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003042

Chemical:	**Propiconazole**
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HED File Code	**11000 Chemistry Reviews**
Memo Date:	**09/14/99**
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01/19/2001

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

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

Sept 14, 1999

MEMORANDUM:

SUBJECT: **Propiconazole** (122101): Nature of the Residue Celery (GLN 860.1300) and Magnitude of the Residue in Sugar Cane (GLN 860.1500). **DP Barcode #** D233755. **Case 3125. MRID** # 44049601, 44142401.

FROM: Thurston G. Morton, Chemist
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THROUGH: Susan V. Hummel, Branch Senior Scientist
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TO: Mark Hartman/Kathy Monk, PM #52
Reregistration Section
Special Review & Reregistration Division (7508W)

EXECUTIVE SUMMARY:

Novartis has submitted data on celery and sugar cane in support of reregistration. All submitted studies (cited above) were reviewed by Dynamac Corporation under contract to EPA. The attached Dynamac review was modified to reflect Agency policies. Based on these studies and previously reviewed studies, HED makes the following conclusions:

- The celery metabolism study is adequate. Data gap satisfied for GLN 860.1300. Total radioactive residues were 0.854 ppm in celery collected 77 days following one foliar application of [phenyl-^{14}C]propiconazole at 0.5 lb ai/A (1x the maximum seasonal rate). Total radioactive residues were 3.124 ppm in celery collected 61 days following two foliar applications of [phenyl-^{14}C]propiconazole at 1.3 lb ai/A/application (2.66 lb/A seasonal application, 5x the maximum seasonal rate). Metabolite identification was performed on the extracts from the 5X treated samples. For celery treated at 5x, 89.7% of the TRR was identified. Extraction with MeOH:H_2O released 93.4% of the TRR (2.918 ppm), the majority of which was organosoluble (88.7% TRR) and was comprised of parent,

propiconazole (85.3% TRR, 2.664 ppm).

- The submitted data fulfill reregistration requirements for use of propiconazole on sugarcane. Total residues of [^{14}C]-propiconazole in the aerial portions of sugarcane grown in FL from seed pieces treated at 1x the maximum seasonal rate (cold dip or hot dip for 30 minutes) and harvested at ~11 months were non-detectable (<0.010 ppm). These data indicated that treatment of sugarcane seed pieces can be considered a non-food use. Data requirements are satisfied, no further residue data for sugarcane are required.

cc : Chem F, Chron F. Morton

RDI:Team: 9/14/99; SVH:9/15/99

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

PROPICONAZOLE
Shaughnessy No. 122101; Case 3125
(DP Barcode D233755)

Registrant's Response to Residue Chemistry Data Requirements

January 9, 1998

Contract No. 68-D4-0010

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

Submitted by:
Dynamac Corporation
1910 Sedwick Road
Durham, NC 27713

PROPICONAZOLE

Shaughnessy No. 122101; Case 3125

(DP Barcode D233755)

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 celery (1996; MRID 44049601) and to the magnitude of [^{14}C]propiconazole residue in sugarcane (1996; MRID 44142401). 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 the residue in celery and the magnitude of the residue in sugarcane. Other residue chemistry data requirements stated in the Propiconazole Phase 4 Review are not addressed herein.

The nature of the residue in animals is adequately understood. Adequate poultry and ruminant metabolism studies have been submitted (DP Barcode D198815, CBRS No. 13166, 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 or 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 are available for determination of propiconazole and its metabolites in plant and animal commodities, respectively. Both methods use a single moiety detection in which residues are converted to 2,4-dichlorobenzoic acid methyl ester and reported as propiconazole equivalents. Both methods have been successfully validated by the Agency and have been forwarded to FDA for publication in PAM Vol. II. 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

1a. The celery metabolism study is adequate. Total radioactive residues were 0.854 ppm in celery collected 77 days following one foliar application of [phenyl-^{14}C]propiconazole at 0.5 lb ai/A (1x the maximum seasonal rate). Total radioactive residues were 3.124 ppm in celery collected 61 days following two foliar applications of [phenyl-^{14}C]propiconazole at 1.3 lb ai/A/application (2.66 lb/A seasonal application, 5x the maximum seasonal rate) .

