13544

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Memo Date:	**12/14/99**
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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

December 14, 1999

MEMORANDUM:

SUBJECT: **Propiconazole** (122101): Nature of the Residue in Spring Wheat (GLN 860.1300). **DP Barcode #** D245249. **Case 3125. MRID #** 44381402.

FROM: Thurston G. Morton, Chemist
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Thurston G Morton
12/14/99

THROUGH: Susan V. Hummel, Branch Senior Scientist
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Susan V. Hummel

TO: Mark Hartman/Kathy Monk, PM #52
Reregistration Section
Special Review & Reregistration Division (7508W)

EXECUTIVE SUMMARY:

Novartis has submitted additional data concerning the nature of the residue in spring wheat. All submitted studies (cited above) were reviewed by Dynamac Corporation under contract to EPA. The attached Dynamac review was modified to reflect current Agency policies. Based on these studies and previously reviewed studies, HED makes the following conclusions:

- The qualitative nature of propiconazole residues in wheat is adequately understood. Total radioactive residues were 0.844 ppm in/on forage, 3.450 ppm in/on straw, 0.156 ppm in/on chaff, and 0.119 ppm in/on grain harvested at 12 days (forage) or 77 days following a single foliar application of [phenyl-U-^{14}C]propiconazole at 0.1 lb ai/A/application (~1x the maximum seasonal rate). Following a similar application at 5x, TRRs were 3.78, 16.88, and 0.154 ppm in/on wheat forage, straw, and grain, respectively.

- Identified or characterized ^{14}C-residues in RACs from wheat treated at 1x accounted for 75-92% of the TRR in/on wheat forage and straw and 25-70% of the TRR in/on wheat chaff and grain. The majority of TRR in forage and straw was identified/characterized as

glucose and malonyl glucose conjugates of CGA-118244 and CGA-118245 (56-62% of the TRR in forage and straw and 10% of the TRR in chaff). The parent, propiconazole, was identified as a minor component in forage (7.3% TRR, 0.062 ppm), straw (3.9% TRR, 0.134 ppm), chaff (1.7% TRR, 0.003 ppm), and grain (0.4% TRR, <0.001 ppm). Free Phase I Metabolites (CGA-91304, CGA-91305, GB-XLIII-42, CGA-118244, CGA-118245, and CGA-136735) were also minor components, with each accounting for ≤1.2% of the TRR (<0.001-0.042 ppm). With the exception of Unknown fraction U1 (40% TRR; 0.047 ppm) in wheat grain, isolated unknown fractions accounted for <10% of the TRR in each matrix. The majority (35% TRR) of fraction U1 in wheat grain was released by cellulase hydrolysis and was very polar in nature, eluting with endogenous sugars.

cc : Chem F, Chron F. Morton

RDI:Team: 12/2/99; SVH:12/14/99

TM, Thurston Morton, Rm. 816D CM2, 305-6691, mail code 7509C

PROPICONAZOLE
PC Code 122101; Case 3125
(DP Barcode D245249)

Registrant's Response to Residue Chemistry Data Requirements

December 14, 1999

Contract No. 69-W-99-053

Submitted to:
U.S. Environmental Protection Agency
Arlington, VA

Submitted by:
Dynamac Corporation
The Dynamac Building
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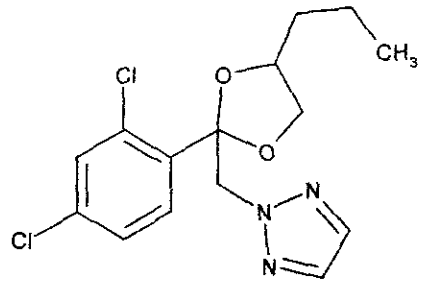
PROPICONAZOLE



PC Code 122101; Case 3125

(DP Barcode D245249)

REGISTRANT'S RESPONSE TO RESIDUE CHEMISTRY DATA REQUIREMENTS

BACKGROUND

Novartis Crop Protection, Inc. has submitted data pertaining to the metabolism of [^{14}C]propiconazole in spring wheat (1997; MRID 44381402). The submitted data are evaluated herein for adequacy in fulfilling residue chemistry data requirements for the reregistration of propiconazole. The Conclusions and Recommendations stated below pertain only to the nature of propiconazole residues in wheat. Other residue chemistry data requirements stated in the Propiconazole Phase 4 Review (6/25/92) are not addressed herein.

The Propiconazole Phase 4 Review, dated 6/25/92 required data depicting the metabolism of phenyl-[^{14}C]propiconazole in wheat, bananas, and pecans. Subsequently, the Agency concluded that a metabolism study on celery would satisfy the nature of the residue in plants (DP Barcode D198815, CBRS No. 13166, F. Fort, 4/26/94). An adequate celery metabolism study has been submitted (T. Morton, D233755, 9/14/99) and the residues of concern in/on plant commodities were determined to be parent propiconazole and its metabolites determined as 2,4-DCBA. Because only a small portion of propiconazole was metabolized in celery, a wheat metabolism study was proposed by Novartis to further investigate the nature of phenyl-[^{14}C]-propiconazole in plants.

The nature of the residue in animals is adequately understood. Adequate poultry and ruminant metabolism studies have been submitted (F. Fort, 4/26/94).

Tolerances, regional tolerances, and interim tolerances are established for residues of

propiconazole (1-[[2-(2,4-dichlorophenyl)-4-propyl-1,3-dioxolan-2-yl]methyl]-1H-1,2,4-triazole) and its metabolites determined as 2,4-dichlorobenzoic acid and expressed as parent compound in/on various plant and animal commodities [40 CFR §180.434]. No tolerances have been established for residues in processed food/feed commodities.

