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

FEB 11 1993

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OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

MEMORANDUM:

SUBJECT: Propiconazole - Review of metabolism study.

EPA IDENTIFICATION NUMBERS: Caswell No.: 323EE
P.C. Code: 122101
DP Barcode: D181156
MRID No.: 424039-01

FROM: Robert F. Fricke, Ph.D. Robert F. Fricke 25 Jan 93
Toxicology Branch II, Section IV
Health Effects Division (H7509C)

TO: Bruce Sidwell
Product Manager (53)
Registration Division (H7505C)

THRU: Elizabeth Doyle, Ph.D. E. A. Doyle 1/25/93
Toxicology Branch II, Head Section IV
Health Effects Division (H7509C)

and

Marcia van Gemert, Ph.D. concurred 1/26/93
Chief, Toxicology Branch II
Health Effects Division (H7509C)

Registrant: Ciba-Geigy Corp.

Chemical: Propiconazole

Action Requested: The registrant, Ciba-Geigy Corp, has requested a review of the submitted metabolism study in rats.

Study: The Metabolism of CGA-64250 in the Rat

Study No./Date: PR 24/83/1 September 1983

Results: This study focused on the identification of the urinary and fecal metabolites resulting from a single dose of ^{14}C-labeled test compound. Excretion is rapid, with 80.7, 94 and 95.6% of the radioactivity appearing in the urine and feces 1, 2, and 3 days, respectively, after dosing. Metabolic profiles were determined on pooled urinary and fecal samples. The parent

(1)

compound is extensively metabolized; only a small percentage remained unabsorbed and appeared in the feces. The n-propyl side chain is first metabolized to α-, β- and γ-hydroxy derivatives and then to α, β- and β, γ-diols. The α, β- diol is further metabolized to α-hydroxy carboxy acid derivative, a major (11%) metabolite (metabolite U8) appearing in the urine. The side chain is sequentially decarboxylated to yield acetic and formic acid derivatives. Once the dioxolane ring is cleaved, a wide variety of metabolic reactions occurs, leading, in general to the hydroxylation of the dichlorophenyl and triazole rings. Sulfation appeared to be the preferential route of secondary metabolism and accounted for 5.5% of the dose.

CORE Grade/Doc No.: Supplementary (not a guideline study)

This study does not satisfy guideline requirements (85-1) for a metabolism study in rats.

RIN 1067-98

Propiconazole (Tilt) Tox Review

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Pages _____ through _____ are not included.

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____ Description of quality control procedures.

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Reviewed by: Robert F. Fricke, Ph.D. Robert F. Fricke 25/Jan 93
Section IV, Tox. Branch II (H7509C)
Secondary Reviewer: Elizabeth A. Doyle, Ph.D. E A. Doyle 1/25/9
Section IV, Tox. Branch II (H7509C)

DATA EVALUATION REPORT

STUDY TYPE: Metabolism - Pharmacokinetics in rats (85-1)

P.C. CODE: 122101 **CASWELL NO.:** 323EE

MRID NO.: 424039-01 010014

TEST MATERIAL: CGA-64250, Propiconazole

SYNONYMS: 1-[2-(2',4'-dichlorophenyl)-4-propyl-1,3-dioxolane-2-yl]-methyl-1H-1,2,4-triazole

STUDY NUMBER: PR 24/83

SPONSOR: Agricultural Division
CIBA-GEIGY Corporation
P.O. Box 18300
Greensboro, NC 27419

TESTING FACILITY: Biochemistry
Department R & D Plant Protection
Agricultural Division
CIBA-GEIGY Corporation
Basle, Switzerland

