABLE C.2.2.1. Distribution of the Parent and the Metabolites in Peanut Hay Following Foliar Application of [Phenyl-UL-^{14}C]-Prothioconazole at 0.800 lb a.i./A (897 g a.i./ha).

Metabolite Fraction	Hay	
	TRR = 107.51 ppm	
	%TRR	ppm
ACN/water	77.5	83.34
Combined ACN/water + ASE	83.5	89.76
DCM	51.2	55.06
Prothioconazole	1.6	1.74
JAU6476-desthio	27.8	29.96
JAU6476-3-OH-desthio	7.3	7.81
JAU6476-4-OH-desthio	2	2.19
JAU6476-triazolinone	1.5	1.66
JAU6476 sulfonic acid	1.4	1.51
JAU6476 disulfide [1]	2.6	2.81
JAU6476-dihydroxydiene sulfonic acid/JAU6476-dihydroxyolefin sulfonic acid	2.5	2.65
JAU6476-desthio-hydroxydienyl-cysteine	1	1.03
JAU6476-desthio-dihydroxyolefin glucosides [1]	1.5	1.6
Unknowns [2]	1.1	1.17
TLC origin	0.2	0.21
Unassigned	0.7	0.77
Aqueous	32.3	34.7
JAU6476 sulfonic acid	0.7	0.77
JAU6476-dihydroxydiene sulfonic acid/JAU6476-dihydroxyolefin sulfonic acid	4.9	5.24
JAU6476-desthio-hydroxydienyl-cysteine	4.2	4.56
JAU6476-desthio-dihydroxyolefin glucosides [1]	12.6	13.49
JAU6476-OH-desthio glucosides [1]	0.9	0.97
Unknowns [3]	3.7	3.98
TLC origin	2	2.11
Unassigned [4]	3.3	3.59

TABLE C.2.2.1. Distribution of the Parent and the Metabolites in Peanut Hay Following Foliar Application of [Phenyl-UL-^{14}C]-Prothioconazole at 0.800 lb a.i./A (897 g a.i./ha).

Metabolite Fraction	Hay	
	TRR = 107.51 ppm	
	%TRR	ppm
Non-extractable	22.5	24.17
ACN/water ASE 50°C [5]	2.7	2.91
ACN/water ASE 100°C [5]	3.3	3.51
Non-extractable	16.5	17.75
Dioxane/HCl	8	8.58
Prothioconazole	0.2	0.24
JAU 6476-desthio	0.4	0.41
JAU6476-3-OH-desthio	0.3	0.31
JAU6476-4-OH-desthio		
JAU6476-triazolinone	0.1	0.12
JAU6476-disulfide [1]	0.6	0.67
Unassigned	0.3	0.28
TLC origin	6.1	6.55
Aqueous	2.3	2.43
Non-extractable	6.3	6.73

[1] Tentative identification.
[2] Consisting of 2 components, each ≤0.8% of the TRRs (≤0.86 ppm).
[3] Consisting of 2 components, each ≤2.8% of the TRRs (≤3.03 ppm).
[4] Consisting of 2 components, each ≤2.2% of the TRRs (≤2.39 ppm).
[5] Combined with initial ACN/water extract.

TABLE C.2.2.2. Distribution of the Parent and the Metabolites in Peanut Nutmeat Following Foliar Application of [Phenyl-UL-^{14}C]-Prothioconazole at 0.800 lb a.i./A (897 g a.i./ha). [1]

Metabolite Fraction	Nutmeat			
	Hexane reflux		MSPD	
	TRR = 0.30 ppm		TRR = 0.29 ppm	
	%TRR	ppm	%TRR	ppm
n-Hexane	42.6 [2]	0.13		
Hexane 1	41.4	0.13		
Hexane 2 (saponified)	38.8	0.12		
Aqueous 1	NR	NR		
Hexane 3	0.2	<0.01		
Aqueous 2	2.4	0.01		
ACN	1.2	<0.01		
n-Heptane			43.9 [2]	0.13
n-Hexane			0.8 [2]	<0.01
DCM			3.1 [2]	0.01
ACN			0.8	<0.01
Methanol/water			23.4	0.07
JAU6476-desthio-hydroxydienyl-cysteine			8.5	0.03
JAU6476-desthio-dihydroxyolefin glucosides [3]			6.5	0.02
JAU6476-OH-desthio glucosides [3]			1	<0.01
Unknowns [4]			6.5	0.02
TLC origin			0.9	<0.01
Water/tetrahydrofuran			2.3	0.01
JAU6476-desthio-hydroxydienyl-cysteine			0.5	<0.01
JAU6476-desthio-dihydroxyolefin glucosides [3]			1.1	<0.01
Unknown			0.2	<0.01
TLC origin			0.5	<0.01

TABLE C.2.2.2. Distribution of the Parent and the Metabolites in Peanut Nutmeat Following Foliar Application of [Phenyl-UL-^{14}C]-Prothioconazole at 0.800 lb a.i./A (897 g a.i./ha). [1]

Metabolite Fraction	Nutmeat			
	Hexane reflux		MSPD	
	TRR = 0.30 ppm		TRR = 0.29 ppm	
	%TRR	ppm	%TRR	ppm
Non-extractable	57.4	0.17	25.7	0.07
ACN/water	24.1	0.07		
JAU6476 sulfonic acid	1.5	<0.01		
JAU6476-desthio-hydroxydienyl-cysteine	5.4	0.02		
JAU6476-desthio-dihydroxyolefin glucosides [3]	12.2	0.04		
JAU6476-OH-desthio glucosides [3]	3.4	0.01		
Unknown	1.7	<0.01		
DCM	7.1	0.02		
Aqueous	17	0.05		
Microwave extracts			5.8	0.02
Non-extractable	33.3	0.1	19.9	0.05
Diastase hydrolysate	17	0.05		
Microwave extracts			7.1	0.02
Non-extractable	16.3	0.05	12.8	0.04
5N HCl	8	0.02		
Non-extractable	8.3	0.03		

[1] Shading indicates that the extraction step and/or characterization analysis was not conducted for the matrix in question. NR = Not reported.
[2] This fraction was characterized as peanut oil fatty acids.
[3] Tentative identification.
[4] Consisting of 2 components, each 3.3% of the TRRs (0.01 ppm).

TABLE C.2.2.3 Distribution of the Parent and the Metabolites in Peanut Nutmeat Following Foliar Application of [Triazole-UL-^{14}C]-Prothioconazole at 0.799 lb a.i./A (895 g a.i./ha).

Metabolite Fraction	Nutmeat	
	TRR = 1.40	
	%TRR	ppm
n-Heptane (peanut oil) [1]	1.2	0.02
n-Heptane (saponified)	1	0.02
Aqueous	0.2	<0.01
n-Hexane (peanut oil) [1]	0.2	<0.01
DCM (peanut oil) [1]	1.6	0.02
ACN [2]	4.5	0.06
Triazolylacetic acid (TAA) [3]	1.2	0.02
Triazolylhydroxypropionic acid (THPA) [3]	0.2	<0.01
Triazolyl unknown	3	0.04
Unknown	0.1	<0.01
Methanol/water [4]	69.1	0.97
JAU6476-desthio	6.2	0.09
Triazolylalanine (TA) [3]	42.4	0.59
Triazolylhydroxypropionic acid (THPA) [3]	14.9	0.21
Unknowns [5]	5.2	0.07
TLC origin	0.4	0.01
Water/tetrahydrofuran [2]	3.3	0.05
Triazolylalanine (TA) [3]	2	0.03
Triazolyl Unknowns [6]	1.29	0.03
Non-extractable	20	0.28
ACN/0.1 N HCl Microwave #1	15	0.21
Triazolylalanine (TA) [3]	5.4	0.08
Triazolylhydroxypropionic acid (THPA) [3]	9.6	0.13
ACN/0.1 N HCl Microwave #2	3.1	0.04
Non-extractable	1.9	0.03

[1] This fraction was characterized as peanut oil fatty acids.
[2] Residues quantitated by HPLC.
[3] Tentative identification.
[4] Residues quantitated by TLC.
[5] Consisting of 3 components, each ≤2.9%of the TRRs (≤0.04 ppm).
[6] Consisting of 2 components, each ≤1.2% of the TRRs (≤0.02 ppm).

TABLE C.2.2.4 Distribution of the Parent and the Metabolites in Peanut Hay Following Foliar Application of [Triazole-UL-^{14}C]-Prothioconazole at 0.799 lb a.i./A (895 g a.i./ha).

Metabolite Fraction	Hay	
	TRR = 47.38 ppm	
	%TRR	ppm
ACN/water	85	40.15
Combined ACN/water + ASE	89.6	42.33
DCM [1]	55.5	26.24
Prothioconazole	2.4	1.13
JAU6476-desthio	22.8	10.79
JAU6476-3-OH-desthio	5.9	2.8
JAU6476-4-OH-desthio	3	1.4
JAU6476-triazolinone	3.5	1.63
JAU6476 sulfonic acid	2.1	1
JAU6476-disulfide [2]	5.2	2.46
Polar unknowns [3]	2.7	1.3
TLC Origin	7.9	3.73
Prothioconazole [2]	4	1.89
Unknowns [4]	3.9	1.84
Aqueous [5]	34.1	16.09
JAU6476-desthio	0.6	0.29
JAU6476-3-OH-desthio	0.7	0.33
JAU6476 sulfonic acid	0.6	0.26
JAU6476-desthio-phenyl-cysteine	1.7	0.78
Triazolylalanine (TA)	1.2	0.56
Triazolylacetic acid (TAA)	0.7	0.33
Triazolylhydroxypropionic acid (THPA)	0.6	0.3
JAU6476-triazolyl-ethanol [2]	0.5	0.26
JAU6476-triazolyl-ethanol-glucoside	1.5	0.71
JAU6476-3-OH-desthio glucoside [2]	1.5	0.73
JAU6476-4-OH-desthio glucoside [2]	5.4	2.57
JAU6476-OH-desthio glucoside isomers [2, 6]	3.6	1.65
JAU6476-OH-desthio-malonyl glucoside isomers [2,7]	6	2.82
JAU6476-dihydroxyolefin sulfonic acid [2]	1	0.47
JAU6476-dihydroxydiene sulfonic acid [2]	0.5	0.25
JAU6476-desthio-dihydroxyolefin-glucoside [2]	2.4	1.13
JAU6476-desthio-dihydroxydiene-glucoside [2]	1.8	0.87
Unknowns [8]	3.8	1.78

TABLE C.2.2.4 Distribution of the Parent and the Metabolites in Peanut Hay Following Foliar Application of [Triazole-UL-^{14}C]-Prothioconazole at 0.799 lb a.i./A (895 g a.i./ha).

Metabolite Fraction	Hay	
	TRR = 47.38 ppm	
	%TRR	ppm
Non-extractable	15.2	7.22
ACN/water ASE [9]	4.6	2.18
Non-extractable	10.6	5.04
Dioxane/HCl	5.2	2.49
Prothioconazole	0.2	0.09
JAU6476-desthio	0.2	0.07
JAU6476-triazolinone	0.1	0.03
JAU6476-disulfide [2]	0.2	0.09
TLC origin	4.6	2.2
Non-extractable	5.4	2.55

[1] Residues quantitated by TLC.
[2] Tentative identification.
[3] Consisting of 2 components, each ≤1.6% of the TRRs (≤0.77 ppm).
[4] Consisting of 2 components, each ≤3.0% of the TRRs (≤1.42 ppm).
[5] Residues quantitated by HPLC.
[6] Consisting of 3 components, each ≤1.5% of the TRRs (≤0.69 ppm).
[7] Consisting of 2 components, each ≤4.1% of the TRRs (≤1.92 ppm).
[8] Consisting of 4 components, each ≤1.2% of the TRRs (≤0.56 ppm).
[9] Combined with initial ACN/water extract.