1b. For celery treated at 5x, 89.7% of the TRR was identified. Extraction with MeOH:H_2O released 93.4% of the TRR (2.918 ppm), the majority of which was organosoluble (88.7% TRR) and was comprised of parent, propiconazole (85.3% TRR, 2.664 ppm). Aqueous soluble residues accounted for 4.7% of the TRR and consisted of polar sugar conjugates. Metabolites isolated after acid hydrolysis of the aqueous fraction were CGA-91304, CGA-91305, and CGA-118244, each at ≤1.9% TRR (≤0.058 ppm). 6.6% of the TRR (0.206 ppm) remained in nonextractable solids.

1c. Similar characterization of the ^{14}C-residues in the 1x celery samples was noted. Extraction with MeOH:H_2O released 97.6% of the TRR (0.834 ppm), the majority of which was organosoluble (95% TRR, 0.811 ppm). Aqueous soluble residues accounted for 2.7% of the TRR (0.023 ppm) and nonextractable solids accounted for 2.4% TRR (0.020 ppm). Metabolite identification analyses were not performed on these sample extracts.

2. Aqueous soluble ^{14}C-residues in sample extracts of the 5x treated celery were analyzed by method AG-454B. ^{14}C-Residues in the aqueous fraction were converted to 2,4-dichlorobenzoic acid (2,4-DCBA) by method AG-454B. HPLC chromatograms and 2D-TLC profiles showed total conversion of the polar metabolites with an intact phenyl ring to 2,4-DCBA. Quantitative data were not provided.

3. The submitted data fulfill reregistration requirements for use of propiconazole on sugarcane. Total residues of [^{14}C]-propiconazole in the aerial portions of sugarcane grown in FL from seed pieces treated at 1x the maximum seasonal rate (cold dip or hot dip for 30 minutes) and harvested at ~11 months were non-detectable (<0.010 ppm). Sugarcane samples were stored frozen and analyzed within 3 days of harvest; therefore no storage stability data are required. These data indicated that treatment of sugarcane seed pieces can be considered a non-food use. Data requirements are satisfied, no further residue data for sugarcane are required.

DETAILED CONSIDERATIONS

Celery

Use patterns registered to Novartis

A REFS search dated 10/8/97 listed two propiconazole end-use products, two 3.6 lb/gal ECs (EPA Reg. Nos. 100-617 and 100-737), registered to Novartis for use on celery. The 3.6 lb/gal EC formulations are registered for foliar application to celery at 0.11 lb ai/A/application at a minimum retreatment interval of 7 days using ground or aerial equipment. A maximum of four applications may be made (0.45 lb ai/A/season) and a 14-day PHI has been established.

In-life phase

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 an adequate metabolism study on celery would satisfy the nature of the residue in plants (F. Fort, 4/26/94). In response, Novartis submitted data (1997; MRID 44049601) depicting the metabolism of [phenyl-^{14}C]propiconazole in celery. The biological and analytical phases of the study were conducted by Novartis (formerly Ciba-Giegy Corporation at Greensboro, NC and at Verno Beach, FL).

The test substance, [^{14}C]propiconazole, was formulated in at a ratio of 41.8/58.2 (wt. propiconazole/wt. inert ingredients), to the amounts that would be typically be found in a final spray solution of a 3.6 lb/gal EC formulation. The specific activity of the test substance was 31.8 μCi/mg with a radiochemical purity or 99.5%. Celery plants (one plant/pot) were grown in greenhouse cubicles with treated and control plants grown separately. Celery plants were treated with one foliar application at 0.5 lb ai/A (1x the maximum seasonal rate) at 138 days post-planting or with two foliar applications at 138 and 154 days post-planting at 1.3 lb ai/A/application (2.66 lb/A seasonal application, 5x). Control plants were treated with two applications of a formulation of the inerts only at 138 and 154 days post-planting.

Mature celery was harvested 61 or 77 days following treatment for the 5x and 1x plants, respectively. Whole plants were collected by cutting plants just above the soil surface. Samples were pooled according to treatment level, cut into 1-2 inch segments, bagged and transferred to freezers. Samples were held in frozen storage (~-20° C) until analysis. All laboratory analyses were completed within ~2.5 months of sample harvests.

Total radioactive residue (TRR)

Samples from each treatment 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 TRRs were 0.854 ppm and 3.124 ppm in celery samples treated at 1x and 5x, respectively.

