272 E
CASWELL FILE

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

OCT 17 1990

008129

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MEMORANDUM

OFFICE OF PESTICIDES AND TOXIC SUBSTANCES

SUBJECT: CYPROCONAZOLE - RAT METABOLISM STUDY IN SUPPORT OF COFFEE IMPORT TOLERANCE PETITION

TO: LEWIS/GRABLE
PRODUCT MANAGER (21)
REGISTRATION DIVISION (H7508C)

FROM: LINDA L. TAYLOR, PH.D. Linda Lee Taylor 10/3/90
TOXICOLOGY BRANCH II, SECTION II
HEALTH EFFECTS DIVISION (H7509C)

THRU: K. CLARK SWENTZEL K. Clark Swentzel 10/4/90
SECTION II HEAD, TOXICOLOGY BRANCH II
HEALTH EFFECTS DIVISION (H7509C)

AND

MARCIA VAN GEMERT, PH.D. Marcia Van Gemert 10/9/90
CHIEF, TOXICOLOGY BRANCH/HFAS/HED (H7509C)

REGISTRANT: SANDOZ CROP PROTECTION
CHEMICAL: -(4-CHLOROPHENYL)- -(1-CYCLOPROPYLETHYL)-1H-1,2,4-TRIAZOLE-1-ETHANOL
SYNONYM: SAN 619 F
PROJECT: 0-1531
CASWELL NO.: 272E
RECORD NO.: 266808
IDENTIFYING NO.: 0E3875
MRID NO.: 415349-01
ACTION REQUESTED: RAT METABOLISM STUDY SUBMITTED IN SUPPORT OF COFFEE IMPORT TOLERANCE PETITION. PLEASE CONNECT UP WITH PETITION SENT FOR REVIEW 6/26/90.

COMMENT: THE REGISTRANT HAS SUBMITTED A REPORT, WHICH DESCRIBES THE NATURE AND AMOUNTS OF THE METABOLITES OF SAN 619 F IN THE EXCRETA OF RATS ADMINISTERED THE TEST MATERIAL IN A PREVIOUSLY-REPORTED STUDY ON THE ABSORPTION, DISTRIBUTION, AND EXCRETION OF SAN 619 F IN THE RAT (REF. SCHWEITZER, A., SAN 619 F: ABSORPTION, DISTRIBUTION, AND EXCRETION IN RATS AFTER SINGLE AND MULTIPLE DOSES OF ^{14}C-SAN 619 F. SANDOZ LTD., BASLE, SWITZERLAND, JANUARY, 1987; REPORT CBK 11738). THIS LATTER STUDY HAS NOT BEEN SUBMITTED TO TB II FOR REVIEW TO DATE, AND EFFORTS TO LOCATE IT IN-HOUSE WERE NOT SUCCESSFUL.

THE CURRENT REPORT PROVIDES DATA ON THE METABOLIC PROFILE OF SAN 619 F, WHICH INDICATE THAT SAN 619 F IS EXTENSIVELY METABOLIZED IN THE RAT, AND METABOLISM IS NOT AFFECTED BY THE ROUTE OF EXPOSURE (I.V. OR ORAL), SEX OF THE ANIMAL, OR DOSE REGIMEN. THIS STUDY DOES NOT SATISFY THE GUIDELINE REQUIREMENTS (85-1) FOR A METABOLISM STUDY, PER SE, BUT IS ADEQUATE FOR DEFINING THE METABOLIC PATHWAY FOR SAN 619 F IN THE RAT. THE DER IS ATTACHED.

008129

REVIEWED BY: LINDA L. TAYLOR, PH.D. Linda Lee Taylor 10/3/90
TOX. BRANCH II, SECTION II, HED (H7509C)
SECONDARY REVIEWER: K. CLARK SWENTZEL K. Clark Swentzel 10/4/90
HEAD SECTION II, TOX. BRANCH II, HED (H7509C)

DATA EVALUATION RECORD

STUDY TYPE: METABOLISM - RATS TOX. CHEM. NO. 272E

MRID NO.: 415349-01

TEST MATERIAL: SAN 619 F

TESTING FACILITY: SANDOZ LTD., BASLE, SWITZERLAND

SPONSOR: NOT STATED SANDOZ CROP PROTECTION CORPORATION SUBMITTED STUDY, BUT ON PAGE 3 IT IS STATED THAT THEY ARE NOT THE SPONSOR

STUDY NO.: CBK 11816/87

AUTHOR: J. KARAPALLY, M. SPIELMANN, AND S. VOLLMIN

TITLE OF REPORT: SAN 619 F METABOLISM IN RAT

REPORT ISSUED: OCTOBER 22, 1987

QUALITY ASSURANCE: A QUALITY ASSURANCE STATEMENT WAS PROVIDED.