Residue methods AG-454 and AG-517 and methods AG-626 and AG-629 (modifications of methods AG-454 and AG-517) are available for determination of propiconazole and its metabolites in plant and animal commodities, respectively. The methods use a single moiety detection in which residues are converted to 2,4-dichlorobenzoic acid (2,4-DCBA) methyl ester and reported as propiconazole equivalents. Methods AG-454 and AG-517 have been successfully validated by the Agency, and because methods AG-626 and AG-629 only include modifications allowing for the use of methyl iodide as the methylation agent, instead of diazomethane, no independent or Agency laboratory validations are required. All methods have been forwarded to FDA for publication in PAM Vol. II for enforcement purposes.

Codex MRLs have been established for residues of propiconazole in various plant and animal commodities; issues of compatibility between Codex MRLs and U.S. tolerances will be addressed when the reregistration eligibility decision for propiconazole is made.

CONCLUSIONS AND RECOMMENDATIONS

1. The qualitative nature of propiconazole residues in wheat is adequately understood. Total radioactive residues were 0.844 ppm in/on forage, 3.450 ppm in/on straw, 0.156 ppm in/on chaff, and 0.119 ppm in/on grain harvested at 12 days (forage) or 77 days following a single foliar application of [phenyl-U-^{14}C]propiconazole at 0.1 lb ai/A/application (~1x the maximum seasonal rate). Following a similar application at 5x, TRRs were 3.78, 16.88, and 0.154 ppm in/on wheat forage, straw, and grain, respectively.

2. Identified or characterized ^{14}C-residues in RACs from wheat treated at 1x accounted for 75-92% of the TRR in/on wheat forage and straw and 25-70% of the TRR in/on wheat chaff and grain. The majority of TRR in forage and straw was identified/characterized as glucose and malonyl glucose conjugates of CGA-118244 and CGA-118245 (56-62% of the TRR in forage and straw and 10% of the TRR in chaff). The parent, propiconazole, was identified as a minor component in forage (7.3% TRR, 0.062 ppm), straw (3.9% TRR, 0.134 ppm), chaff (1.7% TRR, 0.003 ppm), and grain (0.4% TRR, <0.001 ppm). Free Phase I Metabolites (CGA-91304, CGA-91305, GB-XLIII-42, CGA-118244, CGA-118245, and CGA-136735) were also minor components, with each accounting for ≤1.2% of the TRR (<0.001-0.042 ppm). With the exception of Unknown fraction U1 (40% TRR; 0.047 ppm) in wheat grain, isolated unknown fractions accounted for <10% of the TRR in each matrix. The majority (35% TRR) of fraction U1 in wheat grain was released by cellulase hydrolysis and was very polar in nature, eluting with endogenous sugars.

3. The registrant proposed that propiconazole is metabolized in wheat primarily by hydroxylation of the α-, β-, or γ-carbon on the n-propyl group of the dioxolane ring to form CGA-136735, CGA-118244, or CGA-118245, respectively. These metabolites subsequently undergo conjugation with glucose or malonyl glucose. Other minor metabolic pathways involve the deketalization of the dioxolane ring to the ketone (CGA-91304) which is then hydrolyzed to the alkanol (CGA-91305) or dehalogenated and hydroxylated on the phenyl ring to yield GB-XLIII-42-1.

DETAILED CONSIDERATIONS

OPPTS GLN 860.1300: Nature of the Residue in Plants

Wheat

Novartis submitted data (1997; MRID 44381402) depicting the metabolism of [phenyl-U-^{14}C]propiconazole in spring wheat. The biological and analytical phases of the study were conducted by Novartis; the in-life phase was conducted in Greensboro, NC and the sample homogenization and TRR determinations were conducted at Novartis' Vero Beach Research Center (VBRC, Vero Beach, FL). Metabolite characterization and identification were conducted at the Novartis Laboratory in Greensboro, NC.

Use directions: Novartis included a specimen label for the 3.6 lb/gal EC (EPA Reg. No. 100-617) formulation of propiconazole registered for use on cereal grains. The 3.6 lb/gal EC is registered for a single foliar application to wheat during flag leaf emergence at up to 0.11 lb ai/A using ground or aerial equipment. No pre-harvest interval is specified, but application is prohibited after the flag leaf emergence (Feekes growth stage 8). The label also prohibits the feeding or grazing of treated wheat by livestock or the cutting of the green crop for hay or silage. The maximum seasonal application rate is 0.11 lb ai/A.

In-life phase: The [phenyl-U-^{14}C]propiconazole had a specific activity of 46.5 μCi/mg (98.6% radiochemical purity) and was formulated as a 3.6 lb/gal EC for application. Wheat plants were grown in the greenhouse in pots containing a sandy loam soil. Thirty-four days following planting, wheat plants were treated with one foliar application at 0.1 lb ai/A (~1x the maximum application rate) or 0.5 lb ai/A (~5x). Twelve pots each were treated at the 1x and 5x rate; five additional pots were treated with a blank formulation for controls.

Wheat forage (50% maturity) and mature wheat plants were harvested 12 and 77 days, respectively, following treatment. Whole wheat plants were cut ~1 inch above the soil, and mature wheat was separated into straw, chaff, and grain. Straw and forage samples were cut into 1-2 inch segments. Samples were bagged, frozen, and shipped to VBRC for homogenization and TRR analysis. Frozen homogenized samples were then shipped to the Greensboro, NC laboratory for characterization and identification of the residues. Samples were maintained in frozen storage (~-20 C) at all sites.