TITLE OF REPORT: The Metabolism of CGA-64250 in the Rat

AUTHOR: W. Mucke

REPORT ISSUED: 1 September 1983

CONCLUSIONS: This study focused on the identification of the urinary and fecal metabolites resulting from a single dose of ^{14}C-labeled test compound. Excretion is rapid, with 80.7, 94 and 95.6% of the radioactivity appearing in the urine and feces 1, 2, and 3 days, respectively, after dosing. Metabolic profiles were determined on pooled urinary and fecal samples. The parent compound is extensively metabolized; only a small percentage remained unabsorbed and appeared in the feces. The n-propyl side chain is first metabolized to α-, β- and γ-hydroxy derivatives and then to α, β- and β, γ-diols. The α, β- diol is further metabolized to α-hydroxy carboxy acid derivative, a major (11%) metabolite (metabolite U8) appearing in the urine. The side chain is sequentially decarboxylated to yield acetic and formic acid derivatives. Once the dioxolane ring is cleaved, a wide variety of metabolic reactions occurs, leading, in general, to the hydroxylation of the dichlorophenyl and triazole rings. Sulfation appeared to be the preferential route of secondary metabolism and accounted for 5.5% of the dose.

Classification: core - Supplementary (not a guideline study) 4

This study does not satisfy guideline requirements (85-1) for a metabolism study in rats.

I. MATERIALS AND METHODS

A. Test compound: Triazole-[3,5-^{14}C]-CGA-64250
Lot No.: not given
Radiochemical and chemical purity: >98%
Specific Activity: 23.1 μCi/mg

B. Test animals: Male rats (TIF: RIA f (SPF)) were obtained from CIBA-GEIGY Animal Farm (CIBA-GEIGY Ltd., Stein, Switzerland). The mean bodyweight was 175 g with a range of 167-186 g. The age of the animals was not given.

C. Study Design: Fasted rats were orally gavaged with a single dose of labeled compound (31.4 mg/kg) dissolved in water/ethanol/polyethylene glycol 200 (50/30/20 v/v/v). Animals were housed individually in metabolism cages.

D. Analysis of Fecal and Urinary Samples: Fecal and urinary samples were collected and pooled at 24-hour intervals. Radioactivity of either urine and extracts or combusted fecal samples was determined using liquid scintillation counting.

E. Tissue Distribution of Radioactivity: Not determined

F. Identification of Metabolites: Initial separation of metabolites was achieved using 2-dimensional thin layer chromatography (TLC). Urinary samples were extracted using one of six different solvent systems designed to separate acidic, nonpolar and polar residues (Table 1). Urine was also treated with either β-glucuronidase or arylsulfatase to aid in the identification of these conjugates. Pooled, 24-hour fecal samples were first lyophilized and then sequentially extracted. The fecal sample was first extracted using methanol/water (80/20). The extract was evaporated to dryness and taken up in water (pH 2), which was extracted with ethyl ether. The ether extract was chromatographed on 2-dimensional TLC. The ether extract was applied to a SiO_2 column and eluted with an ethyl acetate-methanol gradient. The resulting radioactive peaks were further extracted and separated (Table 3).

Several analytical techniques were used to separate the various urinary and fecal metabolites. High performance liquid chromatography (HPLC), using both UV and radioactivity monitors, was used to separate metabolites. In some cases, separation was achieved using sequential elution by reversed phase (R18) and SiO_2 HPLC. Liquid chromatography (LC) and preparative thin layer chromatography (TLC) were also used. Where appropriate, metabolites were methylated, acetylated or sylilated to achieve separation. Some metabolites were derivatized with 1-fluoro-2,4-dinitrobenzene or phenylboronic acid.

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Table 1: Extraction and separation of urinary metabolites (Data taken from study Figures 2 to 8)

Fraction	Extraction	Separation	Metabolites
E_1 (3.1%)	Adjustment of urine to pH 9 Extraction with ether	HPLC (RP18 and SiO_2)	$Zu_1, Zu_{17}, Zu_{29}, Zu_{32}$, Zu_{33} < 1% each Zu_{16} 2%
E_2 (4.9%)	Adjustment of urine to pH 5 Treatment with β-glucuronidase Adjustment of urine to pH 9 Extraction with ether	LC (XAD-4) HPLC (RP18 and SiO_2)	Yu <1% and Zu_{27} < 1% $Zu_{20}, Zu_{23}, Zu_{25}$ 1% each Zu_{24} 2%
E_3 (4.5%)	Same as above but treated with arylsulfatase	HPLC (RP18 and SiO_2)	Yu^* < 1% Zu_{22}, Zu_{34} < 1% each Zu_{18}, Zu_{19} 2% each
E_4 (19.1%)	Adjustment of urine to pH 2 Extraction with ether	LC (DEAE Sephadex) HPLC (RP18 and SiO_2)	Hu, Lu, Pu < 1% each Ku 1%, Cu 7%, Du 11% Eu_1, Eu_2, Eu_3 6%
W_{41} (2.0%)	LC (XAD-4), Eluted with water	Urease treatment LC (Sephadex) Derivatization HPLC (RP18 and SiO_2)	Zu_{35} < 1% Zu_{31} 2% $Zu_2, Zu_3, Zu_4, Zu_6, Zu_7$, $Zu_9, Zu_{10}, Zu_{11}, Zu_{12}, Zu_{13}$ Zu_{14}, Zu_{15}, Tu, Wu, Xu, Zu all < 1% each Ru 4%
W_{42} (10.9%)	LC (XAD-4), Eluted with methanol	LC (XAD-4 and SiO_2) HPLC (SiO_2 and RP18)	