TABLE C.2.3. Summary of Characterization and Identification of Radioactive Residues in Peanut Matrices Following Foliar Application of [Phenyl-UL-^{14}C]-Prothioconazole at 0.800 lb a.i./A (897 g a.i./ha).

Compound	Hay [1]		Nutmeat			
			Hexane reflux		MSPD	
	TRR = 107.51 ppm		TRR = 0.30 ppm		TRR = 0.29 ppm	
	%TRR	ppm	%TRR	ppm	%TRR	ppm
Identified						
Prothioconazole	1.8	1.98	--	--	--	--
JAU6476-desthio	28.2	30.37	--	--	--	--
JAU6476-3-OH-desthio	7.3	7.81	--	--	--	--
JAU6476-4-OH-desthio	2	2.19	--	--	--	--
JAU6476-triazolinone	1.6	1.78	--	--	--	--
JAU6476 sulfonic acid	2.1	2.28	1.5	<0.01*	--	--
JAU6476-dihydroxydiene sulfonic acid/JAU6476-d hydroxyolefin sulfonic acid	7.4	7.89	--	--	--	--
JAU6476-desthio-hydroxydienyl-cysteine	5.2	5.59	5.4	0.02	9	0.04
Tentatively identified						
JAU6476-disulfide	3.2	3.48	--	--	--	--
JAU6476-3-OH-desthio	0.3	0.31	--	--	--	--
JAU6476-4-OH-desthio			--	--	--	--
Glucosides of JAU6476-hydroxy-desthio isomers	14.1	15.09	12.2	0.04	7.6	0.03
JAU6476-OH-desthio glucosides	0.9	0.97	3.4	0.01	1	<0.01
Peanut oil determined as fatty acids (n-hexane extract/MSPD fractions 1-3)	--	--	42.6	0.13	47.8	0.14
Characterized						
Unknowns [2]	4.8	5.15	1.7	<0.01	6.8	0.03
Aqueous	2.3	2.43	--	--	--	--
Diastase hydrolysate	--	--	17	0.05	--	--
Acid hydrolysate (5N HCl)	--	--	8	0.02	--	--
Microwave extracts	--	--	--	--	12.9	0.04
ACN	--	--	--	--	0.8	<0.01
TLC origins	8.3	8.87	--	--	1.4	0.02
Unassigned	4.3	4.64	--	--	--	--
Total identified	74.1	79.74	65.1	0.21	65.4	0.22
Total characterized	19.7	21.09	26.7	0.07	21.9	0.10
Total extractable	93.8	100.83	91.8	0.28	87.3	0.32
Unextractable (PES) [3]	6.3	6.73	8.3	0.03	12.8	0.04
Accountability [4]	100.0		103.0		124.0	

[1] The reported values for each metabolite/fraction reflect the total amount found in all extracts and hydrolysates.
[2] Refer to TABLES C.2.2.1 and C.2.2.2 for distribution.
[3] Residues remaining after exhaustive extractions.
[4] Accountability = (Total extractable + Total unextractable)/(absolute values of the TRRs (ppm)) * 100.

*Values <0.01 ppm were included in the calculations for total identified and/or total characterized as 0.01 ppm. This resulted in high accountability values (>100%).

TABLE C.2.3.1 Summary of Characterization and Identification of Radioactive Residues in Peanut Matrices Following Foliar Application of [Triazole-UL-^{14}C]-Prothioconazole at 0.800 lb a.i./A (897 g a.i./ha). [1]

Compound	Hay		Nutmeat	
	TRR = 47.38 ppm		TRR = 1.40	
	% of the TRRs	ppm	% of the TRRs	ppm
Identified				
Prothioconazole	6.6 [2]	3.11	--	--
JAU6476-desthio	23.6	11.15	6.2	0.09
JAU6476-3-OH-desthio	6.6	3.13	--	--
JAU6476-4-OH-desthio	3	1.4	--	--
JAU6476-triazolinone	3.6	1.66	--	--
JAU6476 sulfonic acid	2.7	1.26	--	--
JAU6476-desthio-phenyl-cysteine	1.7	0.78	--	--
Triazolylalanine (TA)	1.2	0.56	49.8 [2]	0.7
Triazolylacetic acid (TAA)	0.7	0.33	1.2 [3]	0.02
Triazolylhydroxypropionic acid (THPA)	0.6	0.3	24.7 [2]	0.35
JAU6476-triazolyl-ethanol-glucoside	1.5	0.71	--	--
Tentatively identified				
JAU6476-disulfide	5.4	2.55	--	--
JAU6476-triazolyl-ethanol	0.5	0.26	--	--
JAU6476-3-OH-desthio glucoside	1.5	0.73	--	--
JAU6476-4-OH-desthio glucoside	5.4	2.57	--	--
JAU6476-OH-desthio glucoside isomers	3.6	1.65	--	--
Σ: malonic acid glucosides of the OH-desthio isomers	6	2.82	--	--
Σ: isomers of dihydroxydiene/dihydroxyolefin sulfonic acid	1.5	0.72	--	--
JAU6476-desthio-dihydroxyolefin-glucoside	2.4	1.13		
JAU6476-desthio-dihydroxydiene-glucoside	1.8	0.87	--	--
Peanut oil determined as fatty acids (n-heptane, n-hexane, and DCM fractions)	--	--	3	0.05
Characterized				
Unknowns [4]	10.4	4.92	9.59	0.14
ACN/0.1N HCl microwave	--	--	3.1	0.04
TLC origin	4.6	2.2	0.4	0.01
Total identified	79.9	37.69	84.9	1.21
Total characterized	15.0	7.12	13.1	0.19
Total extractable	94.6	44.82	98.0	1.40
Unextractable (PES) [5]	5.4	2.55	1.9	0.03
Accountability [6]	100.0		102.0	

[1] The reported values for each metabolite/fraction reflect the total amount found in all extracts and hydrolysates.

[2] Includes tentatively identified material.
[3] Tentatively identified.
[4] Refer to TABLES C.2.2.2.1 and C.2.2.2.2 for relative distribution of unknowns.
[5] Residues remaining after exhaustive extractions.
[6] *Accountability = (Total extractable + Total unextractable)*/(absolute values of the TRRs (ppm)) * 100.
*Values <0.01 ppm from the distribution tables were included in the calculations for total identified and/or total characterized as 0.01 ppm. This resulted in high accountability values (>100%).

C.3. Proposed Metabolic Profile

Based on the results of the phenyl and triazole-label peanut metabolism studies, the applicant concluded that prothioconazole is initially metabolized in peanut by: (1) oxidation and loss of sulfur to form JAU6476-desthio; (2) hydroxylation of the chlorobenzyl ring of JAU6476-desthio at positions 3 and 4 to form the hydroxy desthio metabolites; (3) conjugation of the hydroxylated metabolites; (4) exchange of oxygen for sulfur; and (5) release of the triazole moiety to form triazolylalanine (TA) and triazolylhydroxypropionic acid (THPA). Free triazole (1*H*-1,2,4-triazole) was not detected in any of the peanut matrices.

FIGURE C.3.1. Proposed Metabolic Profile of Prothioconazole in Peanut

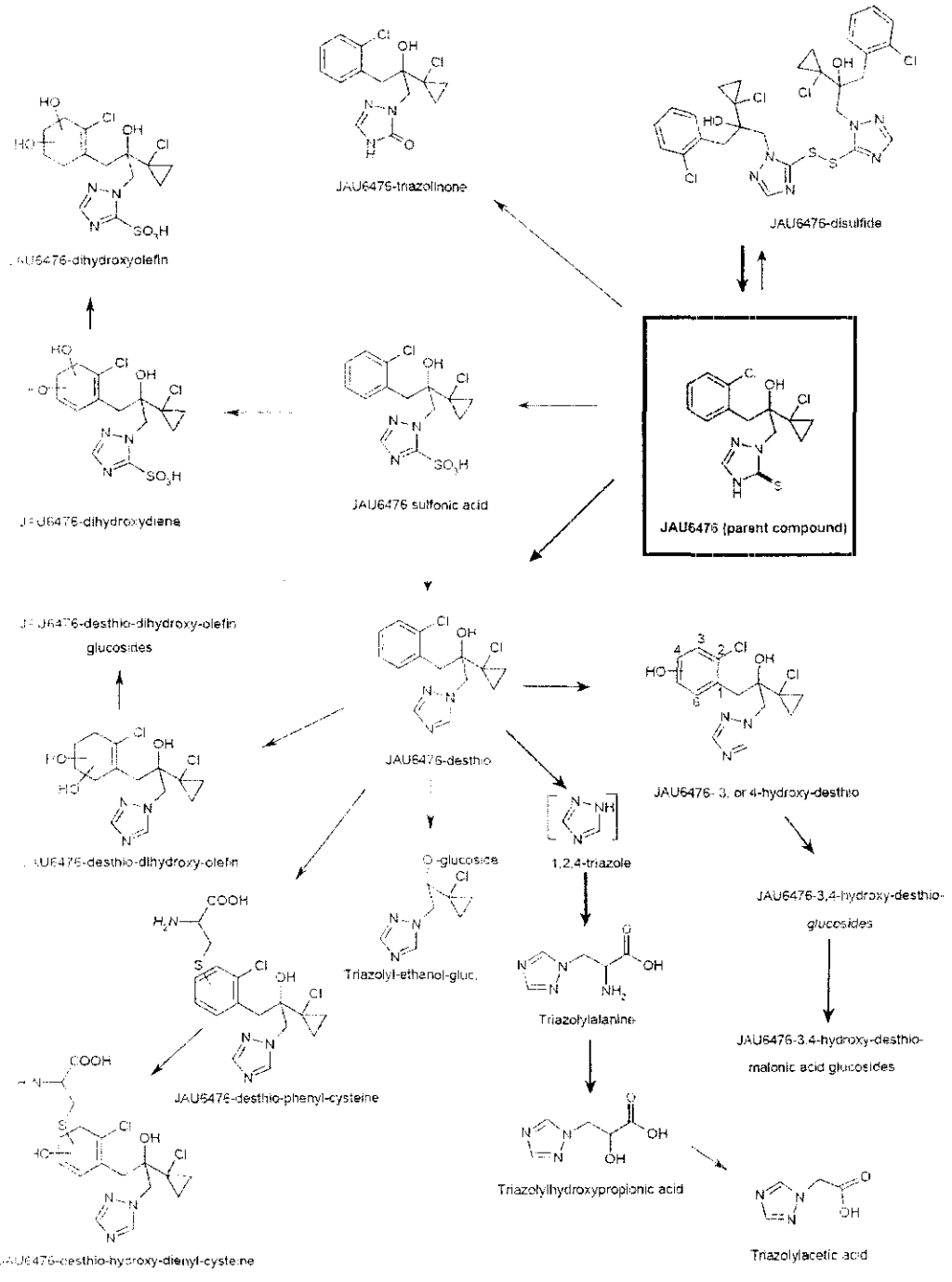


TABLE C.3.1 Identification of Compounds.