Total radioactive residue (TRR)

At VBRC, samples were homogenized with dry ice, combusted, and radioassayed by liquid scintillation counting (LSC) in triplicate. The limit of quantitation (LOQ) for the radioassays was implied to be 0.001 ppm. The TRR, expressed in [^{14}C]propiconazole equivalents, are presented in Table 1.

Table 1. Total radioactive residues found in/on wheat RACs harvested following a single foliar application of [phenyl-^{14}C]propiconazole at 0.1 lb ai/A (1x) or 0.5 lb ai/A (5x).

Matrix	Sampling interval (DAT)	Total Radioactive Residues (ppm) [a]	
		1x-treatment	5x-treatment
Wheat, forage (50% maturity)	12	0.844	3.780
Wheat, straw	77	3.450	16.882
Wheat, chaff	77	0.156	0.280
Wheat, grain	77	0.119	0.154

[a] TRR values are expressed in [^{14}C]propiconazole equivalents and are the average of triplicate analyses.

Extraction and hydrolysis of residues

Homogenized wheat samples were subjected to extraction and hydrolysis procedures for residue characterization at the Novartis Laboratory in Greensboro, NC. During the fractionation procedures, aliquots of extracts and nonextractable residues were analyzed for radioactivity by LSC or combustion/LSC. The general extraction procedures are summarized below.

Homogenized wheat samples were extracted (2x) with methanol:water (9:1, v:v) and filtered. The filtercake was rinsed with additional MeOH:water (9:1, v:v) and the rinsate and filtrates were combined. The combined extracts were applied to a flash C_{18} column, and ^{14}C-residues were eluted with MeOH:water (9:1, v:v) followed by MeOH. The eluates were combined, concentrated by rotary evaporation, partitioned 3x with ethyl acetate (EtOAc), and centrifuged. Aliquots of the organic and aqueous phases were each analyzed by HPLC and 2D-TLC. Additional aliquots of the aqueous phase were subjected to hydrolysis with enzyme (cellulase, 0.1 M NaOAc buffer, pH 4.6, at 37 C for 12 hours) and acid (3 N HCl at 95 C for 1 hour). The resulting hydrolysates were analyzed by HPLC and 2D-TLC.

^{14}C-Residues remaining in post-extraction solids (PES) of 1x-treated wheat straw and grain were sequentially extracted and/or hydrolyzed by (i) refluxing in MeOH:water (9:1, v:v) for 4 hours; (ii) refluxing in 1% NaCl for 4 hours; (iii) incubating with cellulase (0.1 N NaOAc buffer, pH 4.6) at 37 C for 24 hours; (iv) incubating with protease (0.2 N TRIS/HCl buffer, pH 7) at 37 C for 24-42 hours; (v) stirring in 0.5 N HCl for 17-23 hours; (vi) stirring in 0.5 N NaOH for 18-67

hours; (vii) refluxing in 6 N HCl for 4 hours; and (viii) refluxing in 6 N NaOH for 4 hours.

Selected fractions of radioactivity released from the PES of 1x wheat straw and grain were cleaned-up by flash C_{18} chromatography, as above, and analyzed by HPLC and 2D-TLC. For 1x wheat straw PES, the MeOH:water reflux, 1% NaCl reflux, cellulase/protease hydrolysates, and the weak base hydrolysate were further analyzed. The mild acid hydrolysate and the strong acid and base hydrolysates from straw were not further analyzed as each of these fractions contained only minor amounts of radioactivity (<1% TRR, <0.03 ppm). For the wheat grain PES, only the cellulase hydrolysate (35.4% TRR, 0.42 ppm) was analyzed; radioactivity in each of the remaining fractions accounted for <10% of the TRR (≤0.012 ppm).

The distribution of ^{14}C-activity in the extracts and hydrolysates of 1x-treated wheat matrices is presented in Table 2. As ^{14}C-residues released by solvent extraction of 1x and 5x samples were similar, only a summary table for ^{14}C-residues in 5x wheat RAC is presented in this report.

Table 2. Distribution and characterization of radioactive residues in wheat treated once with a foliar application of [^{14}C]propiconazole at 0.1 lb ai/A (~1x).

Fraction	% TRR [a]	ppm	Characterization/Identification [b]		
Wheat, forage (1x; TRR = 0.844)					
Organic	30.9	0.261	HPLC and TLC analyses resolved:		
			Propiconazole	7.3% TRR	0.062 ppm
			CGA-91304	0.3% TRR	0.003 ppm
			CGA-91305	0.3% TRR	0.003 ppm
			GB-XLIII-42	0.3% TRR	0.003 ppm
			CGA-118244	0.4% TRR	0.003 ppm
			CGA-118245	0.2% TRR	0.002 ppm
			A1b	3.4% TRR	0.029 ppm
			A2a	0.8% TRR	0.007 ppm
			A2b and A6	8.6% TRR	0.073 ppm
			A3b	1.4% TRR	0.012 ppm
			A3c	6.8% TRR	0.057 ppm
			Unknowns U1, U2, and U4	1.1% TRR	0.009 ppm
Aqueous	48.1	0.406	HPLC and TLC analyses resolved:		
			A1a	1.0% TRR	0.008 ppm
			A5	1.1% TRR	0.009 ppm
			A1b	5.7% TRR	0.048 ppm
			A2a	1.9% TRR	0.016 ppm
			A2b	7.5% TRR	0.063 ppm
			A6	6.5% TRR	0.055 ppm
			A3b and A3c	11.8% TRR	0.100 ppm
			Unknown U1 and U2	4.2% TRR	0.035 ppm
			Unknown U3	3.8% TRR	0.032 ppm
			Unknown U4	1.0% TRR	0.008 ppm