Table 2: Separation of fecal metabolites (Data taken from study Figures 10 to 15)

Fecal Fraction	Separation	Metabolites
E_{10} 3%	LC (SiO_2) HPLC (RP18 and SiO_2)	A_F 1%, B_F 2%
E_{131} 5%	Preparative TLC HPLC (RP18 and SiO_2)	C_F, D_F, S_F < 1% each H_F, L_F < 1% each K_F 2%
E_{16} 2%	HPLC (SiO_2 and RP18)	U_F, W_F, X_F, Z_F < 1% each Y_F 1%
E_{17} 1%	HPLC (SiO_2 and RP18) Preparative TLC	Z_{F1}, Z_{F7} <1% each
E_{19} 2%	HPLC (SiO_2 and RP18)	T_F 2%

Definitive identification of the metabolites was achieved using a combination of mass spectroscopy and/or nuclear magnetic resonance (NMR). When available, authentic standards were used.

G. Statistics: Not given

H. Quality assurance was documented by signed and dated GLP and quality assurance statements.

I. Flagging Statement: The sponsor did not apply the criteria of 40 CFR 158.34 for flagging studies for potential adverse effects to the results of this study.

II. Results

A. Clinical Observations: Not determined

B. Distribution of Radioactivity in Excreta:
Radioactivity was either measured directly (urine and urine extracts) or after combustion (fecal samples). 1-^{14}C-n-hexadecane was used as an internal standard for quench correction. The cumulative amounts of radioactivity in the feces and urine are summarized in Table 3. After 2 days greater than 90% of the labeled residues were excreted; there was a slight preference for urinary excretion.

Table 3: Cumulative amounts of ^{14}C-labeled residues in feces and urine (Data summarized from Table II of the study)

	Cumulative % in Excreta		
	1 day	2 days	3 day
Feces	36.2	42.7	43.3
Urine	44.5	51.3	52.3
Total	80.7	94.0	95.6

C. Identification of Metabolites:

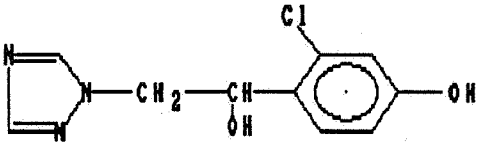
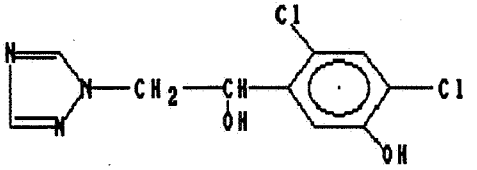
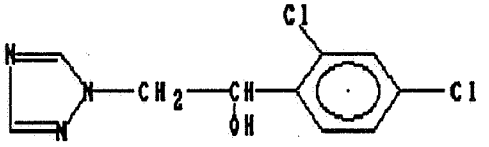
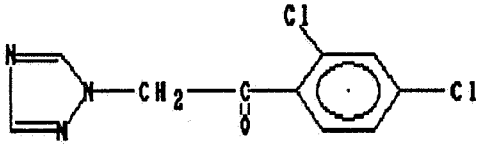
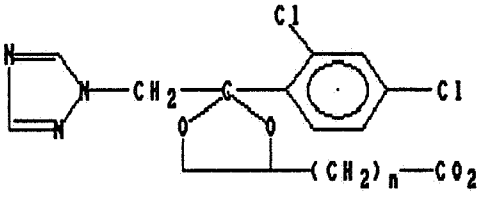
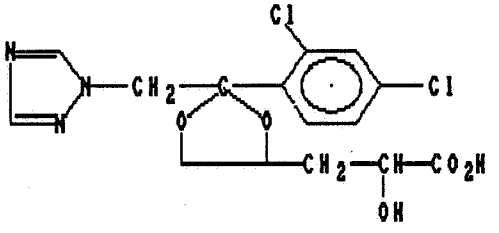
1. Initial separation: Initial separation of the pooled urinary and fecal samples on 2-dimensional thin layer chromatography (TLC) revealed 14 urinary metabolites, seven of which were subsequently identified. The existence of glucuronide and sulfate metabolites present in the urine was confirmed by reacting a urine sample with either β-glucuronidase or arylsulfatase. The resulting reaction mixture was rechromatographed, revealing the disappearance of two metabolites (U15 and U16) and the appearance of four new metabolites (U1 to U4).