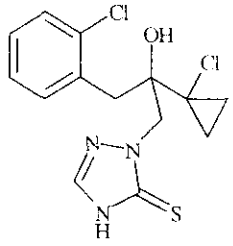
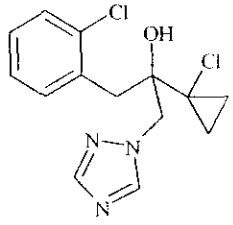
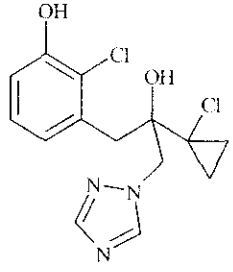
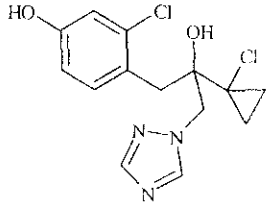
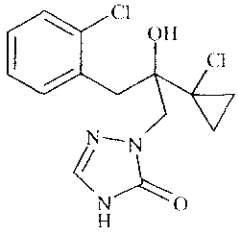
Common name/code FIGURE C.3.1 ID No.	Chemical name	Chemical structure
Prothioconazole JAU6476	2-[2-(1-chlorocyclopropyl)-3-(2-chlorophenyl)-2-hydroxypropyl]-1,2-dihydro-3*H*-1,2,4-triazole-3-thione	
JAU6476-desthio	α-(1-chlorocyclopropyl)-α-[(2-chlorophenyl)methyl]-1*H*-1,2,4-triazole-1-ethanol	
JAU6476-3-OH-desthio	α-(1-chlorocyclopropyl)-α-[(2-chloro-3-hydroxyphenyl)methyl]-1*H*-1,2,4-triazole-1-ethanol	
JAU6476-4-OH-desthio	α-(1-chlorocyclopropyl)-α-[(2-chloro-4-hydroxyphenyl)methyl]-1*H*-1,2,4-triazole-1-ethanol	
JAU6476-triazolinone	2-[2-(1-chlorocyclopropyl)-3-(2-chlorophenyl)-2-hydroxypropyl]-2,4-dihydro-3*H*-1,2,4-triazole-3-one	

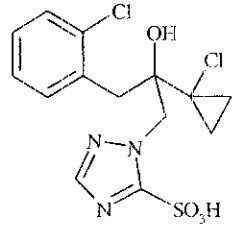
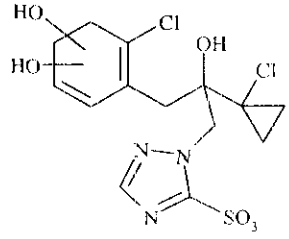
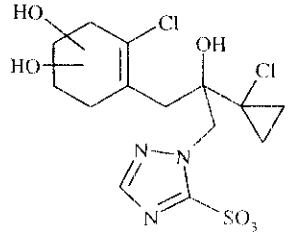
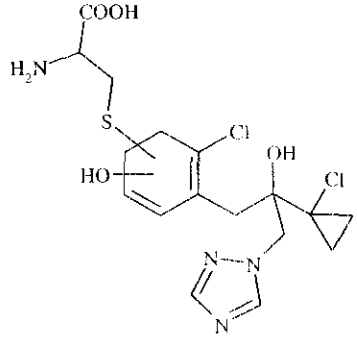
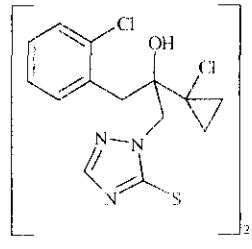
TABLE C.3.1 Identification of Compounds.		
Common name/code FIGURE C.3.1 ID No.	Chemical name	Chemical structure
JAU6476 sulfonic acid	1-[2-(1-chlorocyclopropyl)-3-(2-chlorophenyl)-2-hydroxypropyl]-1*H*-1,2,4-triazole-5-sulfonic acid	
JAU6476-dihydroxy-diene sulfonic acid		
JAU6476-dihydroxyolefin sulfonic acid		
JAU6476-desthio-hydroxy-dienyl-cysteine		
JAU6476-disulfide		

TABLE C.3.1 Identification of Compounds.

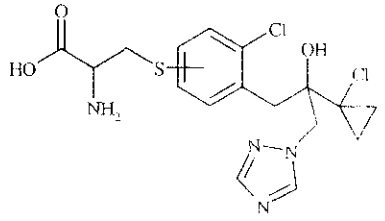
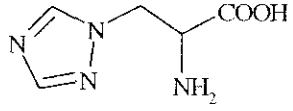
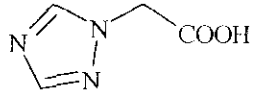
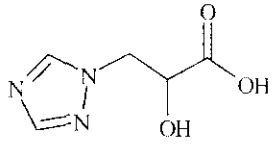
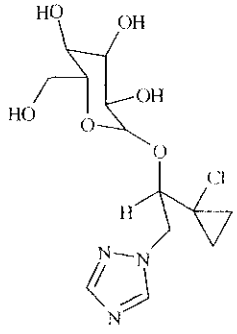
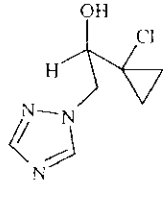
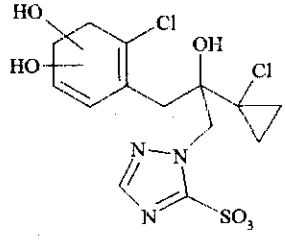
Common name/code FIGURE C.3.1 ID No.	Chemical name	Chemical structure
JAU6476-desthio-phenyl-cysteine		
Triazolylalanine (TA)	α-amino-1*H*-1,2,4-triazole-1-propanoic acid	
Triazolylacetic acid (TAA)	1*H*-1,2,4-triazole-1-acetic acid	
Triazolylhydroxypropionic acid (THPA)	α-hydroxy-1*H*-1,2,4-triazole-1-propanoic acid	
JAU6476-triazolyl-ethanol-glucoside		
JAU6476-triazolyl-ethanol	1-(1-chlorocyclopropyl)-2-(1*H*-1,2,4-triazol-1-yl)ethanol	

TABLE C.3.1 Identification of Compounds.

Common name/code FIGURE C.3.1 ID No.	Chemical name	Chemical structure
JAU6476-dihydroxy-diene sulfonic acid		

D. CONCLUSION

[Phenyl-UL-^{14}C] and [triazole-UL-^{14}C]-prothioconazole was applied three times at approximately 0.267 lb a.i./A (299 g a.i./ha) and 0.265-0.267 lb a.i./A (297-299 g a.i./ha), respectively, for a total seasonal rate of 0.799-0.800 lb a.i./A (895-897 g a.i./ha). Prothioconazole (JAU6476) was extensively metabolized in peanut plants. JAU6476-desthio was formed by oxidation and loss of sulfur. Hydroxylation of the chlorobenzyl ring at positions 3 and 4 led to hydroxy desthio metabolites followed by further conjugation. The parent compound also exchanged sulfur for oxygen resulting in JAU6476-triazolinone. Cleavage of the triazole moiety formed triazole-label specific metabolites. Free triazole (1*H*-1,2,4-triazole) was not detected in any of the peanut matrices.

The major residue found in peanut hay was JAU6476-desthio. The parent compound was less than 10% of the TRRs for both radiolabels. Minor components were isomers of JAU6476-OH-desthio and their glucosides and malonylglucosides, as well as triazolyl-label specific metabolites. In nutmeat, triazolylalanine and triazolylhydroxypropionic acid were the major residues for the triazole-label study. For the phenyl-label study, isomers of JAU6476-desthio-dihydroxy-olefin-glucosides and the sum of fatty acids were the main components. JAU6476 was not found in nutmeat for both radiolabels.

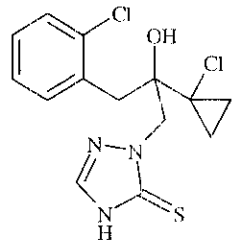
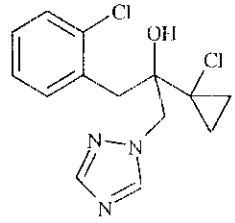
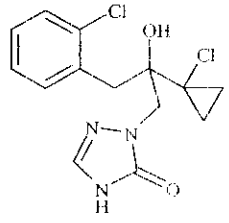
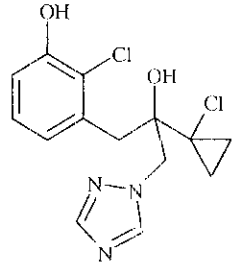
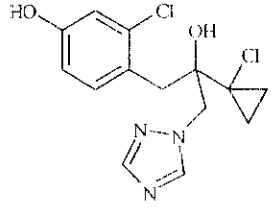
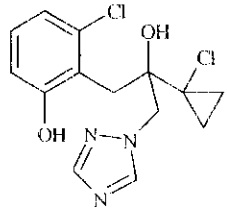
E. REFERENCES

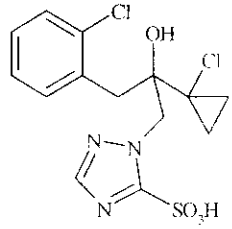
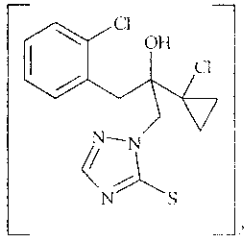
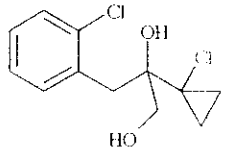
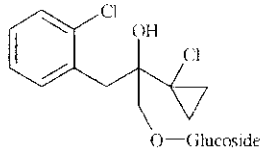
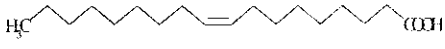
None.

F. DOCUMENT TRACKING

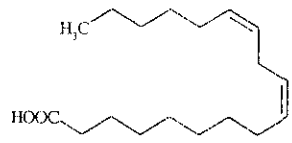
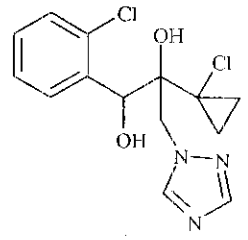
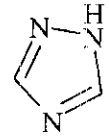
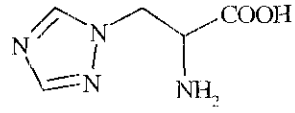
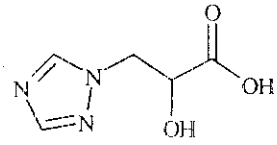
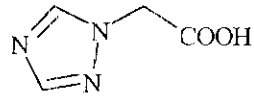
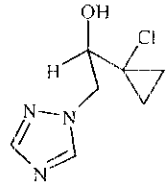
RDI: Louise Croteau, Suzan Mathew (PMRA) 23/01/2006; Henri Bietlot (PMRA) 24/01/2006; Stephen Funk (IO) 13/03/2006; Leung Cheng (RAB3).
Petition Number: PP#4F6830
DP Barcode: D303508
PC Code: 113961