CONCLUSION: SAN 619 F WAS FOUND TO BE EXTENSIVELY METABOLIZED BY THE RAT, WHICH THE ROUTE OF EXPOSURE, TYPE OF DOSING SCHEDULE, OR SEX OF ANIMAL DID NOT INFLUENCE. ALTHOUGH BOTH DIASTEREOMERS WERE WELL METABOLIZED, M1 SEEMED TO BE BIOTRANSFORMED MORE EXTENSIVELY THAN M2. THE MAJOR METABOLIC REACTIONS INCLUDED:

1) OXIDATIVE ELIMINATION OF THE TRIAZOLE RING;
2) HYDROXYLATION OF THE CARBON BEARING THE METHYL GROUP;
3) OXIDATION OF THE METHYL GROUP TO THE CARBINOL AND FURTHER TO THE CARBOXYLIC ACID.

CLASSIFICATION: SUPPLEMENTARY; THIS STUDY DOES NOT SATISFY THE GUIDELINE REQUIREMENT (85-1) FOR A METABOLISM STUDY, PER SE, BUT IS A SUPPLEMENTAL PART OF AN ABSORPTION, DISTRIBUTION, EXCRETION STUDY (ADE) FROM WHICH THE SAMPLES ANALYZED AND REPORTED IN THE CURRENT REPORT WERE TAKEN. TO DATE, THE ADE STUDY HAS NOT BEEN SUBMITTED TO TB II FOR REVIEW.

A. MATERIALS:

1. TEST COMPOUND: SAN 619 F
DESCRIPTION: NO PHYSICAL DESCRIPTION PROVIDED;
BATCH #: LABELED: #3, SYNTHESIS # 652; UNLABELED: OP 1 & 2, EB, PURIFIED FROM BATCH # 8404;
PURITY: LABELED: 98%; RATIO OF DIASTEREOMERS 1:1, ^{14}C-LABEL INTRODUCED IN -POSITION(1-^{14}C-ETHANOL), SPECIFIC GRAVITY: 19.4 uCi/MG (4.31×10^7 DPM/MG)
UNLABELED: >98%; RATIO OF DIASTEREOMERS 1:1.

2. TEST ANIMAL
SPECIES: RAT; STRAIN: KFM, WIST
AGE: NOT PROVIDED
WEIGHT: NOT PROVIDED
SOURCE: MADORIN, FULLINSDORF, SWITZERLAND

STUDY DESIGN: IN THE ABSORPTION/DISTRIBUTION/EXCRETION STUDY (REF. SCHWEITZER, A., SAN 619 F: ABSORPTION, DISTRIBUTION, AND EXCRETION IN RATS AFTER SINGLE AND MULTIPLE DOSES OF ^{14}C-SAN 619 F. SANDOZ LTD., BASLE, SWITZERLAND, JANUARY, 1987; REPORT CBK 11738), FROM WHICH THE EXCRETION SAMPLES WERE COLLECTED FOR THE ANALYSES REPORTED IN THE CURRENT REPORT, FIVE RATS PER SEX WERE ADMINISTERED THE TEST COMPOUND IN EACH OF 5 DIFFERENT EXPERIMENTS.

EXPERIMENT #	EXPOSURE ROUTE	DOSE OF ^{14}C-SAN 619 F
1	INTRAVENOUS	SINGLE (LOW) DOSE - 10 MG/KG
2	ORAL	SINGLE (LOW) DOSE - 10 MG/KG
3	ORAL	SINGLE (LOW) DOSE - 10 MG/KG (BILE DUCT CANNULATED)
4	ORAL	PRETREATMENT FOR 14 DAYS WITH 10 MG/KG UNLABELED SAN 619 F FOLLOWED BY SINGLE (LOW) DOSE 10 MG/KG
5	ORAL	SINGLE (HIGH) DOSE - 130 MG/KG

THE CURRENT REPORT DEALS WITH THE ISOLATION, IDENTIFICATION, AND QUANTIFICATION OF THE METABOLITES OF ^{14}C-SAN 619 F IN THE URINE, FECES, AND BILE OF THE RATS DOSED AS DESCRIBED ABOVE. THE SAMPLES USED WERE THE QUANTITATIVELY AND SEPARATELY COLLECTED URINE AND FECES SAMPLES FROM 0-168 HOURS (EXP. 1, 2, 4, 5) AND THE BILE SAMPLES COLLECTED FROM 1-144 HOURS (EXP. 3). URINE AND FECES OF THE SEXES WERE POOLED FOR EACH EXPERIMENT; THE BILE SAMPLES WERE POOLED FOR MALES AND FEMALES SEPARATELY.