Extraction and hydrolysis of residues

Homogenized celery samples for the 1x and 5x treatment rates were subjected to extraction and hydrolysis procedures for residue characterization. Metabolite identification was performed on the extracts from the 5x treated samples.

Celery samples were homogenized and extracted with MeOH:H_2O (9:1, v:v). Extractable residues were applied to a flash C-18 column and eluted with MeOH:H_2O (9:1, v:v) followed by 100% MeOH. Nonextractable solids were not processed further. The MeOH:H_2O extract was concentrated by rotary evaporation and partitioned 3x with ethyl acetate (EtOAc); organosoluble

residues were analyzed by HPLC and 2D-TLC. Aliquots of the aqueous soluble fraction were analyzed directly, characterized using anion exchange chromatography, or cleaned-up on a flash C-18 column, eluting with mixtures of 0.02M H_3PO_4:acetonitrile (ACN), H_2O:ACN, and finally 100% ACN. The cleaned-up aqueous fraction was analyzed directly or subjected to acid hydrolysis (6M HCl, 1 hour, 95° C) or cellulase hydrolysis (enzyme in 0.1M NaOAc, pH 4.6, 12 hours, 37° C) prior to analysis.

Selected aqueous fractions were further processed by sequential cleanup on a flash C-18 column, fractionation using anion exchange chromatography, further C-18 column cleanup, preparative HPLC followed by analysis or hydrolysis (cellulase or acid) prior to analysis. These additional analyses did not further elucidate the nature of the residue in celery, therefore, data from these analyses are not presented in Tables 1 or 2.

The distribution of ^{14}C-activity in the extracts and hydrolysates of celery is presented in Table 1.

Characterization/identification of residues

Radioactive residues in solvent extracts and hydrolysates were analyzed by reverse-phase HPLC and 2D-TLC. Metabolites were identified by comparison with HPLC and TLC chromatograms of the following reference standards: propiconazole, CGA-91304, CGA-91305, CGA-118244, CGA-118245, CGA-136735, CGA-177291, CGA-217495, and GB-XLIII-42-1. See Figure 1 for the chemical structures of these compounds. For organic fractions, HPLC analysis was used to quantify radioactivity with confirmation of metabolite identification performed by TLC. Radioactivity in aqueous fractions was quantitated by TLC with metabolite identification confirmed by HPLC. Adequate 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 gradient mobile phases of ACN and MeOH buffered with 0.02M H_3PO_4; ACN and H_2O; or MeOH buffered with 0.05% Na_2HPO_4. Radioactivity was quantitated using fraction collection/LSC.

2D-TLC analyses were performed on precoated silica gel plates using mobile phases of EtOAc:hexane (3:1, v:v) and chloroform ($CHCl_3$):2-propanol (9:1, v:v); EtOAc:$CHCl_3$:ACN:acetic acid:H_2O (40:40:17:1:2, v:v:v:v:v) and EtOAc:MeOH:ammonium hydroxide (85:10:5, v:v:v); or 1-butanol:acetic acid:H_2O (80:10:10, v:v:v) and methyl ethyl ketone:acetic acid:H_2O (60:10:10, v:v:v). Radioactivity was quantitated using a radioanalytic imaging system.

The distribution and characterization/identification of ^{14}C-activity in the extracts of celery are presented in Table 1. A summary of the identified ^{14}C-residues are presented in Table 2.

Table 1. Distribution and characterization/identification of radioactive residues in celery treated with [^{14}C]propiconazole at 2.6 lb ai/A (5x the maximum seasonal rate).

Fraction	% TRR	ppm[a]	Characterization/Identification
Celery (TRR = 3.124 ppm)			
MeOH:H_2O	93.4	2.918	Partitioned with EtOAc
EtOAc	88.7	2.772	HPLC analysis resolved, 2D-TLC confirmed: propiconazole 85.3% TRR, 2.664 ppm
Aqueous	4.7	0.146	2D-TLC and HPLC resolved highly polar components; subsamples subjected to anion exchange chromatography or cleaned up by prep C-18 and hydrolyzed using cellulase or acid reflux.
Neutral fraction from anion exchange chromatography	0.3	0.008	Not processed further.
Acidic fraction from anion exchange chromatography	4.4	0.138	Not processed further.
Cellulase hydrolysate	2.2	0.068	2D-TLC analysis resolved, HPLC confirmed: CGA-91305 1.6% TRR, 0.049 ppm CGA-118244 0.6% TRR, 0.018 ppm
Acid hydrolysate	4.4	0.137	2D-TLC analysis resolved, HPLC confirmed: CGA-91304 1.1% TRR, 0.035 ppm CGA-91305 1.9% TRR, 0.058 ppm CGA-118244 1.4% TRR, 0.043 ppm
Solids	6.6	0.206	Not processed further.