Template Version September 2003

APPENDIX I Chemical Names and Structures of Reference Standards Used in the Phenyl-Label Study Peanut Metabolism Study.		
Common name/Company code	Chemical name	Chemical structure
Prothioconazole; JAU6476	2-[2-(1-chlorocyclopropyl)-3-(2-chlorophenyl)-2-hydroxypropyl]-1,2-dihydro-3*H*-1,2,4-triazole-3-thione	
JAU6476-desthio	α-(1-chlorocyclopropyl)-α-[(2-chlorophenyl)methyl]-1*H*-1,2,4-triazole-1-ethanol	
JAU6746 triazolinone	2-[2-(1-chlorocyclopropyl)-3-(2-chlorophenyl)-2-hydroxypropyl]-2,4-dihydro-3*H*-1,2,4-triazole-3-one	
JAU6476-3-hydroxy-desthio	α-(1-chlorocyclopropyl)-α-[(2-chloro-3-hydroxyphenyl)methyl]-1*H*-1,2,4-triazole-1-ethanol	
JAU6476-4-hydroxy-desthio	α-(1-chlorocyclopropyl)-α-[(2-chloro-4-hydroxyphenyl)methyl]-1*H*-1,2,4-triazole-1-ethanol	
JAU6476-6-hydroxy-desthio	α-(1-chlorocyclopropyl)-α-[(2-chloro-6-hydroxyphenyl)methyl]-1*H*-1,2,4-triazole-1-ethanol	

APPENDIX I. Chemical Names and Structures of Reference Standards Used in the Phenyl-Label Study Peanut Metabolism Study.		
Common name/Company code	Chemical name	Chemical structure
JAU6476 sulfonic acid (a radiolabeled standard was also used)	1-[2-(1-chlorocyclopropyl)-3-(2-chlorophenyl)-2-hydroxypropyl]-1*H*-1,2,4-triazole-5-sulfonic acid	
JAU6476-disulfide; dimer of prothioconazole		
JAU6476-benzylpropyldiol	2-(1-chlorocyclopropyl)-3-(2-chlorophenyl)propane-1,2-diol [1]	
Benzylpropyldiol glucoside [2]		
JAU6476-OH-desthio-glucosides (Isomers 1-3) [2]		No structure provided
Oleic acid [3]	octadeca-9-enoic acid	

APPENDIX I. Chemical Names and Structures of Reference Standards Used in the Phenyl-Label Study Peanut Metabolism Study.

Common name/Company code	Chemical name	Chemical structure
Linoleic acid [3]	octadeca-9,12-dienoic acid	
JAU6476-α-OH-desthio	2-(1-chlorocyclopropyl)-1-(2-chlorophenyl)-3-(1*H*-1,2,4-triazol-1-yl)-1,2-propanediol	
1*H*-1,2,4-triazole [1] Free triazole		
Triazolylalanine (TA) [2]	α-amino-1*H*-1,2,4-triazole-1-propanoic acid	
Triazolylhydroxypropionic acid (THPA) [6]	α-hydroxy-1*H*-1,2,4-triazole-1-propanoic acid	
Triazolylacetic acid (TAA) [4]	1*H*-1,2,4-triazole-1-acetic acid	
JAU6476-triazolyl ethanol [5]	1-(1-chlorocyclopropyl)-2-(1*H*-1,2,4-triazol-1-yl)ethanol	

[1] Chemical name generated using ACD chemical naming software.
[2] Isolated and identified in wheat metabolism study (refer to the DER for MRID 46246141).
[3] Standard was radiolabeled. We note that the structures provided by the petitioner for oleic acid and linoleic acid were reversed (i.e, the structure provided for oleic acid was actually linoleic acid); the correct structures are presented in this table.
[4] Standard was radiolabeled.
[5] Isolated and identified in hen metabolism study (refer to the DER for MRID 46246203).
[6] Radiolabeled standard was isolated and identified in a cell culture experiment.

Primary Evaluators

Stephen Funk, Ph.D., Senior Chemist
Immediate Office
Date: Mar 13 2006

Louise G Croteau, Senior Evaluation Officer
FREAS, HED
Date: Jan 30/06

Approved by

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HED/RAB3
Date:

Henri P Bietlot, Acting Section Head
FREAS, HED
Date: Apr 13/06

In the absence of signatures, this document is considered to be a draft with deliberative material for internal use only.

This DER was originally prepared under contract by Dynamac Corporation (20440 Century Boulevard, Suite 100; Germantown, MD 20874; submitted 05/12/2005). The DER has been reviewed by the Health Effects Division (HED) and Health Canada's Pest Management Regulatory Agency (PMRA). The DER has been revised to reflect current Office of Pesticide Programs (OPP) policies, and Directive 98-02.

STUDY REPORTS:

46477701 Duah, F. K. (2005) JAU6476 - Crop Residues Storage Stability Deficiency Study. Project Number: J6131603: RAJAY037: 201262. Unpublished study prepared by Bayer CropScience. 138 p.

Gould T. and Timberlake B. (2005) Storage stability of JAU6476 and desthio JAU6476 in canola, wheat, mustard greens, turnip root, and tomato fruit, processed products, and solutions, Bayer CropScience Interim Report.

EXECUTIVE SUMMARY:

The field crop residue studies which were included in the registration submission were initiated in 2000. The analysis of samples from these studies for the residues of JAU6476 and the metabolite JAU6476-desthio were initiated in 2001. Bayer also initiated a study (in 2001) to address the freezer storage stability of JAU6476 and JAU6476-desthio in crop matrices. After completion of the analysis of crop samples in 2003, Bayer discovered that the JAU6476 crop residue analytical method used (Method No. 00598 or 00598/M001) was able to extract weathered JAU6476-desthio residues, but the extraction efficiency for weathered JAU6476 residues (using the same method) was not fully optimized. Subsequently, Bayer developed a new and more stringent crop residue analytical method (RPA JA/03/01) which provided excellent extraction efficiencies for both JAU6476 and JAU6476-desthio.

As required by OPPTS 860.1380 and Directive 98-02 (Section 5), Bayer initiated a new or second storage stability study (August 2004) using the new analytical method. The applicant chose the tested matrices to be representative of five diverse crops [an oilseed (canola), a non-oily grain (wheat), a leafy vegetable (mustard greens), a root crop (turnip), and a fruiting vegetable (tomato)] as well as the processed commodities of three crops [an oilseed, a fruiting vegetable, and a non-oily grain]. Bayer re-analysed all field crop residue samples with the new analytical method after 17 to 42 months of freezer storage. Bayer has also analysed samples from the earlier (first) storage stability study after 35 to 36 months of frozen storage. Bayer has recently (October 2005) conducted analysis of samples from the second storage stability study after 12.5 to 12.7 months of freezer storage, and plans to conduct additional analysis of samples at time intervals that will address the longest storage intervals (42 months) for the field crop residue samples.

The maximum storage intervals of crop samples from harvest to analysis for total prothioconazole-derived residues were 1234 days (40.6 months) for barley grain and 1269 days (41.7 months) for barley hay and straw; 542 days (17.8 months) for dried beans and peas; 1263 days (41.5 months) for mustard greens, 1243 days (40.8 months) for turnip tops and roots; 1285 days (42 months) for peanut nutmeat and hay, and 911 days (31 months) for processed peanut commodities; 1261 days (41 months) for canola seed and 918 days (30 months) for processed canola commodities; 1240 days (40.8 months) for rice grain and straw, and 902 days (30 months) for processed rice commodities; 469 days (15.4 months) for wheat forage, 1214 days (39.9 months) for wheat grain, 1221 days (40.1 months) for wheat hay, and 1203 days (39.5 months) for wheat straw, and 909 days (30 months) for processed wheat commodities.

Prothioconazole-derived residues were found to be stable for 12.5 to 12.7 months in canola oil (14% decline), canola seed (20% decline), mustard green (22% decline), tomato fruit (14% decline), turnip roots (0% decline), wheat flour (12% decline), wheat forage (16% decline), wheat grain (27% decline), and wheat straw (15% decline). JAU6476 showed 33% and 36% decline in tomato paste and wheat bran, respectively. However, the JAU6476 plant metabolism studies in three dissimilar crops have shown that JAU6476 is expected to contribute only 0 to 7% (0 to 20% normalized) of the total residues measured in the field crop residue studies. Therefore,

the apparent slight instability of prothioconazole-derived residues in tomato paste and wheat bran would not be expected to have any significant effect on the total JAU6476 (JAU6476 plus JAU6476-desthio) residue levels measured in the field crop residue studies. JAU6476-desthio, the major residue anticipated in crop matrices, was found to be stable in all matrices after 12.5 to 12.7 months of freezer storage. Percent decline of JAU6476-desthio was equal to or less than 5% in all matrices. JAU6476-desthio would be expected to contribute 6 to 58% (80 to 100% normalized) of the residues measured in the JAU6476 field crop residue trials.

Even though JAU6476 appears to be slightly unstable in two matrices (wheat bran, tomato paste), the overall impact on the crop residues will not be significant. Based on OPPTS 860.1380 and Directive 98-02 (Section 5-10), the Agency will consider corrections on a case-by-case basis, taking into account factors such as the absolute (ppm) and relative (% ROC) residue levels of the component that is unstable in storage. Therefore, correction for dissipation of prothioconazole-derived residues during freezer storage will not be necessary. Bayer has volunteered to submit interim data on analyses planned at 24-, 36-, and 45-month storage intervals from the study initiated in August 2004.

According to the 12.5 to 12.7-month data (interim report) in comparison with the previously submitted storage stability data, it is expected that the total prothioconazole-derived residues would be stable in crop matrices for up to 42 months in frozen storage. Therefore, the field crop residue data which were obtained after 17 to 42 months of freezer storage would be representative of residue levels which were present in the various crop matrices at harvest.

STUDY/WAIVER ACCEPTABILITY/DEFICIENCIES/CLARIFICATIONS:

Under the conditions and parameters used in the study, the storage stability data are classified as scientifically acceptable. The acceptability of this study for regulatory purposes is addressed in the forthcoming U.S. EPA Residue Chemistry Summary Document, DP Barcode D303508, and in Canada's Regulatory Decision Document. Interim and final reports will be submitted to both Agencies as confirmatory data.

COMPLIANCE:

Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided. No deviations from regulatory requirements were reported.

A. BACKGROUND INFORMATION

Prothioconazole is a broad-spectrum systemic fungicide intended for the control of many diseases in different crops such as cereals, oilseed rape or peanuts. The biochemical mode of action is the inhibition of the demethylation of lanosterol or 24-methylene-dihydrolanosterol, which are precursors of sterols in fungi. The chemical has been submitted for joint EPA and PMRA review by Bayer CropScience. The crops proposed for joint review include barley, the

dried shell and bean subgroup, the oilseed crop group, and wheat. Uses on peanuts and rice are being proposed for the U.S. only.