IT WAS STATED THAT, SINCE THE MAJOR AMOUNT OF THE ADMINISTERED DOSE WAS EXCRETED VIA FECES (TABLE 1), THE METABOLITES WERE ISOLATED FROM A MIXTURE OF ALL FECES SAMPLES (MALE & FEMALE) OF THE HIGH-DOSE EXPERIMENT.

SEPARATION AND PURIFICATION OF THE METABOLITES WERE ACHIEVED MAINLY BY PREPARATIVE TLC. FOR FINAL SEPARATION AND PURIFICATION, THE SAMPLES WERE SUBJECTED TO REVERSED-PHASE HPLC. THE METABOLITES WERE IDENTIFIED BY SPECTROSCOPIC METHODS, AND THE SPECTROSCOPIC DATA WERE COMPARED WITH THOSE OF SYNTHESIZED REFERENCE MATERIAL (AS AVAILABLE) FOR VERIFICATION.

FIRST INFORMATION ABOUT THE STRUCTURES OF THE METABOLITES WAS OBTAINED BY FOURTIER TRANSFORM PROTON MAGNETIC RESONANCE SPECTROSCOPY (NMR) AT 360 MHz. TO VERIFY PROPOSED STRUCTURES, THE FOLLOWING MASS SPECTRA WERE OBTAINED:

1) FAST ATOM BONBARDMENT (FAB) MASS SPECTRA TO VERIFY MOLECULAR ION MASS;
2) ELECTRON IMPACT (EI) MASS SPECTRA TO CONFIRM STRUCTURES ON BASIS OF FRAGMENTATION.

QUALITATIVE AND QUANTITATIVE COMPOSITION OF THE METABOLITES IN THE VARIOUS EXTRACTS WAS DETERMINED BY THIN LAYER CHROMATOGRAPHY. SAMPLES WERE MIXED WITH A SOLUTION OF AUTHENTIC STANDARDS (THOSE AVAILABLE: M3, M4, M(, M11, M14, M15, M18) AND APPLIED ON TLC-PLATES. THE PLATES WERE DEVELOPED IN A TWO-DIMENSIONAL (2D) TLC SYSTEM IN A SATURATED TANK. THE RADIOACTIVE SPOTS ON THE TLC PLATES WERE DETECTED BY PHOTOGRAPHING THE PLATES IN A RADIOCHROMATOGRAM SPARK CHAMBER FITTED WITH A POLAROID CAMERA. FOR THE IDENTIFICATION OF THE METABOLITES, THE C-14 SPOTS ON THE PHOTOGRAPH WERE PROJECTED ONTO THE ORIGINAL TLC PLATE BY MEANS OF AN ANTISKOP. THE PROJECTED SPOTS WERE DRAWN ON THE PLATE AND COMPARED WITH THE SPOTS OF THE VISUALIZED REFERENCE MATERIAL. THE UNKNOWN METABOLITES WERE DESIGNATED BY MAKING USE OF A PREVIOUSLY-PREPARED MODEL OF THE TLC CONTAINING ALL METABOLITES ON ACETATE FOIL. THE QUANTIFICATION OF THE METABOLITES WAS ACHIEVED BY SCRAPING THE SILICA GEL LAYERS CONTAINING THE CORRESPONDING SPOTS FROM THE GLASS PLATE AND COUNTING IN THE LIQUID SCINTILLATION COUNTER.

RESULTS

THE C-14 LABEL WAS STATED TO BE METABOLICALLY STABLE, WHICH WAS DETERMINED IN A PILOT STUDY IN WHICH NO $^{14}CO_2$ WAS DETECTED FOLLOWING ORAL EXPOSURE TO LABELED SAN 619 F. IN THE PREVIOUS ADE STUDY, IT WAS DETERMINED THAT SAN 619 F IS ELIMINATED VIA THE URINE, FECES, AND BILE (SEE TABLE 1). ON AVERAGE, ABOUT 90% OF THE ADMINISTERED DOSE IS EXCRETED VIA THE URINE AND FECES WITHIN 168 HOURS POST DOSING. THE ABSORBED FRACTION OF THE DOSE WAS SHOWN TO BE ELIMINATED MAINLY VIA THE BILE (EXPERIMENT 3).