[a] PPM values were normalized to 100% recovery.

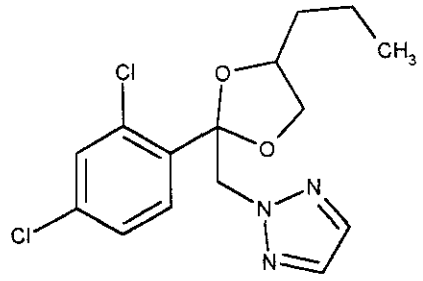
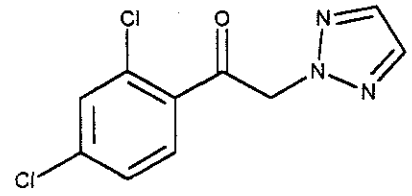
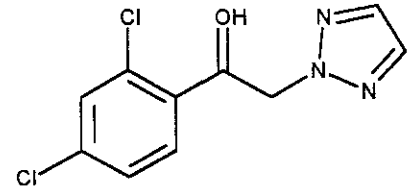
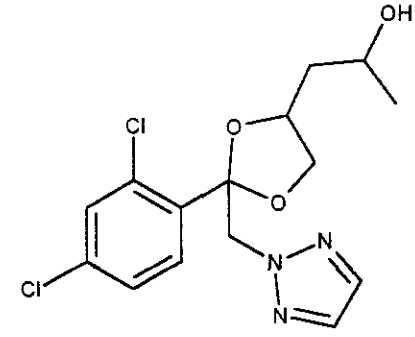
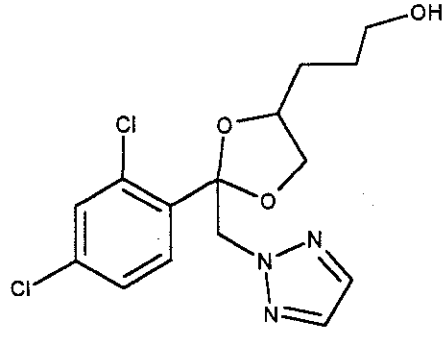
Table 2. Summary of characterized/identified ^{14}C-residues in celery treated with 2 applications of [^{14}C]propiconazole at 1.3 lb ai/A/application (2.66 lb/A seasonal application, 5x).

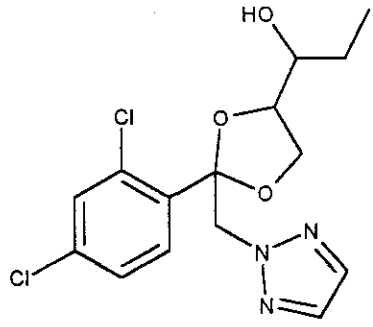
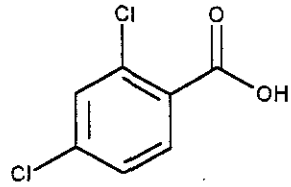
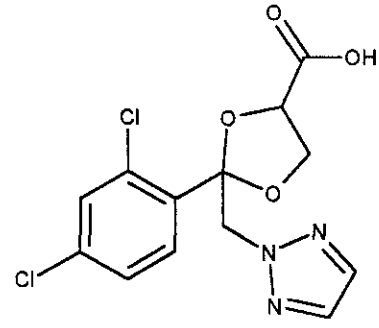
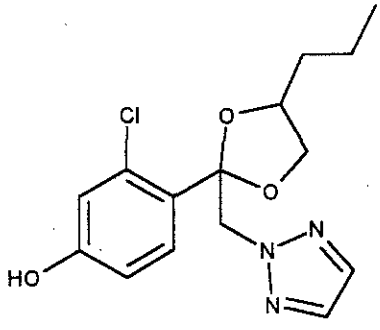
Metabolite	% TRR	ppm
Identified [a]		
Propiconazole	85.3	2.664
CGA-91304 [b]	1.1	0.035
CGA-91305 [b]	1.9	0.058
CGA-118244 [b]	1.4	0.043
Total identified	**89.7**	**2.800**
Nonextractable	6.6	0.206

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

[b] These metabolites were released after acid hydrolysis of the aqueous soluble residues.