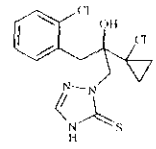
TABLE A.1. Test Compound Nomenclature.	
Chemical structure	
Common name	*Prothioconazole* (ISO accepted)
Company experimental name	JAU6476
IUPAC name	2-[(2*RS*)-2-(1-chlorocyclopropyl)-3-(2-chlorophenyl)-2-hydroxypropyl]-2*H*-1,2,4-triazole-3(4*H*)-thione
CAS *name*	2-[2-(1-chlorocyclopropyl)-3-(2-chlorophenyl)-2-hydroxypropyl]-1,2-dihydro-3*H*-1,2,4-triazole-3-thione
CAS registry number	178928-70-6
PC Code	113961
End-use product (EP)	Proline® 480 SC (4 lb/gal suspension concentrate), EPA File Symbol 264-IEL

TABLE A.2. Physicochemical Properties of Prothioconazole			
Parameter	Value		Reference
Melting range	139.1 to 144.5°C		MRID 46246003 / CES[1]
pH	5.8 (1% solution)		MRID 46246003 / CES
Density at 20°C	1.36 g/mL		MRID 46246003 / CES
Water solubility at 20°C	pH 4 8 9	mg/L 5 300 2000	MRID 46246003 / CES
Solvent solubility at 20°C	Solvent Acetone Acetonitrile Dichloromethane Dimethysulfoxide Ethyl acetate n-Heptane 1-Octanol Polyethylene glycol 2-Propanol Xylene	g/L >250 69 88 126 >250 <0.1 58 >250 87 8	MRID 46246003 / CES
Vapor pressure at 20 or 25°C	$<4 \times 10^{-7}$ Pa (calculated from determinations at 70 °C)		MRID 46246003 / CES
Dissociation constant, pK_a	6.9 (calculated from K_{OW})		MRID 46246003 / CES
Octanol/water partition coefficient, Log(K_{OW}), at 20°C	pH unbuffered water pH 4 pH 7 pH 9	Log Kow 4.05 4.16 3.82 2.00	MRID 46246003 / CES
UV/visible absorption spectrum	Peak maxima at 257 nm		MRID 46246003 / CES

[1] CES: Chemistry Evaluation Section of PMRA

B. EXPERIMENTAL DESIGN

B.1. Sample Handling and Preparation

The first study (which will referred to in this DER as Study 1) was initiated January 2001 at Battelle-AgriFood Laboratories. We note that partial results for this study were submitted as an appendix to the wheat crop field trial study submitted under DP Barcode D303508 (in MRID 46246219) and that some of the information pertaining to sample preparation and storage conditions that was not included in the current submission was obtained from that appendix. At Battelle, samples of untreated canola seed, mustard greens, tomato, turnip root, and wheat forage, straw, hay, and grain were spiked with a mixed standard of prothioconazole and prothioconazole-desthio in a 1:1 ratio at a total of 0.200 ppm, expressed as parent equivalents; the spiking standard was prepared in methanol. Samples were then placed in frozen storage (≤-10°C). No information pertaining to the form of the sample (whole, chopped, or homogenized) or the storage containers was provided. No zero-time analyses were conducted. Samples were analyzed at Battelle after approximately 3 months of storage. After approximately two years of storage, the samples were shipped frozen to Bayer (Stilwell, KS), where they were stored frozen

(≤-15°C). Samples were removed from storage after ~1050-1080 days (34.5-35.5 months) of total storage and analyzed.

Because Study 1 did not include any zero-time analyses, Bayer initiated a second storage stability study (Study 2) in August 2004. In Study 2, samples of untreated canola seed, canola oil, mustard greens, tomato, tomato paste, turnip root, and wheat forage, grain, straw, bran and flour were separately spiked with prothioconazole or prothioconazole-desthio at 0.250 ppm. Samples were placed in frozen storage (≤-15°C) and analyzed after storage intervals of 0, 2, 4, 6 and 12.7 months. The interim results of Study 2 are included in this submission; the applicant intends the study to reflect additional storage intervals of 24, 36, and 45 months.

To address the stability of weathered prothioconazole residues, Bayer also reanalyzed certain crop field trial samples in January-February 2005, after 30 to 46 months of frozen storage (Study 3). The samples chosen for reanalysis were: canola seed (from Ashton, ID field trial); barley hay, grain, and straw (from Maricopa and Wilcox, AZ field trials); dried peas (from Ephrata, WA and Jerome, ID trials); wheat forage (from Louisville, NE field trial, 7-day PHI); and wheat hay and straw (from East Bernard, TX and Knightdate, NC field trials). These samples were originally analyzed at Battelle within 3 months of collection; however, the results of the original analyses were not reported to EPA/PMRA because the applicant determined that the analytical methods used did not adequately extract weathered residues. The results that were reported in the crop field trial submissions (refer to the DERs for MRIDs 46246215 and 46246219-46246221) reflected analyses conducted at Bayer within 18-32 months of sample collection using the proposed enforcement method. For Study 3, the samples were reanalyzed at Bayer approximately 12-15 months after the initial analyses at Bayer.

B.2. Analytical Methodology

Samples of canola seed, mustard greens, tomato, turnip root, and wheat forage, hay, straw, and grain from the ~3-month storage interval from Study 1 and samples of canola seed, barley hay, straw, and grain, and wheat forage, hay, and straw from the ~1- to 3-month storage interval from Study 3 were analyzed for combined residues of prothioconazole and prothioconazole-desthio (JAU6476-desthio) at Battelle using LC-MS/MS Method 00598 or its modification Method 00598/M001. The applicant did not include any description of the methods in the current submission, and no information pertaining to which method was used for individual samples was provided. Descriptions of these methods, including validation data, have been submitted separately; refer to the DER for MRIDs 46477702 and 46477703. Briefly, cysteine hydrochloride was added to samples to stabilize prothioconazole, and then samples were extracted with acetonitrile (ACN)/water. The extract was partitioned with n-hexane, and the ACN/water phase was partitioned with dichloromethane. The dichloromethane phase was concentrated and mixed with ACN and water for LC-MS/MS analysis. The LOQs reported in the methods were 0.01 ppm for cereal grain and canola seed and 0.05 ppm for all other matrices.

Samples of canola seed, mustard greens, tomato, turnip root, and wheat forage, hay, straw, and grain from the ~35-month storage interval from Study 1, all samples from Study 2, and samples

of canola seed, barley hay, straw, and grain, dried peas, and wheat forage, hay, and straw from storage intervals greater than 18 months from Study 3 were analyzed at Bayer for total prothioconazole-derived residues (prothioconazole and its metabolite prothioconazole-desthio) using LC-MS/MS method RPA JA/03/01. A brief description of the method was included in the submission. For a complete description of the method, refer to the DER for MRID 46246206. Briefly, crop matrices were 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 was spiked 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. Residue levels were quantitated from a calibration curve of mixed reference standards of prothioconazole as sulfonic acid and prothioconazole-desthio. The method was modified for Study 1 to increase the amount of extraction solvents and the volume of internal standard used to account for the larger sample sizes used in the study. The results for prothioconazole sulfonic acid and prothioconazole-desthio are reported in prothioconazole equivalents and then totaled to yield "total prothioconazole-derived residues." The applicant did not report any LOQs for the storage stability samples. The reported LODs were 0.003-0.004 ppm.

C. RESULTS AND DISCUSSION

Bayer CropScience has submitted the results of three storage stability studies with prothioconazole and the metabolite prothioconazole-desthio. The first study (which will referred to in this DER as Study 1) was initiated January 2001 at Battelle-AgriFood Laboratories. We note that partial results for this study were submitted as an appendix to the wheat crop field trial study submitted under DP Barcode D303508 (in MRID 46246219). In Study 1, samples of untreated canola seed, mustard greens, tomato, turnip root, and wheat forage, straw, hay, and grain were fortified with a mixed standard of prothioconazole and prothioconazole-desthio (in a 1:1 ratio) at a total of 0.200 ppm expressed as parent equivalents. Samples were stored frozen (≤-10°C) for up to ~35 months. Only limited information pertaining to sample preparation prior to storage was submitted. At the 3-month storage interval, samples were analyzed at Battelle, and at the final storage interval, samples were analyzed by Bayer. No zero-time analyses were conducted.

Because Study 1 did not include any zero-time analyses, Bayer initiated a second storage stability study (Study 2) in August 2004. In Study 2, samples of untreated canola seed, canola oil, mustard greens, tomato, tomato paste, turnip root, and wheat forage, grain, straw, bran and flour were separately spiked with prothioconazole or prothioconazole-desthio at 0.250 ppm. Samples were stored frozen (≤-15°C) for up to 12.7 months. The interim results of Study 2 are included in this submission; the applicant intends the study to reflect additional storage intervals of 24, 36, and 45 months.

To address the stability of weathered prothioconazole residues, Bayer also reanalyzed certain crop field trial samples after 30 to 46 months of frozen storage (Study 3). Samples of canola seed, barley hay, grain, and straw, dried peas, wheat forage, and wheat hay and straw from crop field trials submitted in conjunction with DP Barcode D303508 (refer to the DERs for MRIDs 46246215 and 46246219-46246221) were used. These samples were originally analyzed at Battelle within 3 months of collection and were reanalyzed at Bayer after 18-32 months of frozen storage because it was determined that the original analytical methods (LC-MS/MS Method 00598 or its modification Method 00598/M001) did not adequately extract weathered residues. For Study 3, Bayer reanalyzed these samples 12-15 months after the initial analyses at Bayer.

Samples that were analyzed at Bayer (canola seed, mustard greens, tomato, turnip root, and wheat forage, hay, straw, and grain from the ~35-month storage interval from Study 1, all samples from Study 2, and samples of canola seed, barley hay, straw, and grain, dried peas, and wheat forage, hay, and straw from the ~30- to 42-month storage interval from Study 3) were analyzed for total prothioconazole-derived residues (prothioconazole, sulfonic acid and the metabolite prothioconazole-desthio) using LC-MS/MS method RPA JA/03/01. Because the applicant has indicated that LC-MS/MS method JA/03/01 is the preferred method for analysis of crop samples, sample results that were generated using Method No. 00598 or 00598/M001 will not be used to evaluate storage stability. Adequate concurrent method recovery data were submitted for both methods.

Based on the reported results from Study 1, combined residues of prothioconazole and prothioconazole-desthio appear to be stable in/on wheat forage, hay, and straw stored frozen for up to ~35 months. Combined residues of prothioconazole and prothioconazole-desthio were found to decline during frozen storage for ~35 months by ~18% in/on canola seed, ~13% in/on mustard greens, ~20% in/on tomato, ~17% in/on turnip root, and ~32% in/on wheat grain.

Based on the reported results from Study 2, total prothioconazole-derived residues were found to be stable for 12.5 to 12.7 months in canola oil (14% decomposition), canola seed (20% decline), mustard green (22% decline), tomato fruit (14% decline), turnip roots (0% decline), wheat flour (12% decline), wheat forage (16% decline), wheat grain (27% decline), and wheat straw (15% decline). Total prothioconazole-derived residues showed 33% and 36% apparent decline in tomato paste and wheat bran, respectively. It appears that over a longer period of time, the amount of total prothioconazole-derived residues is recovered in lower amounts in certain plant matrices. However, plant metabolism studies in three dissimilar crops have shown that JAU6476 is expected to contribute only 0 to 7% (0 to 20% normalized) of the total residues measured in the field crop residue studies. Therefore, the apparent slight instability of JAU6476 in tomato paste and wheat bran would not be expected to have any significant effect on the total JAU6476 (JAU6476 plus JAU6476-desthio) residue levels measured in the field crop residue studies. JAU6476-desthio, the major residue anticipated in crop matrices, was found to be stable in all matrices after 12.5 to 12.7 months of freezer storage. Percent decline of JAU6476-desthio was equal to or less than 5% in all matrices. JAU6476-desthio would be expected to contribute 6 to 58% (80 to 100% normalized) of the residues measured in the JAU6476 field crop residue trials.

Based on the reported results from Study 3, weathered total prothioconazole-derived residues appear to be stable in/on barley hay, straw, and grain stored frozen for ~13 months beyond initial analyses at Bayer, in/on dried peas stored frozen for 12 months beyond initial analyses, and in/on wheat hay and straw stored frozen for ~15 months beyond initial analyses. Residues were found to decline ~14% in/on canola seed stored frozen for ~12 months beyond initial analyses at Bayer and ~13% in/on wheat forage stored frozen for ~15 months beyond initial analyses. Initial analyses at Bayer were conducted 29-32 months after sample collection for barley, canola, and wheat commodities and 18 months after sample collection for dried peas.