Of the radioactivity appearing in the feces, approximately half (16.2% of the administered dose) was present in the final ether extract. TLC revealed 16 fecal metabolites, ten of which were positively identified. Radioactivity (15.6%) remaining in the fecal solids was not analyzed.

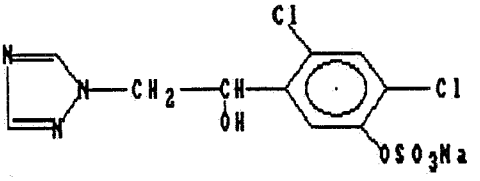
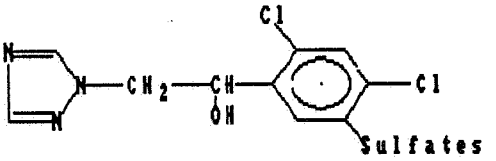
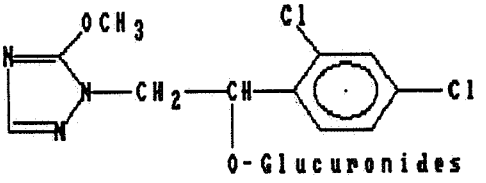
2. Identification of Metabolites: Extensive extraction and separation of both urinary and fecal samples revealed that the parent compound was extensively metabolized. Approximately 21 urinary and fecal metabolites were identified (Table 4). Many of the metabolites were present in very low levels (< 1%); the major metabolite was present at only 11%. Very little (< 3%) of the parent compound was present in the feces and was attributed to incomplete absorption.

3. Proposed Metabolic Pathway: With the structures of many of the metabolites known, a metabolic pathway was proposed (Appendix 1). Essentially all of the parent compound is absorbed and metabolized by a variety of divergent pathways. The n-propyl side chain is sequentially metabolized to mono- and di-hydroxy derivatives, which were preferentially excreted via the feces. The α,β and β,γ dihydrodiols were further oxidized to α carboxylic acid derivatives. Following formation of the formic acid derivative, oxidative attack of the dioxolane ring lead to the formation of

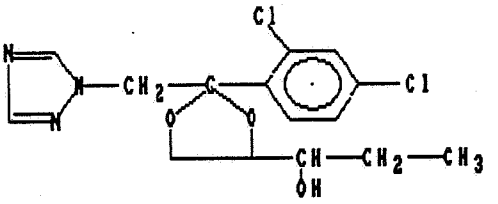
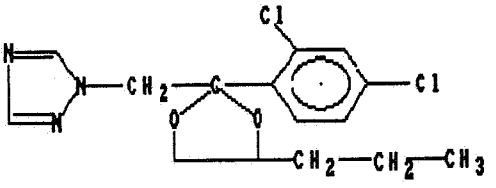
Table 4: Major Urinary and Fecal Metabolites: Percent Distribution and Structures (Data taken from study Table III and Figure 1)