Fraction	% TRR [a]	ppm	Characterization/Identification [b]		
PES	21.0	0.177	Not further analyzed.		
Wheat, straw (1x; TRR = 3.450)					
Organic	36.6	1.263	HPLC and TLC analyses resolved:		
			Propiconazole	3.9% TRR	0.134 ppm
			CGA-91304	1.2% TRR	0.042 ppm
			CGA-91305	0.1% TRR	0.004 ppm
			GB-XLIII-42	0.1% TRR	0.005 ppm
			CGA-118244	1.0% TRR	0.034 ppm
			CGA-118245	0.4% TRR	0.013 ppm
			CGA-136735	0.1% TRR	0.005 ppm
			A1a	0.8% TRR	0.028 ppm
			A5	0.3% TRR	0.010 ppm
			A1b	5.5% TRR	0.190 ppm
			A2a	1.6% TRR	0.055 ppm
			A2b	4.2% TRR	0.145 ppm
			A6	7.0% TRR	0.242 ppm
			A3b	1.1% TRR	0.038 ppm
			A3c	7.2% TRR	0.248 ppm
			Unknowns U1-U4	2.0% TRR	0.069 ppm
Aqueous	26.4	0.911	HPLC and TLC analyses resolved:		
			U1	2.3% TRR	0.079 ppm
			U2	0.6% TRR	0.021 ppm
			U3	2.4% TRR	0.083 ppm
			U4	0.5% TRR	0.017 ppm
			A1a	1.1% TRR	0.038 ppm
			A5	0.4% TRR	0.014 ppm
			A1b	2.7% TRR	0.093 ppm
			A2a	1.0% TRR	0.035 ppm
			A2b	5.3% TRR	0.183 ppm
			A6	2.0% TRR	0.069 ppm
			A3b	2.8% TRR	0.097 ppm
			A3c	2.4% TRR	0.083 ppm
PES	37.0	1.277	Subjected to sequential enzyme and chemical hydrolyses.		
MeOH:water reflux	16.4	0.567	HPLC analyses resolved:		
			A1a	0.1% TRR	0.004 ppm
			A5	0.2% TRR	0.007 ppm
			A1b	0.2% TRR	0.005 ppm
			A2a and A2b	0.7% TRR	0.024 ppm
			A6	3.3% TRR	0.114 ppm
			A3b	1.7% TRR	0.006 ppm
			A3c	6.0% TRR	0.206 ppm
			Unknowns U1-U4	4.4% TRR	0.152 ppm

Fraction	% TRR [a]	ppm	Characterization/Identification [b]		
1% NaCl reflux	6.3	0.218	HPLC analyses resolved:		
			Phase I Metabolites	4.0% TRR	0.139 ppm
			A2b	0.2% TRR	0.007 ppm
			A6	0.6% TRR	0.019 ppm
			A3b	0.4% TRR	0.013 ppm
			A3c	1.1% TRR	0.038 ppm
			Unknown U4	0.1% TRR	0.004 ppm
Cellulase	2.1	0.072	HPLC analyses resolved:		
			Phase I Metabolites	2.3% TRR	0.078 ppm
			A6	0.2% TRR	0.008 ppm
Protease	1.6	0.055	A3b	0.1% TRR	0.004 ppm
			A3c	0.4% TRR	0.015 ppm
			Unknowns U2, U3, U4	0.6% TRR	0.020 ppm
0.5N HCl	0.8	0.029	Not further analyzed.		
0.5N NaOH	4.3	0.148	HPLC analyses resolved:		
			A3b and A3c	3.8% TRR	0.131 ppm
			Unknowns U1and U2	0.4% TRR	0.011 ppm
6N HCl	0.6	0.021	Not further analyzed.		
6N NaOH	0.3	0.011			
Nonextractable	2.7	1.0			
Wheat, chaff (1x; TRR = 0.156)					
Organic	10.7	0.017	HPLC and TLC analyses resolved:		
			Propiconazole	1.7% TRR	0.003 ppm
			CGA-91305	0.7% TRR	0.001 ppm
			CGA-118244 and CGA-118245	0.4% TRR	0.001 ppm
			U4	1.2% TRR	0.002 ppm
			A1a	0.9% TRR	0.001 ppm
			A5	0.3% TRR	<0.001 ppm
			A1b	0.9% TRR	0.001 ppm
			A2b and A6	2.0% TRR	0.003 ppm
			A3b	0.8% TRR	0.001 ppm
			A3c	1.8% TRR	0.003 ppm
Aqueous	14.5	0.023	HPLC and TLC analyses resolved:		
			U1 and U2	6.2% TRR	0.010 ppm
			U3	1.0% TRR	0.002 ppm
			U4	2.5% TRR	0.004 ppm
			A1a	0.5% TRR	0.001 ppm
			A5	1.6% TRR	0.002 ppm
			A1b	0.2% TRR	<0.001 ppm
			A3b	1.0% TRR	0.002 ppm
			A3c	1.1% TRR	0.002 ppm
PES	74.8	0.117	Not further analyzed.		