BY MEANS OF THE VARIOUS CHROMATOGRAPHIC TECHNIQUES, THE DIASTEREOMERS A (M1) AND B (M2) OF UNCHANGED SAN 619 F AND THIRTEEN METABOLITES WERE ISOLATED AND IDENTIFIED THROUGH THEIR NMR. THE IDENTITY WAS CONFIRMED FURTHER BY EI AND/OR FAB SPECTRA. ADDITIONALLY, THE STRUCTURES OF 9 METABOLITES (M1, M2, M3, M4, M9, M11, M14, M15, AND M18) WERE CONFIRMED BY COMPARISON OF THEIR SPECTROSCOPIC DATA WITH THOSE OF THE SYNTHESIZED REFERENCE MATERIAL. THE CHEMICAL NAMES AND STRUCTURES OF THE METABOLITES ARE PRESENTED IN TABLE 21, COPY ATTACHED).

DISCUSSION

THE AUTHOR STATED THAT ^{14}C-SAN 619 F WAS EXTENSIVELY METABOLIZED IN THE RAT. IN ADDITION TO UNCHANGED PARENT COMPOUND, 35 METABOLITES WERE DETECTED IN THE EXCRETA. ON AVERAGE, 10% OF THE UNCHANGED SAN 619 F WAS RECOVERED IN THE URINE AND THE FECES; THE MAJOR PART OF THE PARENT COMPOUND WAS EXCRETED VIA FECES AND ONLY TRACES (<1%) WERE ELIMINATED VIA URINE. THE MAJOR METABOLITES ARE M1, M2, M3, M4, M9, M10, M11, M14, M15, M16, M18, M20, M30, M33.

THE COURSE OF METABOLISM IS SAID TO BE STEREOSELECTIVE. DIASTEREOMER A (M1) WAS METABOLIZED TO A GREATER EXTENT THAN DIASTEREOMER B (M2). THE STRUCTURAL RELATIONSHIP OF THE VARIOUS METABOLITES TO A AND B (PRESENTED IN TABLE 20, COPY ATTACHED) WAS ESTABLISHED IN ANOTHER STUDY (VOLLMIN, S., SAN 619 F: METABOLISM OF THE DIASTEREOMERS A AND B IN THE RAT. SANDOZ LTD., BASLE, SWITZERLAND, JUNE 2, 1987: REPORT CBK 11730/87). THE FOLLOWING METABOLITE PAIRS WERE FOUND TO BE DIASTEREOMERS: M3/M4, M9/M14, M11/M18, AND M30/M33. FOR METABOLITES M15 AND M16, NO DIASTEREOMERS ARE SAID TO EXIST BECAUSE ONLY ONE CHIRAL CENTER IS LEFT IN THEIR CHEMICAL STRUCTURES.

AS DESCRIBED BY THE AUTHOR, THE MAJOR METABOLITE M3 AND ITS DIASTEREOMER M4 ARE FORMED BY OXIDATIVE ELIMINATION OF THE TRIAZOLE RING. ADDITIONALLY, A MAJOR METABOLIC REACTION IS THE HYDROXYLATION OF THE CARBON BEARING THE METHYL GROUP, WHICH LEADS TO THE DIASTEREOMERS M9/M14. METABOLITES M30/M33 COULD BE REGARDED AS FORMED FROM M3/M4 BY HYDROXYLATION OF THE CARBON BEARING THE METHYL GROUP OR FROM M9/M14 BY ELIMINATION OF THE TRIAZOLE RING. ANOTHER SIGNIFICANT METABOLIC PATHWAY IS THE OXIDATION OF THE METHYL GROUP TO THE CARBINOL (M11/M18) AND FURTHER OXIDATION TO THE CARBOXYLIC ACID (M10). M20 MIGHT HAVE BEEN FORMED FROM M14 BY OXIDATION OF THE METHYL GROUP OR FROM M18 BY HYDROXYLATION OF THE CARBON BEARING THE METHYL GROUP. THE REDUCTIVE ELIMINATION OF THE CARBON BEARING THE METHYL GROUP FORMING M15, FOLLOWED BY OXIDATION OF THE HYDROXYL GROUP TO THE KETONE (M16) IS A FURTHER METABOLIC ROUTE OF SAN 619 F IN THE RAT (PROPOSED METABOLIC PATHWAY IS PRESENTED IN FIGURE 4, COPY ATTACHED).