Metabolites[a]	% of Total	Structure
Urinary Metabolites		
U1 (Zu_{18}, Zu_{19})	< 0.5	
U2 (Zu_{20})	< 0.5	
U3	< 0.5	(--)[b]
U4	< 0.5	(--)
U5 (Zu_{17})	3.5	
U6 (Zu_{36})	2.0	
U7 (Eu_1, n = 1) (Eu_2, n = 3) (Eu_3, n = 2)	1.5	
U8 (Du,Cu,Wu,Xu)	11.0	
U9	1.0	(--)

(Table 4 continued)

U10 (Ru)	4.5	
U11	1.5	(--)
U12	3.0	(--)
U13	2.5	(--)
U14	1.5	(--)
U15 (Sulfates)	5.5	
U16	4.0	(--)
U17 (Glucuronides)	4.0	

Fecal Metabolites

F1 (C_F,D_F)	1.0	Same as U1
F2 (S_F)	0.5	Same as U2
F3 (K_F)	1.5	Same as U5
F4 (H_F)	0.5	
F5 (A_F,B_F) (Parent Coumpound)	3.0	

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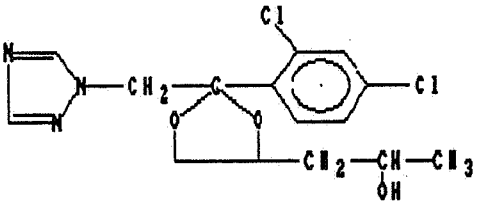
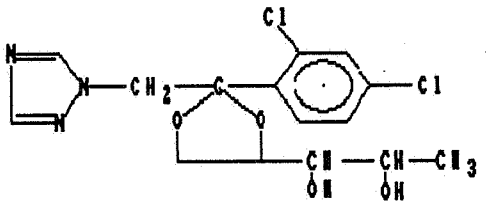
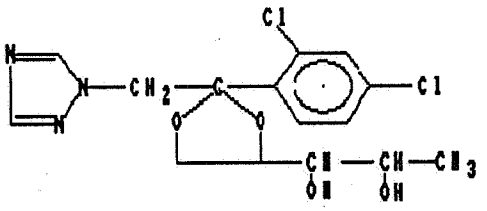
(Table 4 continued)

F6 (W_F, X_F, Y_F)	1.0	
F7 (U_F)	0.5	
F8 (Z_F)	1.5	
F10 (T_F)	3.5	Same as U8

[a] The letters in parenthesis represent the metabolites separated and identified using the different extraction systems. In most cases, the same metabolite was isolated and identified in more than one extraction scheme; for example, Du, Cu, Wu, Xu all proved to be the same urinary metabolite, U8.

[b] (--), Structure not determined.

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the keto metabolite, Zu_{16}, which was further reduced to the corresponding alcohol (K_F, 2%; Zu_{13}, < 1%; Zu_{14} < 1%). A small portion (<1%) of the alcohol derivative appeared in the urine as a glucuronide conjugate (Zu_4). Following removal of the dioxolane ring, the dichlorophenyl and triazole rings were hydroxylated. The hydroxyl metabolites were further metabolized to various sulfate and glucuronide derivatives; there was a preference towards sulfation reactions.

III. Discussion and Comments: The scope of this study was limited to identification of the urinary and fecal metabolites resulting from a single dose of ^{14}C-labeled test compound. The test compound is rapidly excreted with 80.7, 94 and 95.6% of the radioactivity appearing in the urine and feces 1, 2, and 3 days, respectively, after dosing. The urinary and fecal samples of the study were pooled and metabolic profiles determined. Although many metabolites were visualized on the TLC plate, only the major TLC metabolites were identified.

To more fully evaluate the metabolic profile, various extraction and separation procedures were used to isolate and subsequently identify both the major and very minor (< 1%) metabolites. A small percentage of unmetabolized parent compound present in the feces was attributed to incomplete absorption. Because the parent compound was extensively metabolized, none of the metabolites was present in a high percentage. Urinary metabolite, U8, was the major metabolite identified and corresponded to 11% of the administered dose. Although the percentage was low (5.5%), sulfation appeared to be the preferential route of secondary metabolism.

Classification: core - Supplementary (not a guideline study)

This study does not satisfy guideline requirements (85-1) for a metabolism study in rats.

RIN 1067-98

Propiconazole (Tilt) Tox Review

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