Fraction	% TRR [a]	ppm	Characterization/Identification [b]		
Wheat, grain (1x; TRR = 0.119)					
Organic	2.3	0.003	HPLC and TLC analyses resolved:		
			Propiconazole	0.4% TRR	<0.001 ppm
			CGA-91304/CGA-91305	0.1% TRR	<0.001 ppm
			CGA-118244/CGA-118245	0.4% TRR	<0.001 ppm
			A5	0.3% TRR	<0.001 ppm
			A1b	0.1% TRR	<0.001 ppm
			A3b	0.1% TRR	<0.001 ppm
			A3c	0.3% TRR	<0.001 ppm
			Unknowns U3 and U4	0.7% TRR	0.001 ppm
Aqueous	5.3	0.006	HPLC and TLC analyses resolved:		
			Unknown U1	4.6% TRR	0.005 ppm
			A1a	0.7% TRR	0.001 ppm
Nonextractable	92.4	0.110	Subjected to sequential extraction and hydrolysis.		
MeOH:water reflux	4.3	0.005	N/A.		
1% NaCl reflux	9.9	0.012	N/A.		
Cellulase	35.4	0.042	HPLC analyses resolved:		
			Unknown U1	35.4% TRR	0.042 ppm
Protease	5.3	0.006	Not further analyzed.		
0.5N HCl	<0.1	<0.001			
0.5N NaOH	1.8	0.002			
6N HCl	<0.1	<0.001			
6N NaOH	5.8	0.007			
Nonextractable	1.2	0.001			

[a] Percent TRR values were normalized by the registrant; total percent recoveries from extractable and nonextractable residues were ~103% for immature wheat (1x), ~108% for wheat forage (1x), ~114% for wheat chaff (1x), ~111% for wheat grain (1x), ~99% for immature wheat (5x), and ~106% for wheat forage (5x).

[b] Initial identifications were made using HPLC, and confirmation was made using TLC. Percent TRR were calculated by the study from the % residues in the fraction, and ppm values were calculated from the % TRR.

[c] Phase I Metabolites (CGA-91304, CGA-91305, GB-XLIII-42, CGA-118244, CGA-118245, and CGA-136735) were reported as a group for the enzyme and chemical hydrolysates of forage and grain nonextractable residues.

Characterization/identification of residues

Radioactive residues in solvent extracts and hydrolysates were analyzed by reverse-phase HPLC and 2D-TLC. Radioactivity in metabolite fractions was quantified using HPLC analysis and metabolite identities were confirmed by TLC and GC/MS. Metabolites were identified by cochromatography with nonlabeled reference standards which included: propiconazole, CGA-91304, CGA-91305, CGA-118244, CGA-118245, CGA-136735, CGA-177291, CGA-217495, and GB-XLIII-42-1. Representative chromatograms and example calculations were submitted.

HPLC analyses were conducted using an ODS-2 reverse-phase column with a UV detector (225 nm) and mobile phase gradients of either MeOH:0.02 M H_3PO_4 (1:1v/v) to ACN, or 0.05% Na_2HPO_4 to MeOH. Radioactivity was quantitated by LSC for collected fractions.

1D- and 2D-TLC analyses were performed using normal-phase silica gel plates with one or more

of the following solvent systems: EtOAc:$CHCl_3$:ACN:acetic acid:water (40:40:17:1:2, v:v:v:v:v); EtOAc:MeOH:NH_4OH (85:10:5, v:v:v); 1-butanol:acetic acid:water (80:10:10, v:v:v); and methyl ethyl ketone:acetic acid:water (60:10:10, v:v:v). Radioactivity was quantitated using a radioanalytic imaging system.

Fourteen regions of common polar metabolites were distributed through the organic and aqueous extracts of the various wheat matrices; these regions were initially characterized by HPLC and 2D-TLC. The aqueous extracts were then subjected to acid hydrolysis (3N HCl) and the hydrolysates were analyzed by co-chromatography with chemical standards by HPLC and 2D-TLC. Aglycones released by acid hydrolysis included CGA-118244, CGA-118245, and CGA-136735 in wheat forage and straw, and CGA-118244 and CGA-118245 in wheat chaff. None of the aqueous soluble residues in 1x-treated wheat grain were hydrolyzed to Phase 1 metabolites (CGA-91304, CGA-91305, GB-XLIII-42, CGA-118244, CGA-118245, and CGA-136735); however, aqueous soluble ^{14}C-residues from 5x-treated grain released aglycones of CGA-118244 and CGA-118245 upon acid hydrolysis. The organic extracts were not subjected to acid hydrolysis because the organic and aqueous extracts contained generally the same metabolites.

To further characterize aqueous soluble ^{14}C-residues, separate aliquots of the aqueous phase from the 5x-treated wheat forage were further fractionated and analyzed by HPLC and A-25 anion exchange chromatography. The aqueous phase was initially separated into three fractions (A1, A2, and A3) using preparative HPLC, and each fraction was further fractionated by preparative HPLC. Separate subsamples of the resulting fractions A1, A2, A3, A1a, A1b, A2a, A2b, A3b, A3c, and A6 (combined A2c, A2d, A2e, and A3a fractions) were analyzed directly by HPLC, or subjected to enzyme hydrolysis, acid hydrolysis (excluding fraction A6), A-25 anion exchange chromatography (fraction A6 only), or further fractionated by HPLC for MS analysis (fractions A2a, A2b, A3b, and A3c only). Each of the hydrolysates were also analyzed by HPLC and 2D-TLC.

- Fraction A1a was determined to be a complex mixture of metabolites of which 86% is acidic as demonstrated by A25 anion exchange chromatography. Enzyme hydrolysis produced limited release of phase 1 metabolites indicating that the components of A1a were not malonyl glucose conjugates.

- Fraction A1b consisted of two main regions, with ~88% of the fraction being acidic in nature. Enzyme hydrolysis released mainly CGA-118244 indicating that the main component of A1b was a malonyl glucose conjugate of CGA-118244.