Figure 1. Propiconazole and its metabolites in celery (MRID 44049601).

Code Name Chemical Name	Structure
CGA-64250 Propiconazole	
CGA-91304	
CGA-91305	
CGA-118244	
CGA-118245[a]	

Code Name Chemical Name	Structure
CGA-136735[a]	
CGA-177291[a]	
CGA-217495[a]	
GB-XLIII-42-1[a]	

[a] These compounds were used as reference standards, but were not detected in celery.

Storage stability

All celery samples and extracts were stored frozen (-20° C) prior to analysis and all samples were extracted and analyzed within 2.5 months of harvest.

To assess the storage stability of ^{14}C-residues in frozen celery, extracts of celery samples treated at 1x were analyzed by 2D-TLC and HPLC at 2.5 and ~15 months after harvest. In addition, subsamples of celery treated at 5x were extracted and analyzed by HPLC at 49 and 414 days after harvest.

The 2D-TLC profiles and HPLC chromatograms of the 1x sample extracts before and after storage were similar. The extractability of ^{14}C-residues from the 5x sample was similar after 12 months of storage and the HPLC chromatograms of each analysis showed similar qualitative and quantitative patterns of metabolite distribution from the two storage intervals. No additional storage stability data are required to support the present metabolism study.

Summary

The celery metabolism study is adequate. Total radioactive residues were 0.854 ppm in celery collected 77 days following one foliar application of [phenyl-^{14}C]propiconazole at 0.5 lb ai/A (1x). Total radioactive residues were 3.124 ppm in celery collected 61 days following two foliar applications of [phenyl-^{14}C]propiconazole at 1.3 lb ai/A/application (2.66 lb/A seasonal application, 5x).

For celery treated at 5x, 89.7% of the TRR was identified. Extraction with MeOH:H_2O released 93.4% of the TRR (2.918 ppm), the majority of which was organosoluble (88.7% TRR) and was comprised of parent, propiconazole (85.3% TRR, 2.664 ppm). Aqueous soluble residues accounted for 4.7% of the TRR and consisted of polar sugar conjugates. Metabolites isolated after acid hydrolysis of the aqueous fraction were CGA-91304, CGA-91305, and CGA-118244, each at ≤1.9% TRR (≤0.058 ppm). 6.6% of the TRR (0.206 ppm) remained in nonextractable solids.

Similar characterization of the ^{14}C-residues in the 1x celery samples was noted, however, metabolite identification analyses were not performed on the sample extracts. Extraction with MeOH:H_2O released 97.6% of the TRR (0.834 ppm), the majority of which was organosoluble (95% TRR, 0.811 ppm). Aqueous soluble residues accounted for 2.7% of the TRR (0.023 ppm) and nonextractable solids accounted for 2.4% TRR (0.020 ppm).

Proposed metabolic pathway

Based on the results of the metabolism studies, the registrant proposed that propiconazole is metabolized in celery either by hyroxylation of the β-carbon from the n-propyl group on the dioxolane ring to form CGA-118244 or deketalization of the dioxolane ring to the alkanol to form CGA-91305. Both of these metabolites are subsequently readily conjugated to sugars.

Radiolabel Method Validation

Aqueous soluble ^{14}C-residues in sample extracts of the 5x treated celery were analyzed by method AG-454B. Celery samples were extracted with MeOH:H_2O and partitioned with EtOAc as described above. ^{14}C-Residues in the aqueous fraction were converted to 2,4-dichlorobenzoic acid (2,4-DCBA) by method AG-454B. Briefly, an aliquot of the aqueous fraction was refluxed with $KMnO_4$ and 1M NaOH (75 min.). After reflux, the $KmnO_4$ was deactivated and the extract was acidified using water, sodium meta-bisulfite, and 6M HCl. Residues were partitioned into 10% ethyl ether in hexane, filtered, concentrated, and redissolved in ACN and H_2O prior to analysis by HPLC and 2D-TLC.