IT IS STATED THAT IN ALL EXPERIMENTS, THE METABOLIC PROFILES FOR URINE, FECES, AND BILE WERE SIMILAR. THE MAJOR METABOLITES WERE DETECTED IN ALL URINE, FECES, AND BILE SAMPLES.

CONCLUSION

SAN 619 F WAS FOUND TO BE EXTENSIVELY METABOLIZED BY THE RAT, WHICH THE ROUTE OF EXPOSURE, TYPE OF DOSING SCHEDULE, OR SEX OF ANIMAL DID NOT INFLUENCE. THE FEMALE SHOWED A GREATER TENDENCY TO ELIMINATE SAN 619 F VIA URINE THAN DID THE MALE. ALTHOUGH BOTH DIASTEREOMERS WERE WELL METABOLIZED, M1 SEEMED TO BE BIOTRANSFORMED MORE EXTENSIVELY THAN M2. THE MAJOR METABOLIC REACTIONS INCLUDED:

1) OXIDATIVE ELIMINATION OF THE TRIAZOLE RING;
2) HYDROXYLATION OF THE CARBON BEARING THE METHYL GROUP;
3) OXIDATION OF THE METHYL GROUP TO THE CARBINOL AND FURTHER TO THE CARBOXYLIC ACID.

ALTHOUGH THIS REPORT/STUDY PROVIDES ADEQUATE INFORMATION ON THE METABOLIC PROFILE OF SAN 619 F, IT DOES NOT SATISFY THE GUIDELINE REQUIREMENTS (85-1) FOR A METABOLISM STUDY, PER SE. THE PREVIOUSLY PERFORMED ABSORPTION, DISTRIBUTION, AND ELIMINATION STUDY FROM WHICH THE EXCRETION SAMPLES WERE DERIVED AND REFERENCED BY THE AUTHOR SHOULD BE SUBMITTED FOR TB II REVIEW IN ORDER TO FULFILL THE DATA REQUIREMENT FOR A METABOLISM STUDY.

TABLE 1

Excretion of radioactivity in urine, feces, and bile[1]

Experiment #	Sex	Time of Sample Collection (hrs.)	Excreted Radioactivity % Administered Dose (mean) Urine	Feces	Bile	Total
1	M	0-168	32.9	68.5	*	101.4
	F	0-168	38.4	50.8	*	89.2
2	M	0-168	27.8	58.6	*	86.4
	F	0-168	41.0	53.9	*	94.9
3	M	0-144	9.5	4.5	75.5	89.5
	F	0-144	26.8	4.8	59.6	91.2
4	M	0-168	27.8	60.1	*	87.9
	F	0-168	34.9	49.2	*	84.1
5	M	0-168	28.4	60.2	*	88.6
	F	0-168	41.7	43.0	*	84.7

[1] results from ADE study
* not determined

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Page_____ is not included in this copy.

Pages 8 through 13 are not included.

The material not included contains the following type of information:

____ Identity of product inert ingredients.

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____ Description of the product manufacturing process.

____ Description of quality control procedures.

____ Identity of the source of product ingredients.

____ Sales or other commercial/financial information.

X A draft product label.

____ The product confidential statement of formula.

____ Information about a pending registration action.

____ FIFRA registration data.

____ The document is a duplicate of page(s) _____.

____ The document is not responsive to the request.

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

Tox Chem No. 272E Cyproconazole | File Last Updated ____ | Current Date 10/2/90

Study/Lab/Study #/Date	Material	EPA Accession No.	Results: LD_{50}, LC_{50}, PIS, NOEL, LEL	TOX Category	CORE Grade/ Doc. No.
Metabolism Species: rat Sandoz Ltd., Basle, Swit. CBK 11816/87 10/22/87	SAN 619 F 98%	415349-01	SAN 619 F found to be extensively metabolized by rat; diastereomers A (M1) & B (M2) of unchanged SAN 619 F & 13 metabolites were isolated & identified; 35 metabolites were detected; metabolic profiles for urine, feces, & bile were stated to be similar; major metabolic reactions include: (1) oxidative elimination of triazole ring, (2) hydroxylation of C bearing methyl group, (3) oxidation of methyl group to carbinol & further to carboxylic acid. Study does not satisfy guideline requirement (86-1) for a metabolism study, per se.		Supplementary