- Fraction A2a consisted of two main regions, with ~77% of the fraction being acidic in nature. Enzyme hydrolysis released CGA-118245 as the main aglycone, along with minor amounts of CGA-118244. MS analysis confirmed that the two main regions of A2a are malonyl glucose and glucose conjugates of CGA-118245.

- Fraction A2b consisted of two main regions with ~69% of the fraction being acidic in

nature. Enzyme hydrolysis released CGA-118244, and MS analysis confirmed that the two regions of A2b are the malonyl glucose and glucose conjugates of CGA-118244.

- Fraction A3b consisted of one neutral region, as demonstrated by A25 anion exchange chromatography. Enzyme hydrolysis released CGA-118244 as the main aglycone, along with minor amounts of CGA-118245 and CGA-136735. MS analysis confirmed that the main component of A3b is the glucose conjugate of CGA-118244.

- Fraction A6 consisted of one neutral region, as demonstrated by A25 anion exchange chromatography. Following enzyme hydrolysis, CGA-118244 and CGA-118245 were released as the major aglycones, along with a minor amount of CGA-136735. Therefore, the main metabolites in A6 were characterized as glucose conjugates of CGA-118244 and CGA-118245.

To further characterize the residues in the organic phase, the 5x-treated mature wheat forage organic phase was further purified by preparative HPLC. Region A5 was isolated and further separated into three fractions (A5a, A5b, and A5c). Subsamples were analyzed directly by HPLC or subjected to A-25 anion exchange chromatography to determine if residues were glucose conjugates (neutral) or malonyl glucose conjugates (acidic). Additional subsamples were subjected to enzyme (cellulose) hydrolysis. Each of the hydrolysates was analyzed by HPLC and 2D-TLC.

- Fractions A5a and A5b were each comprised of several regions, with ~80-88% being acidic in nature. Enzyme hydrolysis released CGA-118244 as the major aglycone. Therefore, the main metabolites in A5a and A5b were considered to be the various isomers of malonyl glucose conjugates of CGA-118244.

- Fraction A5c was comprised of one neutral region that released CGA-118244 as the major component upon enzyme hydrolysis. Therefore, A5c was considered to be the glucose conjugate of CGA-118244.

A summary of the characterized and identified ^{14}C-residues in 1x and 5x-treated wheat is presented in Tables 3 and 4, respectively. The chemical structures for identified metabolites are presented in Figure 1.

Table 3. Summary of radioactive residues characterized/identified in wheat RACs from plants treated with a single foliar application of [^{14}C]propiconazole at 0.1 lb ai/A (~1x maximum application rate). [a]

Compound/Fraction (*HPLC fractions*)	Wheat, forage (TRR = 0.844 ppm)		Wheat, straw (TRR = 3.450 ppm)		Wheat, chaff (TRR = 0.156 ppm)		Wheat, grain (TRR = 0.119 ppm)	
	% TRR	ppm [b]	% TRR	ppm	% TRR	ppm	% TRR	ppm
Propiconazole	7.3	0.062	3.9	0.134	1.7	0.003	0.4	<0.001
CGA-91304	0.3	0.003	1.2	0.042	ND	--	0.1	<0.001
CGA-91305	0.3	0.003	0.1	0.004	0.7	0.001		
GB-XLIII-42	0.3	0.003	0.1	0.005	ND	--	ND	--
CGA-118244	0.4	0.003	1.0	0.034	0.4	0.001	0.4	<0.001
CGA-118245	0.2	0.002	0.4	0.013				
CGA-136735	ND	--	0.1	0.005	ND	--	ND	--
Phase I Metabolites [c]	NA	--	6.3	0.217	NA	--	NA	--
Malonyl glucose conjugates of CGA-118244 isomers (*A5/A1b/A2b*)	32.7 [d]	0.276	18.8	0.651	5.1 [d]	0.008	0.4	<0.001
CGA-118245 isomers (*A2a*)	2.7	0.023	3.3	0.114	ND	--	ND	--
Glucose conjugates of CGA-118244/CGA-118245 (*A6*)	--	--	13.1	0.453	--	--	ND	--
CGA-118244 isomers (*A3b and A3c*)	20.1	0.169	27.1	0.933	4.7	0.007	0.4	<0.001
Total identified	**64.3**	**0.544**	**75.4**	**2.605**	**12.6**	**0.020**	**1.7**	**0.002**
A1a - acidic complex	1.0	0.009	2.0	0.070	1.5	0.002	0.7	0.001
Unknown U1	4.8 [e]	0.040	5.2	0.180	6.2 [e]	0.010	40.0 [f]	0.047
Unknowns U2, U3, and U4	5.2	0.044	8.1	0.280	4.6	0.008	0.7	0.001
Unanalyzed hydrolysates [g]	NA	--	1.7	0.061	NA	--	27.1	0.032
Total identified/characterized	**75.3**	**0.637**	**92.4**	**3.196**	**24.9**	**0.040**	**70.2**	**0.083**
Unextracted	21.0	0.177	1.0	0.035	74.8	0.117	1.2	0.001

[a] See Figure 1 for structures of identified metabolites.

[b] Expressed in [^{14}C]propiconazole equivalents.

[c] Phase I Metabolites (CGA-91304, CGA-118244, CGA-118245, and CGA-136735) released by enzyme and chemical hydrolysis of wheat straw PES were reported as one group.

[d] Fraction includes unresolved fraction A6 containing Glucose conjugate of CGA-118244/CGA-118245 isomers.