HPLC chromatograms and 2D-TLC profiles showed a total conversion of the polar metabolites with an intact phenyl ring to 2,4-DCBA. Quantitative data were not provided.

Magnitude of the Residue in Sugarcane

No tolerances have been established for propiconazole residues in/on sugarcane [40 CFR §180.434].

A REFS search dated 10/8/97 listed two propiconazole end-use products, two 3.6 lb/gal ECs (EPA Reg. Nos. 100-617 and 100-737), registered to Novartis for use on sugarcane seed. The 3.6 lb/gal ECs are for use in HI only and specify that seed pieces are to be dipped in a cold or hot solution containing 0.75 fl oz of the EC in 100 gal of water (1:17,000). For the cold dip, seed pieces are immersed to give a thorough wetting at ambient temperatures and then removed. For the hot dip, the treatment solution is heated to 52 ° C and seeds are soaked for 20-30 minutes.

The Propiconazole Phase 4 Review, dated 6/25/92, required data depicting the uptake of phenyl-[^{14}C]-propiconazole residues into the aerial portions of sugarcane grown from treated seed pieces in FL and HI. In response, Novartis submitted data (MRID 44142401) depicting the TRR in sugarcane samples grown from seed treated with phenyl-[^{14}C]-propiconazole at 1x the maximum label use rate. Seeds were treated with an aqueous solution containing 25 ppm phenyl-[^{14}C]-propiconazole (specific activity of 44.3 µCi/mg, radiochemical purity of 97.3%) for 1 minute at ambient temperatures (cold dip) or for 30 minutes at 52° C (hot dip).

Three treated seed pieces per treatment method were composited, frozen immediately after treatment, and combusted for radioassay. Control and cold and hot dip treated seeds were grown in pots containing Florida sandy soil with one seed/pot. Eight seeds were grown for each treatment method (cold dip, hot dip, or control). Sugarcane was harvested at maturity (~11 months after planting) by cutting the aerial portions of the stalks. Leaves were trimmed, and cane samples were homogenized by each treatment method in dry ice.

The biological phase was conducted at Ciba Vero Beach Research Center, FL and samples were shipped via freezer truck to Ciba Crop Protection in Greensboro, NC for analysis. Seed and cane samples were held at ≤-5° C for 10 and 3 days, respectively, prior to analysis. All samples were radioassayed in triplicate by LSC and the results are expressed in [^{14}C]-propiconazole equivalents.

TRRs in the cold and hot dip treated seed piece samples were 1.319 and 6.731 ppm, respectively. TRRs in sugarcane grown in FL from cold and hot dip treated seeds and harvested at ~11 months were non-detectable (<0.010 ppm). The TRR in control sugarcane sample was also nondetectable (<0.01 ppm).

The available propiconazole residue data for sugarcane fulfill reregistration requirements. The data indicate that [^{14}C]-propiconazole residues in the aerial portions of sugarcane grown in FL from treated seed pieces are non-detectable. Although tests were not conducted in HI, sugarcane grown in HI is typically harvested 24 months after planting. Therefore, resides in sugarcane grown from treated seed in HI are likely to be lower than those levels found in FL. The data indicated that treatment of sugarcane seed pieces can be considered a non-food use. Data requirements are satisfied, no further residue data for sugarcane are required.

MASTER RECORD IDENTIFICATION NUMBER

The citations for the MRID documents referred to in this review are presented below.

44049601 Simoneaux, B. (1996) Uptake and Metabolism of CGA-64250 in Greenhouse Grown Celery after Spray Treatment with L Phenyl-(carbon 14)-CGA-64250: Lab Project Number: ABR-95100: 271-94: BIOL-94015. Unpublished study prepared by Ciba Crop Protection. 144 p.

44142401 Close, C. (1996) (Carbon 14)-Propiconazole: Uptake and Metabolism in Seed Piece Dipped Sugarcane: (Final Report): Lab Project Number: ABR-96097: 73-95: ANPHI-96005. Unpublished study prepared by Ciba-Geigy Corp. 56 p.

AGENCY MEMORANDA CITED

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