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CASWELL FILE  
272E

1/17/89 memo  
1/17/89  
OFFICE OF  
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Cyproconazole Technical and Cyproconazole 40 WG: : Evaluation of Toxicity Data Submitted to Support Registration for Nonfood Uses.

TO: Lois Rossi PM-21  
Registration Division (TS-767C)

FROM: K. Clark Swentzel  
Section 2  
Toxicology Branch 2 (TS-769C)

THRU: James N. Rowe, Ph.D.  
Acting Section Head  
Toxicology Branch 2 (TS-769C)

and

Marcia van Gemert, Ph.D.  
Acting Branch Chief  
Toxicology Branch 2 (TS-769C)

EPA ID Nos.: 55947-RGG and 55947-RGE  
Project Nos.: 8-0814 and 8-0813  
Caswell No.: 272E  
Registrant: Sandoz Corp.

The registrant has simultaneously submitted applications for the registration of Cyproconazole, for manufacturing use only, and Cyproconazole WG 40, an end-use product turf fungicide product for use on golf courses and sod farms (see attached label and technical information).

The appropriate battery of toxicity studies were submitted for the technical product (Table I) based on the application for nonfood uses (158.35), however, the registrant must address several study deficiencies indicated in the attached summary and DERS. Neither the subchronic feeding studies nor the teratogenicity studies are currently acceptable, however, it is possible to upgrade the core-classification of the subchronic study in dogs and the teratogenicity study in rats from core-supplementar by submitting requested information. Additional information is also required for the 2-generation reproduction study in rats. Also, the primary ocular irritation study in rabbits and the in vivo mouse micronucleus test must be repeated.

The battery of acute studies for the WG 40 formulation (Table II) was also appropriate, however, an acute inhalation study was omitted because the registrant concluded that product does not contain a significant proportion of respirable particles. Although the registrant submitted particle size data to support this conclusion, a detailed description of the method(s) used as well as examples of findings from tests performed to derive this data should be submitted.

The most serious adverse effects observed in these studies were seen in the teratogenicity studies. Hydrocephalus was observed in both rats and rabbits and cleft palate was seen in the rat study. Since a NOEL for developmental toxicity was not attained in either study, the calculation of a safe dose level, based on these findings, is not possible.

Table I

Cyproconazole(TGAI): Toxicity Studies Required under 158.135 for Non-Food Use and Conclusions Regarding the Satisfaction of These Requirements

<u>Test</u>	<u>Required</u>	<u>Satisfied</u>
Acute oral LD50	Yes	Yes
Acute dermal LD50	Yes	Yes
Acute inhalation LC50	Yes	Yes
Primary eye irritation	Yes	No <sup>1/</sup>
Primary dermal irritation	Yes	Yes
Dermal sensitization	Yes	Yes
21-day dermal	Yes	Yes
90-day feeding studies-rodent:	Yes	No <sup>2/</sup>
-nonrodent:	Yes	No <sup>3/</sup>
Teratogenicity-rodent:	Yes	No <sup>4/</sup>
-nonrodent:	Yes	No <sup>5/</sup>
Reproduction, 2-generation:	Yes	No <sup>6/</sup>
Gene mutation	Yes	Yes
Structural aberration	Yes	No <sup>7/</sup>
Other genotoxic effects	Yes	Yes

- 1/ Data are equivocal, study should be repeated in different animals; core-classification: supplemental.
- 2/ A NOEL was not attained, therefore this study is not acceptable for regulatory purposes.
- 3/ Core-classification is supplemental; classification can be upgraded provided an acceptable study audit is provided.
- 4/ A NOEL for developmental toxicity could not be determined; additional data is required; Core-classification: supplemental (can be upgraded).
- 5/ Core-classification is supplemental; a NOEL for developmental toxicity was not attained.
- 6/ Core-classification is a provisional minimum; additional information is required.
- 7/ The only study submitted under this category (in vivo mouse micronucleus test) was not acceptable.

Table II

SAN 619F 40 WDG(EP): Toxicity Studies Required under 158.135 for Non-Food Use and Conclusions Regarding the Satisfaction of These Requirements

Test	Required	Satisfied
Acute oral LD50	Yes	Yes
Acute dermal LD50	Yes	Yes
Acute inhalation LC50	Yes(provisional)	No*
Primary eye irritation	Yes	Yes
Primary dermal irritation	Yes	Yes
Dermal sensitization	Yes	Yes

*Add 21-Dermal*

\* A description of methodology and examples of findings from tests performed to derive the submitted particle size data must be provided before a decision on the possible requirement of this test can be made.

## Conclusions from Individual Studies

### Technical Grade Active Ingredient

#### Acute Toxicity Studies

- 1) Acute Oral LD<sub>50</sub> Study in the Male and Female Rat with SAN 619F  
LD<sub>50</sub> = 1020 ± 290 mg/kg in males; 1330 ± 346 mg/kg in females. Tox. Category-III  
Core classification- minimum
- 2) SAN 619F: Acute Dermal LD<sub>50</sub> in the Male and Female Rabbit  
LD<sub>50</sub> > 2000 mg/kg; Tox. Category- III  
Core classification- minimum
- 3) SAN 619F: Acute Dermal Toxicity in the Male and Female Rat  
LD<sub>50</sub> > 2000 mg/kg; Tox. Category = III  
Core classification- minimum
- 4) Four-Hour Acute Dust Aerosol Inhalation Toxicity (LC<sub>50</sub>) Study with SAN 619F in Rats.  
LC<sub>50</sub> > 5.6 mg/l (includes all airborne particles regardless of size).  
Tox. Category = III; Core classification- minimum
- 5) Irritant Effects on the Rabbit Eye of SAN 619F Technical  
Data are equivocal, study should be repeated in different animals.  
Core classification- supplemental
- 6) Irritant Effects on Rabbit Skin of SAN 619F Technical  
No primary irritation reaction. Tox. Category = IV. Core classification- minimum