[e] Fraction also includes radioactivity from U2.

[f] Cellulase hydrolysis of wheat grain released 35.4% of the TRR (0.042 ppm) as Unknown fraction U1, which was very polar in nature, eluting at the same time as endogenous sugars.

[g] Unanalyzed hydrolysate fractions include 0.5N HCl, 6N HCl, and 6N NaOH hydrolysates from wheat straw, and all hydrolysate fractions except the cellulase supernatant for wheat grain.

Table 4. Summary of radioactive residues characterized/identified in wheat RACs from plants treated with a single foliar application of [^{14}C]propiconazole at 0.5 lb ai/A (~5x).

Compound/Fraction (*HPLC fractions*)	Wheat, forage (TRR = 3.780 ppm)		Wheat, straw (TRR =16.882 ppm)		Wheat, chaff (TRR = 0.280 ppm)		Wheat, grain (TRR = 0.154 ppm)	
	% TRR	ppm [a]	% TRR	ppm	% TRR	ppm	% TRR	ppm
Propiconazole	17.2	0.651	9.0	1.516	3.9	0.011	0.8	0.001
CGA-91304	0.3	0.011	1.5	0.250	0.8	0.002	0.3	<0.001
CGA-91305	0.3	0.012	0.1	0.024	0.8	0.002	<0.1	<0.001
GB-XLIII-42	0.3	0.011	0.1	0.024	ND	--	ND	--
CGA-118244	0.4	0.017	1.1	0.190	1.0	0.003	0.2	<0.001
CGA-118245	0.1	0.003	0.3	0.048	0.2	0.001	0.1	<0.001
CGA-136735	0.1	0.005	0.8	0.143	ND	--	ND	--
Malonyl glucose conjugates of CGA-118244 isomers (*A5/A1b/A2b*)	33.8 [b]	1.280	21.1	3.621	5.6	0.016	1.4	0.002
CGA-118245 isomer (*A2a*)	3.0	0.115	3.7	0.621	<0.1	<0.001	0.1	<0.001
Glucose conjugates of CGA-118244/CGA-118245 (*A6*)	--	--	3.8	0.636	3.0	0.008	~ 0.3	<0.001
CGA-118244 isomers (*A3b/A3c*)	18.8	0.712	12.8	2.158	6.0	0.017	2.6	0.004
Total identified	**74.3**	**2.817**	**54.3**	**9.231**	**21.4**	**0.060**	**5.9**	**0.009**
A1a - acidic complex	1.0	0.038	0.4	0.067	0.5	0.001	1.2	0.002
Unknown U1	2.0	0.076	2.0	0.330	6.8	0.019	3.7	0.006
Unknowns U2, U3, and U4	4.0	0.150	3.7	0.622	7.1	0.020	2.7	0.004
Total identified/characterized	**81.3**	**3.081**	**60.4**	**10.250**	**35.8**	**0.100**	**13.5**	**0.021**
Unextracted [c]	17.2	0.650	36.3	6.128	64.4	0.180	86.5	0.133

[a] Expressed in [^{14}C]propiconazole equivalents.
[b] Includes unresolved fraction A6 containing Glucose conjugate of CGA-118244/CGA-118245 isomers.
[c] Samples from 5x treated plants were only subjected to solvent extraction.

Storage stability: Dates for harvest, extraction, and analysis of the wheat metabolism samples were included with the submission. The petitioner stated that all samples were initially extracted, partitioned, and analyzed within 6 months of harvest; however, total storage intervals were 120-267 days (~4-9 months) for immature wheat, wheat forage, chaff, and grain, and up to 524 days (~17 months) for the second extraction of 1x-treated wheat forage. To demonstrate storage stability of residues of propiconazole, a separate sub-sample of 1x-treated wheat grain was extracted and analyzed by HPLC 524 days (~17 months) following harvest. Comparison of the 6-month and 17-month analyses of wheat grain demonstrated that residues of propiconazole were stable for up to 17 months in wheat grain; no additional storage stability data are required to support the storage conditions and intervals of the wheat metabolism study.

Conclusions: The qualitative nature of propiconazole residues in wheat is adequately understood. Total radioactive residues were 0.844 ppm in/on forage, 3.450 ppm in/on straw, 0.156 ppm in/on chaff, and 0.119 ppm in/on grain harvested 12 days (forage) or 77 days following a single foliar application of [phenyl-U-^{14}C]propiconazole at 0.1 lb ai/A/application (~1x the maximum proposed seasonal rate). Following a similar application at 5x, TRRs were 3.78, 16.88, and 0.154 ppm in/on wheat forage, straw, and grain, respectively.

In the 1x-treated wheat samples, identified or characterized ^{14}C-residues accounted for 75-92% of the TRR in/on wheat forage and straw and 25-70% of the TRR in/on wheat chaff and grain. The majority of TRR in forage and straw was identified/characterized as glucose and malonyl glucose conjugates of CGA-118244 and CGA-118245 (56-62% of the TRR in forage and straw and 10% of the TRR in chaff). The parent, propiconazole, was identified as a minor component in forage (7.3% TRR, 0.062 ppm), straw (3.9% TRR, 0.134 ppm), chaff (1.7% TRR, 0.003 ppm), and grain (0.4% TRR, <0.001 ppm). The free Phase I Metabolites (CGA-91304, CGA-91305, GB-XLIII-42, CGA-118244, CGA-118245, and CGA-136735) were also minor components, with each accounting for ≤1.2% of the TRR (<0.001-0.042 ppm). With the exception of Unknown fraction U1 (40% TRR; 0.047 ppm) in wheat grain, isolated unknown fractions accounted for <10% of the TRR in each matrix. The majority (35% TRR) of fraction U1 in wheat grain was released by cellulase hydrolysis and was very polar in nature, eluting with endogenous sugars.