Concurrent method recovery data for Storage Stability Studies 1, 2, and 3 are presented in TABLES C.1.1, C.1.2, and C.1.3, respectively. The data indicate that LC-MS/MS methods 00598 and 00598/M001 are adequate for the determination of prothioconazole and prothioconazole-desthio in/on canola seed, mustard greens, tomato, turnip root, and wheat forage, hay, straw, and grain, and that LC-MS/MS method RPA JA/03/01 is adequate for the determination of combined residues of prothioconazole and prothioconazole-desthio in barley hay, barley straw, barley grain, canola seed, canola oil, dried peas, mustard greens, tomato, tomato paste, turnip root, and wheat forage, hay, straw, grain, bran, and flour. All concurrent recoveries were acceptable.

The results of Storage Stability Studies 1, 2, and 3 are presented in TABLES C.2.1, C.2.2, and C.2.3, respectively. Because the applicant has indicated that LC-MS/MS method JA/03/01 is the preferred method for analysis of crop samples, sample results that were generated using Method No. 00598 or 00598/M001 will not be used to evaluate storage stability. No raw data for analyses conducted using Method No. 00598 or 00598/M001 were included in the submission.

For Study 1, apparent total prothioconazole-derived residues were reported for unspiked samples of all matrices analyzed using method JA/03/01 at levels ranging 0.00018-0.00226 ppm. Even though these results were below the reported LOD for each matrix, the applicant used the results to correct the residue results in spiked samples prior to calculating percent recoveries for concurrent method recovery samples and prior to calculating corrected recoveries for storage stability samples.

For Study 2, apparent total prothioconazole-derived residues greater than LOD were reported in/on one sample each of unspiked canola seed (0.00579 ppm) and wheat straw (0.00772), and two samples of unspiked mustard greens (0.00436 and 0.00570 ppm). Residues were less than LOD in/on three samples each of unspiked canola oil, tomato, tomato paste, and wheat forage, grain, bran, and flour; two samples each of unspiked canola seed, turnip root, and wheat straw; and one sample of unspiked mustard greens.

For Study 3, apparent total prothioconazole-derived residues greater than LOD were reported in/on barley grain (0.0070 ppm), canola seed (0.0091 ppm), dried peas (0.0078 ppm), and wheat straw (0.0040 ppm). Residues were less than LOD in/on one sample each of barley hay and straw and wheat forage and hay.

TABLE C.1.1. Summary of Concurrent Recoveries of Prothioconazole and Prothioconazole-Desthio (Combined) from Various Crop Matrices from Storage Stability Study 1.[1]

Matrix	Spike level (ppm) [2]	Storage interval (days)	Sample size (n)	Recoveries (%) [3]	Mean
Canola seed	0.200	89	3	92, 93, 94	93
		1079	2	97, 98	98
Mustard greens	0.200	90	3	90, 91, 96	92
		1078	2	96, 97	96
Tomato	0.200	90	3	96, 96, 98	97
		1079	2	95, 99	97
Turnip root	0.2	89	3	94, 96, 96	95
		1078	2	99, 99	99
Wheat forage	0.200	1049	2	84, 85	85
Wheat hay	0.2	92	3	92, 94, 94	93
		1078	2	92, 93	92
Wheat straw	0.200	92	3	85, 85, 91	87
		1077	2	91, 94	93
Wheat grain	0.200	93	3	85, 87, 87	86
		1077	2	97, 97	97

[1] Samples from the 89- to 93-day storage interval were analyzed for combined residues of prothioconazole and prothioconazole-desthio using LC-MS/MS Method No. 00598 or 00598/M001. Samples from the 1049- to 1079-day storage interval were analyzed for total prothioconazole-derived residues using LC-MS/MS method RPA JA/03/01; concurrent recovery data for these samples were presented in the Appendix to MRID 46246219 only.

[2] Spiked with a mixture of prothioconazole and prothioconazole-desthio at a 1:1 ratio.

[3] Results were corrected for average residue levels in unspiked samples.

TABLE C.1.2 Summary of Concurrent Recoveries of Prothioconazole and Prothioconazole-Desthio from Various Crop Matrices from Storage Stability Study 2.[1]

Matrix	Analyte	Spike level (ppm)	Storage interval (days)	Sample size (n)	Recoveries (%) [2]	Mean
Canola seed	Prothioconazole	0.25	0	3	86, 88, 87	87
		0.25	63	2	87, 86	86
		0.25	129	2	74, 74	74
		0.25	192	2	72, 71	71.5
		0.25	381	2	71, 72	71.5
	Prothioconazole-desthio	0.25	0	3	94, 94, 95	94.3
		0.25	63	2	96, 93	95
		0.25	129	2	95, 95	95
		0.25	192	2	93, 94	93.5
		0.25	381	2	93, 96	94.5
Canola oil	Prothioconazole	0.25	0	3	91, 90, 90	90.3
		0.25	64	2	92, 92	92
		0.25	132	2	94, 91	93
		0.25	190	2	89, 92	90.5
		0.25	380	2	94, 92	93
	Prothioconazole-desthio	0.25	0	3	95, 95, 95	95
		0.25	64	2	99, 99	99
		0.25	132	2	93, 93	93
		0.25	190	2	94, 92	93
		0.25	380	2	94, 91	92.5
Mustard greens	Prothioconazole	0.25	0	3	80, 80, 84	81.3
		0.25	58	2	88, 88	88
		0.25	127	2	90, 87	88
		0.25	185	2	82, 82	82
		0.25	375	2	77, 78	77.5
	Prothioconazole-desthio	0.25	0	3	96, 95, 95	95.3
		0.25	58	2	95, 95	95
		0.25	127	2	100, 97	99
		0.25	185	2	92, 95	93.5
		0.25	375	2	98, 98	98

TABLE C.1.2. Summary of Concurrent Recoveries of Prothioconazole and Prothioconazole-Desthio from Various Crop Matrices from Storage Stability Study 2.[1]

Matrix	Analyte	Spike level (ppm)	Storage interval (days)	Sample size (n)	Recoveries (%) [2]	Mean
Tomato fruit	Prothioconazole	0.25	0	3	81, 83, 80	81.3
		0.25	57	2	105, 104	105
		0.25	126	2	71, 71	71
		0.25	184	2	51, 50	50.5
		0.25	374	2	63, 62	62.5
	Prothioconazole-desthio	0.25	0	3	93, 93, 93	93
		0.25	57	2	95, 95	95
		0.25	126	2	92, 94	93
		0.25	184	2	92, 91	91.5
		0.25	374	2	93, 94	93.5
Tomato paste	Prothioconazole	0.25	0	3	91, 91, 91	91
		0.25	58	2	89, 85	87
		0.25	126	2	89, 89	89
		0.25	184	2	86, 86	86
		0.25	374	2	87, 89	88
	Prothioconazole-desthio	0.25	0	3	92, 93, 95	93.3
		0.25	58	2	97, 98	98
		0.25	126	2	90, 94	92
		0.25	184	2	91, 92	91.5
		0.25	374	2	93, 93	93
Turnip root	Prothioconazole	0.25	0	3	87, 86, 88	87
		0.25	57	2	85, 84	84
		0.25	125	2	74, 74	74
		0.25	183	2	60, 56	58
		0.25	380	2	37, 40	38.5
	Prothioconazole-desthio	0.25	0	3	95, 94, 97	95.3
		0.25	57	2	93, 93	93
		0.25	125	2	96, 94	95
		0.25	183	2	94, 93	93.5
		0.25	380	2	98, 93	95.5

TABLE C.1.2. Summary of Concurrent Recoveries of Prothioconazole and Prothioconazole-Desthio from Various Crop Matrices from Storage Stability Study 2.[1]

Matrix	Analyte	Spike level (ppm)	Storage interval (days)	Sample size (n)	Recoveries (%) [2]	Mean
Wheat forage	Prothioconazole	0.25	0	3	76, 76, 77	76.3
		0.25	59	2	82, 82	82
		0.25	130	2	79, 77	78
		0.25	188	2	68, 71	69.5
		0.25	377	2	56, 57	56.5
	Prothioconazole-desthio	0.25	0	3	90, 93, 92	91.6
		0.25	59	2	95, 96	96
		0.25	130	2	92, 96	94
		0.25	188	2	91, 91	91
		0.25	377	2	94, 93	93.5
Wheat straw	Prothioconazole	0.25	0	3	91, 91, 92	91.3
		0.25	60	2	88, 90	89
		0.25	130	2	86, 87	87
		0.25	188	2	84, 84	84
		0.25	377	2	86, 85	85.5
	Prothioconazole-desthio	0.25	0	3	94, 94, 97	95
		0.25	60	2	91, 90	91
		0.25	130	2	90, 91	91
		0.25	188	2	92, 91	91.5
		0.25	377	2	93, 94	93.5
Wheat grain		0.25	0	3	87, 90, 89	88.7
		0.25	61	2	90, 91	90
		0.25	131	2	86, 87	86
		0.25	189	2	84, 85	84.5
		0.25	378	2	88, 90	89
	Prothioconazole-desthio	0.25	0	3	94, 96, 96	95.3
		0.25	61	2	100, 99	99
		0.25	131	2	90, 91	91
		0.25	189	2	94, 95	94.5
		0.25	378	2	79, 81	80

TABLE C.1.2. Summary of Concurrent Recoveries of Prothioconazole and Prothioconazole-Desthio from Various Crop Matrices from Storage Stability Study 2.[1]

Matrix	Analyte	Spike level (ppm)	Storage interval (days)	Sample size (n)	Recoveries (%) [2]	Mean
Wheat bran	Prothioconazole	0.25	0	3	83, 84, 83	83.3
		0.25	63	2	88, 90	89
		0.25	131	2	79, 89	84
		0.25	189	2	82, 82	82
		0.25	378	2	85, 86	85.5
	Prothioconazole-desthio	0.25	0	3	94, 95, 97	95.3
		0.25	63	2	96, 95	95
		0.25	131	2	96, 98	97
		0.25	189	2	93, 94	85.5
		0.25	378	2	89, 86	95.3
Wheat flour	Prothioconazole	0.25	0	3	90, 86, 89	88.3
		0.25	57	2	89, 89	89
		0.25	127	2	80, 80	80
		0.25	185	2	83, 83	83
		0.25	374	2	79, 80	79.5
	Prothioconazole-desthio	0.25	0	3	99, 98, 95	97.3
		0.25	57	2	99, 97	98
		0.25	127	2	94, 95	95
		0.25	185	2	95, 93	94
		0.25	374	2	95, 96	95.5

[1] Samples were analyzed for using LC-MS/MS method RPA JA/03/01.
[2] Results were corrected for any detectable residue levels in unspiked samples.