The distribution of ^{14}C-residues in RACs from 5x-treated plants were similar, except that parent was present at higher levels (0.8-17.2% TRR).

Proposed metabolic pathway

Based on the results of the wheat metabolism study, the registrant proposed that propiconazole is primarily metabolized in wheat by hydroxylation of the α-, β-, or γ-carbon on the n-propyl group of the dioxolane ring to form CGA-136735, CGA-118244, or CGA-118245, respectively. These metabolites subsequently undergo conjugation with glucose or malonyl glucose. Other minor metabolic pathways involve the deketalization of the dioxolane ring to the ketone (CGA-91304) which is then hydrolyzed to the alkanol (CGA-91305) or dehalogenated and hydroxylated

on the phenyl ring to yield GB-XLIII-42-1.

MASTER RECORD IDENTIFICATION NUMBER

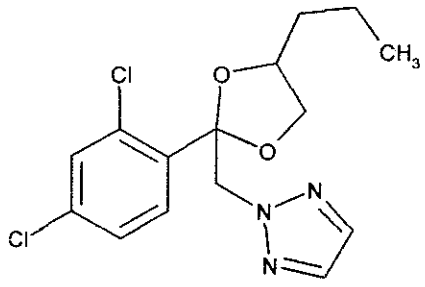
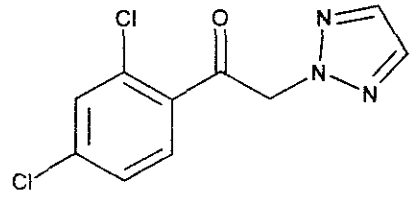
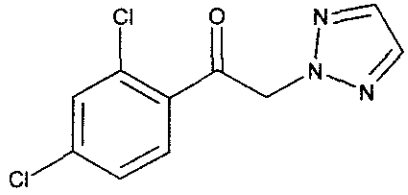
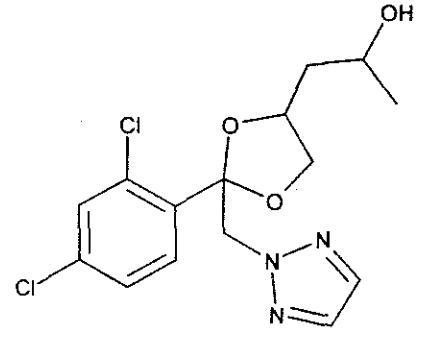
44381402 Swain, W. (1997) Uptake and Metabolism of CGA-64250 in Greenhouse Grown Spring Wheat after Spray Treatment with Phenyl--(carbon 14)-CGA--64250: Lab Project Number: ABR-97039:502-95: BIOL-95018. Unpublished study prepared by Novartis Crop Protection, Inc. 170 p.

AGENCY MEMORANDA CITED

CBRS No.: 13166
DP Barcode: D198815
Subject: Propiconazole. 90 Day Response.
From: F. Fort
To: R. Gebken/B. Sidwell
Dated: 4/26/94
MRID(s): None

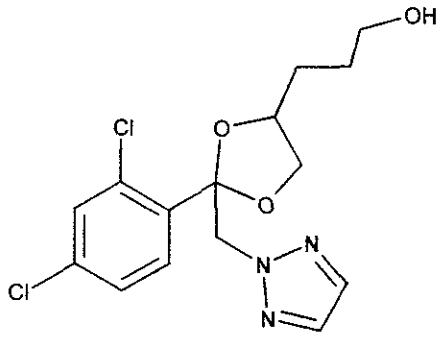
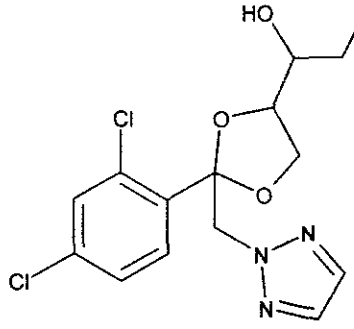
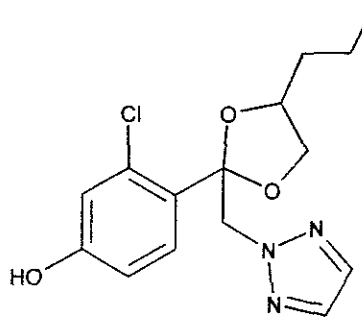
CBRS No.: none
DP Barcode: D233755
Subject: Propiconazole. Celery Metabolism Study.
From: T. Morton
To: M. Hartman/K. Monk
Dated: 9/14/99
MRID(s): 44049601

Figure 1. Propiconazole and its metabolites identified in wheat matrices (MRID 44381402).

Code Name Chemical Name	Chemical Structure	Matrices
CGA-64250 Propiconazole 1-[[2-(2,4-dichlorophenyl)-4-propyl-1,3-dioxolan-2-yl]methyl]-1H-1,2,4-triazole		Wheat forage, straw, chaff and grain
CGA-91304		Wheat forage, straw, chaff, and grain
CGA-91305		Wheat forage, straw and chaff
CGA-118244		Wheat forage, straw, chaff, and grain

(continued)

Figure 1. *Continued.*

Code Name Chemical Name	Chemical Structure	Matrices
CGA-118245		Wheat forage, straw, chaff, and grain
CGA-136735		Wheat straw
GB-XLIII-42-1		Wheat forage and straw