TABLE C.1.3. Summary of Concurrent Recoveries of Prothioconazole, Prothioconazole-Desthio, and Prothioconazole Sulfonic Acid in Various Crop Matrices from Storage Stability Study 3.[1]

Matrix	Analyte	Spike level (ppm)	Storage interval (days)	Sample size (n)	Recoveries (%) [2]
Barley hay	Prothioconazole	0.5	1368/1381	1	79
	Prothioconazole-desthio	0.5		1	91
	Prothioconazole sulfonic acid	0.5		1	71
Barley straw	Prothioconazole	0.5	1312/1347	1	77
	Prothioconazole-desthio	0.5		1	94
	Prothioconazole sulfonic acid	0.5		1	76
Barley grain	Prothioconazole	0.1	1312/1347	1	92
	Prothioconazole-desthio	0.1		1	94
	Prothioconazole sulfonic acid	0.1		1	92
Canola seed	Prothioconazole	0.1	1234	1	92
	Prothioconazole-desthio	0.1		1	93
	Prothioconazole sulfonic acid	0.1		1	93
Dried peas	Prothioconazole	0.1	904/907	1	91
	Prothioconazole-desthio	0.1		1	94
	Prothioconazole sulfonic acid	0.1		1	94
Wheat forage	Prothioconazole	0.5	1360/1367	1	80
	Prothioconazole-desthio	0.5		1	92
	Prothioconazole sulfonic acid	0.5		1	87
Wheat hay	Prothioconazole	0.5	1349/1370	1	87
	Prothioconazole-desthio	0.5		1	92
	Prothioconazole sulfonic acid	0.5		1	84
Wheat straw	Prothioconazole	0.5	1326/1350	1	86
	Prothioconazole-desthio	0.5		1	91
	Prothioconazole sulfonic acid	0.5		1	91

[1] Samples were analyzed using LC-MS/MS method RPA JA/03/01.
[2] Results were corrected for residue levels in unspiked samples.

TABLE C.2.1. Stability of Combined Residues of Prothioconazole and Prothioconazole-Desthio in Various Crop Matrices Following Storage at ≤-10°C (Storage Stability Study 1).[1]

Commodity	Spike level (ppm)	Storage interval (days) [months]	Recovered residues (ppm)	Corrected % recovery [2]	Average % decline [3]
Canola seed	0.200	89 [2.9]	0.150, 0.151, 0.155	81, 81, 83	18
		1079 [35.4]	0.161, 0.161, 0.163	82, 82, 83	18
Mustard greens	0.200	90 [3.0]	0.143, 0.143, 0.145	77, 78, 78	22
		1078 [35.4]	0.164 0.169, 0.171	85, 87, 88	13
Tomato	0.200	90 [3.0]	0.169, 0.169, 0.175	87, 88, 90	12
		1079 [35.4]	0.152, 0.156, 0.158	78, 80, 81	20

TABLE C.2.1. Stability of Combined Residues of Prothioconazole and Prothioconazole-Desthio in Various Crop Matrices Following Storage at ≤-10°C (Storage Stability Study 1).[1]

Commodity	Spike level (ppm)	Storage interval (days) [months]	Recovered residues (ppm)	Corrected % recovery [2]	Average % decline [3]
Turnip root	0.200	89 [2.9]	0.159, 0.165, 0.169	84, 87, 89	13
		1078[35.4]	0.163, 0.164, 0.167	82, 82, 84	17
Wheat forage [4]	0.200	1049 [34.5]	0.158, 0.159, 0.162	92, 93, 95	7
Wheat hay	0.200	92 [3.0]	0.143, 0.151, 0.155	77, 81, 83	20
		1078 [35.4]	0.171, 0.176, 0.181	91, 94, 97	6
Wheat straw	0.200	92 [3.0]	0.156, 0.156, 0.159	90, 90, 91	10
		1077 [35.4]	0.169, 0.173, 0.176	90, 92, 94	8
Wheat grain	0.200	93 [3.1]	0.097, 0.100, 0.101	56, 58, 58	42
		1077 [35.4]	0.127, 0.134, 0.139	65, 68, 71	32

[1] Samples from the 89- to 93-day storage interval were analyzed for combined residues of prothioconazole and prothioconazole-desthio using LC-MS/MS Method No. 00598 or 00598/M001; these data were not used for storage stability evaluations and are presented here for informational purposes only. Samples from the 1049- to 1079-day storage interval were analyzed for total prothioconazole-derived residues using LC-MS/MS method RPA JA/03/01.

[2] Corrected for average concurrent method recovery and average residue levels in unspiked samples.

[3] Calculated by the applicant; average percent decline = 100% - average corrected percent recovery.

[4] Wheat forage samples were not analyzed at the 3-month storage interval.

TABLE C.2.2. Stability of Prothioconazole and Prothioconazole-Desthio Residues in Various Crop Matrices Following Freezer Storage (Storage Stability Study 2).[1]

Matrix	Spiking level (ppm)	Storage interval (days)	Recovered residues		Corrected % recovery[2]		Average % loss[3]
			ppm	%	Individual	Average	
Prothioconazole							
Canola seed	0.250	0	0.216	86	--	--	--
	0.250	0	0.220	88	--	--	--
	0.250	0	0.218	87	--	--	--
	0.250	63	0.209	83	96	95	5
	0.250	63	0.206	82	94		
	0.250	129	0.170	65	88	89	11
	0.250	129	0.172	66	89		
	0.250	192	0.162	64	89	88	12
	0.250	192	0.157	62	86		
	0.250	381	0.145	57	79	80	**20**
	0.250	381	0.146	57	80		
Canola oil	0.250	0	0.227	91	--	--	--
	0.250	0	0.226	90	--	--	--
	0.250	0	0.225	90	--	--	--
	0.250	64	0.223	89	97	96	4
	0.250	64	0.222	89	96		
	0.250	132	0.214	86	93	92	8
	0.250	132	0.213	85	92		
	0.250	190	0.198	79	87	88	12
	0.250	190	0.220	80	88		
	0.250	380	0.197	79	86	86	**14**
	0.250	380	0.198	79	87		
Mustard greens	0.250	0	0.208	80	--	--	--
	0.250	0	0.208	80	--	--	--
	0.250	0	0.217	84	--	--	--
	0.250	58	0.197	77	88	89	11
	0.250	58	0.203	79	90		
	0.250	127	0.190	74	84	85	15
	0.250	127	0.193	76	86		
	0.250	185	0.177	67	82	82	18
	0.250	185	0.179	68	83		
	0.250	375	0.166	61	79	78	**22**
	0.250	375	0.165	60	78		
Tomato fruit	0.250	0	0.202	81	--	--	--
	0.250	0	0.209	83	--	--	--

TABLE C.2.2. Stability of Prothioconazole and Prothioconazole-Desthio Residues in Various Crop Matrices Following Freezer Storage (Storage Stability Study 2).[1]

Matrix	Spiking level (ppm)	Storage interval (days)	Recovered residues		Corrected % recovery[2]		Average % loss[3]
			ppm	%	Individual	Average	
	0.250	0	0.199	80	--	--	--
	0.250	57	0.229	91	87	88	12
	0.250	57	0.236	94	90		
	0.250	126	0.154	61	86	87	13
	0.250	126	0.159	63	89		
	0.250	184	0.122	48	95	94	6
	0.250	184	0.120	47	94		
	0.250	374	0.134	53	85	86	**14**
	0.250	374	0.136	54	86		
Tomato paste	0.250	0	0.229	91	--	--	--
	0.250	0	0.228	91	--	--	--
	0.250	0	0.228	91	--	--	--
	0.250	58	0.212	84	97	97	3
	0.250	58	0.212	84	97		
	0.250	126	0.197	78	88	88	12
	0.250	126	0.197	79	88		
	0.250	184	0.155	61	71	74	26
	0.250	184	0.169	67	78		
	0.250	374	0.148	59	67	67	**33**
	0.250	374	0.147	58	67		
Turnip root	0.250	0	0.221	87	--	--	--
	0.250	0	0.217	86	--	--	--
	0.250	0	0.222	88	--	--	--
	0.250	57	0.202	80	95	97	3
	0.250	57	0.210	83	99		
	0.250	125	0.176	70	94	94	6
	0.250	125	0.176	69	94		
	0.250	183	0.160	63	108	108	0
	0.250	183	0.158	62	107		
	0.250	380	0.122	47	122	124	**0**
	0.250	380	0.124	49	125		
Wheat forage	0.250	0	0.193	76	--	--	--
	0.250	0	0.192	76	--	--	--
	0.250	0	0.194	77	--	--	--
	0.250	59	0.190	75	92	92	8
	0.250	59	0.189	75	91		

TABLE C.2.2. Stability of Prothioconazole and Prothioconazole-Desthio Residues in Various Crop Matrices Following Freezer Storage (Storage Stability Study 2).[1]

Matrix	Spiking level (ppm)	Storage interval (days)	Recovered residues		Corrected % recovery[2]		Average % loss[3]
			ppm	%	Individual	Average	
	0.250	130	0.177	70	90	89	11
	0.250	130	0.174	69	88		
	0.250	188	0.155	61	88	89	11
	0.250	188	0.160	63	90		
	0.250	377	0.120	46	80	84	**16**
	0.250	377	0.129	49	87		
Wheat straw	0.250	0	0.236	91	--	--	--
	0.250	0	0.236	91	--	--	--
	0.250	0	0.239	92	--	--	--
	0.250	60	0.212	84	94	95	5
	0.250	60	0.214	85	96		
	0.250	130	0.191	76	87	87	13
	0.250	130	0.191	76	87		
	0.250	188	0.181	71	84	85	15
	0.250	188	0.184	72	86		
	0.250	377	0.184	72	85	85	**15**
	0.250	377	0.185	73	85		
Wheat grain	0.250	0	0.217	87	--	--	--
	0.250	0	0.225	90	--	--	--
	0.250	0	0.222	89	--	--	--
	0.250	61	0.200	79	88	88	12
	0.250	61	0.202	80	89		
	0.250	131	0.194	76	89	88	12
	0.250	131	0.193	76	88		
	0.250	189	0.182	72	85	85	15
	0.250	189	0.182	72	85		
	0.250	378	0.165	65	73	73	**27**
	0.250	378	0.164	65	73		

TABLE C.2.2. Stability of Prothioconazole and Prothioconazole-Desthio Residues in Various Crop Matrices Following Freezer Storage (Storage Stability Study 2).[1]

Matrix	Spiking level (ppm)	Storage interval (days)	Recovered residues		Corrected % recovery[2]		Average % loss[3]
			ppm	%	Individual	Average	
Wheat bran	0.250	0	0.208	83	--	--	--
	0.250	0	0.211	84	--	--	--
	0.250	0	0.209	83	--	--	--
	0.250	63	0.191	75	85	84	16
	0.250	63	0.185	73	83		
	0.250	131	0.172	68	81	81	19
	0.250	131	0.174	69	82		
	0.250	189	0.161	63	77	76	24
	0.250	189	0.158	62	75		
	0.250	378	0.138	54	63	64	**36**
	0.250	378	0.144	56	65		
Wheat flour	0.250	0	0.226	90	--	--	--
	0.250	0	0.215	86	--	--	--
	0.250	0	0.222	89	--	--	--
	0.250	57	0.218	87	97	98	2
	0.250	57	0.220	88	98		
	0.250	127	0.205	82	102	102	0
	0.250	127	0.204	81	102		
	0.250	185	0.190	75	91	92	8
	0.250	185	0.191	76	92		
	0.250	374	0.229	91	96	96	**4**
	0.250	374	0.229	91	96		
Prothioconazole-desthio							
Canola seed	0.250	0	0.237	94	--	--	--
	0.250	0	0.236	94	--	--	--
	0.250	0	0.239	95	--	--	--
	0.250	63	0.247	98	103	101	0
	0.250	63	0.237	94	99		
	0.250	129	0.229	89	94	94	6
	0.250	129	0.233	90	95		
	0.250	192	0.224	89	96	96	4
	0.250	192	0.228	91	97		
	0.250	381	0.225	90	95	95	5
	0.250	381	0.226	90	95		

TABLE C.2.2. Stability of Prothioconazole and Prothioconazole-Desthio Residues in Various Crop Matrices Following Freezer Storage (Storage Stability Study 2).[1]

Matrix	Spiking level (ppm)	Storage interval (days)	Recovered residues		Corrected % recovery[2]		Average % loss[3]
			ppm	%	Individual	Average	
Canola oil	0.250	0	0.238	95	--	--	--
	0.250	0	0.239	95	--	--	--
	0.250	0	0.237	95	--	--	--
	0.250	64	0.250	100	101	100	0
	0.250	64	0.244	97	98		
	0.250	132	0.226	90	97	97	3
	0.250	132	0.226	90	96		
	0.250	190	0.227	90	97	97	3
	0.250	190	0.228	90	97		
	0.250	380	0.219	88	95	96	4
	0.250	380	0.222	89	96		
Mustard greens	0.250	0	0.245	96	--	--	--
	0.250	0	0.243	95	--	--	--
	0.250	0	0.242	95	--	--	--
	0.250	58	0.241	95	100	101	0
	0.250	58	0.247	97	102		
	0.250	127	0.238	94	95	95	5
	0.250	127	0.239	94	96		
	0.250	185	0.231	90	97	97	3
	0.250	185	0.231	90	97		
	0.250	375	0.241	93	95	96	4
	0.250	375	0.246	95	97		
Tomato fruit	0.250	0	0.232	93	--	--	--
	0.250	0	0.232	93	--	--	--
	0.250	0	0.232	93	--	--	--
	0.250	57	0.238	95	100	101	0
	0.250	57	0.244	97	102		
	0.250	126	0.222	89	95	96	4
	0.250	126	0.228	91	98		
	0.250	184	0.226	90	98	98	2
	0.250	184	0.228	91	99		
	0.250	374	0.231	92	98	98	2
	0.250	374	0.227	91	97		
Tomato paste	0.250	0	0.230	92	--	--	--
	0.250	0	0.234	93	--	--	--
	0.250	0	0.239	95	--	--	--
	0.250	58	0.244	97	99	99	1

TABLE C.2.2. Stability of Prothioconazole and Prothioconazole-Desthio Residues in Various Crop Matrices Following Freezer Storage (Storage Stability Study 2).[1]

Matrix	Spiking level (ppm)	Storage interval (days)	Recovered residues		Corrected % recovery[2]		Average % loss[3]
			ppm	%	Individual	Average	
	0.250	58	0.242	96	99		
	0.250	126	0.223	89	96	98	2
	0.250	126	0.232	93	100		
	0.250	184	0.226	89	97	98	2
	0.250	184	0.228	90	98		
	0.250	374	0.221	88	94	95	5
	0.250	374	0.224	89	96		
Turnip root	0.250	0	0.239	95	--	--	--
	0.250	0	0.237	94	--	--	--
	0.250	0	0.243	97	--	--	--
	0.250	57	0.232	92	99	99	1
	0.250	57	0.233	93	100		
	0.250	125	0.233	93	98	97	3
	0.250	125	0.230	92	96		
	0.250	183	0.233	92	99	98	2
	0.250	183	0.227	90	96		
	0.250	380	0.228	91	95	98	2
	0.250	380	0.242	96	101		
Wheat forage	0.250	0	0.226	90	--	--	--
	0.250	0	0.233	93	--	--	--
	0.250	0	0.232	92	--	--	--
	0.250	59	0.237	95	99	101	0
	0.250	59	0.247	98	103		
	0.250	130	0.225	90	96	96	4
	0.250	130	0.225	90	96		
	0.250	188	0.226	89	98	98	2
	0.250	188	0.226	89	98		
	0.250	377	0.230	90	97	96	4
	0.250	377	0.229	90	96		

TABLE C.2.2. Stability of Prothioconazole and Prothioconazole-Desthio Residues in Various Crop Matrices Following Freezer Storage (Storage Stability Study 2).[1]

Matrix	Spiking level (ppm)	Storage interval (days)	Recovered residues		Corrected % recovery[2]		Average % loss[3]
			ppm	%	Individual	Average	
Wheat straw	0.250	0	0.242	94	--	--	--
	0.250	0	0.243	94	--	--	--
	0.250	0	0.251	97	--	--	--
	0.250	60	0.231	92	102	103	0
	0.250	60	0.240	95	105		
	0.250	130	0.219	86	95	96	4
	0.250	130	0.223	88	97		
	0.250	188	0.221	87	96	96	4
	0.250	188	0.223	88	96		
	0.250	377	0.231	91	97	96	4
	0.250	377	0.228	90	96		
Wheat grain	0.250	0	0.237	94	--	--	--
	0.250	0	0.241	96	--	--	--
	0.250	0	0.241	96	--	--	--
	0.250	61	0.234	93	94	95	5
	0.250	61	0.238	95	96		
	0.250	131	0.225	89	98	98	2
	0.250	131	0.224	89	98		
	0.250	189	0.233	92	98	98	2
	0.250	189	0.234	93	98		
	0.250	378	0.220	79	99	97	3
	0.250	378	0.192	76	95		
Wheat bran	0.250	0	0.236	94	--	--	--
	0.250	0	0.238	95	--	--	--
	0.250	0	0.243	97	--	--	--
	0.250	63	0.251	99	104	103	0
	0.250	63	0.248	98	103		
	0.250	131	0.230	91	94	95	5
	0.250	131	0.234	93	96		
	0.250	189	0.228	90	96	96	4
	0.250	189	0.231	91	97		
	0.250	378	0.227	89	102	100	0
	0.250	378	0.220	87	99		

TABLE C.2.2. Stability of Prothioconazole and Prothioconazole-Desthio Residues in Various Crop Matrices Following Freezer Storage (Storage Stability Study 2).[1]

Matrix	Spiking level (ppm)	Storage interval (days)	Recovered residues		Corrected % recovery[2]		Average % loss[3]
			ppm	%	Individual	Average	
Wheat flour	0.250	0	0.250	99	--	--	--
	0.250	0	0.246	98	--	--	--
	0.250	0	0.238	95	--	--	--
	0.250	57	0.237	95	97	98	2
	0.250	57	0.242	96	99		
	0.250	127	0.228	91	96	98	2
	0.250	127	0.236	94	99		
	0.250	185	0.232	92	98	98	2
	0.250	374	0.229	91	96	96	4
	0.250	374	0.229	91	96		

[1] Samples were analyzed for total prothioconazole-derived residues using LC-MS/MS method RPA JA/03/01.
[2] Corrected for average concurrent method recovery and average residue levels in unspiked samples.
[3] Calculated by the applicant; average percent decline = 100% - average corrected percent recovery.

TABLE C.2.3. Stability of Weathered Total Prothioconazole-Derived Residues in Various Crop Matrices Following Freezer Storage (Storage Stability Study 3).

Matrix	Battelle Residue Data [1]		Bayer Residue Data Submitted to PMRA and EPA [2,3]		Residue Data from Reanalysis of Samples at Bayer [3]		Calculated Storage Stability Recovery [4]	
	Storage Interval (days)	Total Residues (ppm)	Storage Interval (days)	Total Residues (ppm)	Storage Interval (days)	Total Residues (ppm)	Storage Interval (days) [months]	Recovery (%)
Barley hay	77	1.333	972	1.814	1381	1.777	409 [13.4]	98
		1.118		1.741		1.621		93
	79	2.250	959	2.577	1368	2.361		92
		1.290		1.668		1.676		100
Barley straw	43	0.920	952	1.336	1347	1.615	395 [13.0]	121
		0.966		1.321		1.514		115
	24	0.871	917	1.324	1312	1.360		103
		0.971		1.269		1.241		98
Barley grain	41	0.059	947	0.088	1347	0.084	400 [13.2]	95
		0.064		0.082		0.096		117
	22	0.029	912	0.059	1312	0.068		115
		0.048		0.083		0.099		119
Canola seed	89	0.040	866	0.074	1234	0.070	368 [12.1]	95
		0.047		0.097		0.076		78

D. CONCLUSION

Storage stability studies were conducted on plant matrices representative of five diverse crops [an oilseed (canola), a non-oily grain (wheat), a leafy vegetable (mustard greens), a root crop (turnip), and a fruiting vegetable (tomato)] as well as the processed commodities of three crops [an oilseed, a fruiting vegetable, and a non-oily grain]. Even though JAU6476 appears to be slightly unstable in two matrices (tomato paste, wheat bran), the overall impact on the crop residues will not be significant. Based on OPPTS 860.1380 and Directive 98-02 (Section 5-10), the Agency will consider corrections on a case-by-case basis, taking into account factors such as the absolute (ppm) and relative (% ROC) residue levels of the component that is unstable in storage. Therefore, correction for dissipation of prothioconazole-derived residues during freezer storage will not be necessary at this time.

It is expected that total prothioconazole-derived residues would be stable in crop matrices up to 45 months in freezer storage based on the 12.5 to 12.7-month data (interim report) compared with the previously submitted storage stability data. Therefore, the field crop residue data which were obtained after 17 to 42 months of freezer storage would be representative of residue levels which were present in the various crop matrices at harvest.

E. REFERENCES

None.

F. DOCUMENT TRACKING

RDI: Louise G Croteau (23/01/2006); Henri Bietlot (13/02/2006); Stephen Funk (13/03/2006); Leung Cheng
Petition Number: PP#4F6830
DP Barcode: D303508
PC Code: 113961

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TABLE C.2.3. Stability of Weathered Total Prothioconazole-Derived Residues in Various Crop Matrices Following Freezer Storage (Storage Stability Study 3).

Matrix	Battelle Residue Data [1]		Bayer Residue Data Submitted to PMRA and EPA [2,3]		*Residue Data from* Reanalysis of Samples at Bayer [3]		Calculated Storage Stability Recovery [4]	
	Storage Interval (days)	Total Residues (ppm)	Storage Interval (days)	Total Residues (ppm)	Storage Interval (days)	Total Residues (ppm)	Storage Interval (days) [months]	Recovery (%)
Dried peas	Peas were not sent to Battelle for analysis		542	0.120	907	0.110	365 [12.0]	92
				0.122		0.115		94
			539	0.102	904	0.107		105
				0.118		0.109		92
Wheat forage	65	1.205	913	1.827	1367	1.335	454 [14.9]	73
		1.036		1.383		1.426		103
	58	0.159	906	0.273	1360	0.255		93
		0.166		0.325		0.249		77
Wheat hay	66	0.507	922	0.710	1370	0.794	448 [14.7]	112
		0.711		1.063		1.146		108
	45	1.749	914	1.928	1349	2.134	435 [14.3]	111
		1.711		2.501		2.546		102
Wheat straw	47	0.491	910	0.930	1350	0.923	440 [14.5]	99
		0.530		1.053		0.948		90
	39	0.749	886	1.284	1326	1.125		88
		0.992		1.548		1.654		107

[1] Residue results were not submitted to EPA/PMRA as part of crop field trial submissions because samples were analyzed using LC-MS/MS Method 00598 or 00598/M001. These data were not used for storage stability evaluations and are presented here for informational purposes only.

[2] Residues results were submitted to EPA/PMRA with crop field trial submissions (refer to the DERs for MRIDs 46246215 and 46246219-46246221.

[3] Samples were analyzed for total prothioconazole-derived residues using LC-MS/MS method RPA JA/03/01.

[4] Storage interval calculated as the difference between the storage interval for reanalysis and the storage interval for the first analysis at Bayer. Calculated storage stability recovery = (ppm total prothioconazole-derived residues from reanalysis) ÷ (ppm total prothioconazole-derived residues at the 539- to 972-day storage interval) x 100.

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