#### Dermal Sensitization

##### Skin Sensitization Test in Guinea Pigs

Cyproconazole technical did not induce a sensitization reaction in any of the guinea pigs receiving challenge doses (3ml; up to 5%) in a study using the maximization method of Magnusson and Kligman.

Core-classification: minimum

#### Subchronic Toxicity Studies

##### 1) Three-Week Dermal Study in Rabbits

Male and female rabbits received repeated dermal applications of Cyproconazole-technical at dosage levels of 50, 250 and 1250 mg/kg for 3 consecutive weeks. There was no macro- or microscopic evidence of induced skin irritation. Evidence of systemic toxicity included inhibited body weight gain and food consumption in high-dose males, increased AST in high-dose males, increased creatinine in high-dose females and increased cholesterol in high-dose males and females. The only noted difference that was statistically significant (p < 0.05), in comparison to the concurrent control, was the mean creatinine level in high-dose females. Based on these observations, the LEL was 1250 mg/kg and the NOEL was 250 mg/kg.

Classification: core-minimum

## 2) Four-Week Study in Rats

Male and female Han Wistar rats were administered Cyproconazole in the diet at levels of 10, 30, 100, 300 and 1000 ppm for 4 weeks. Evidently, this study was performed to determine the dietary levels that should be administered in a subsequent 13-week feeding study in rats. Changes associated with treatment, which were observed in males and females administered the highest dietary level, included decreased body weight and body weight gain, elevated ALT and LDH, increased absolute and relative (% body weight) liver weight and hepatocytomegaly. Elevated BUN and relative testes weights as well as liver vacuolation were observed in high-dose males. Increased cholesterol and relative adrenal weights were noted for high-dose females. The effects present in high mid-dose (300 ppm) males and females were: elevated LDH, increased absolute and relative liver weight and liver vacuolation. Increased relative testes and adrenal weights were seen in high mid-dose males and females, respectively. The LEL and NOEL were 300 ppm (15 mg/kg) and 100 ppm (5mg/kg), respectively.

Core classification: supplementary data (not a Guideline Study)

## 3) Thirteen-Week Study in Rats

Male and female Han Wistar rats were administered Cyproconazole in the diet at levels of 20, 80 and 320 ppm for 13 weeks; the treatment period was followed by a 4-week recovery period during which additional control and high-dose groups were fed the control diet. Changes associated with treatment, which were observed in rats administered the highest dietary level, include inhibited body weight gain, increased blood levels of creatinine and sodium with a concomitant decrease in calcium, increased liver weights and histological changes in liver. The noted changes in creatinine and calcium were also consistently observed in rats receiving the 20 ppm level but not in those administered 80 ppm. However, since these changes were not seen in treated rats after the recovery period, they should be considered treatment-related effects. A NOEL was not attained, therefore this study is not acceptable for regulatory purposes.

Classification: core-minimum (but NOEL not attained)

## 4) Thirteen-Week Study in Beagle Dogs

Male and female beagle dogs were administered Cyproconazole in the diet at levels of 20, 100 and 500 ppm for 13 weeks. Changes associated with treatment, observed in both sexes administered the highest dietary level, included "slack muscle tone", inhibited body weight gain, increased platelet counts, decreased: bilirubin, total cholesterol, HDL-cholesterol, triglycerides, total protein and albumin and increased alkaline phosphatase and gamma glutamyl transferase; decreased food consumption was seen in high-dose males. Increased absolute and relative liver weights and increased relative kidney weights were noted for high-dose males and females; relative brain weights were increased in high-dose females. Histopathologic evidence of liver toxicity in high-dose males and females included hepatocytomegaly, degeneration of single hepatocytes and cytoplasmic inclusions. Evidence of liver toxicity in mid-dose dogs was increased absolute liver weights in males and hepatocytomegaly in males and females.

The LEL in this study, based on adverse effects in liver, was 100 ppm (approximately 4 mg/kg/day) and the NOEL was 20 ppm (approximately 0.8 mg/kg/day).

Since this study was not inspected by a QAU during the in-life phase, a data audit, signed and dated by a QA Officer, must be submitted to the Agency before this study can be accepted for regulatory purposes.

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Core classification: supplemental (can be upgraded to minimum provided an acceptable QAU audit is provided as noted above)

### Developmental Toxicity

#### 1) Teratogenicity Study in Rats

A suspension of Cyproconazole in distilled water mixed with carboxymethylcellulose sodium salt (CMC, 4%) was administered daily to pregnant Wistar/Han rats (25/group) via oral gavage from day 6 through 15 of gestation at dosage levels of 6, 12, 24 and 48 mg/kg. Evidence of maternal toxicity included inhibited body weight gain during treatment at dosage levels of 12 mg/kg and above and decreased body weight and food consumption among females in the 24 and 48 mg/kg dosage groups. However, since the noted differences in maternal body weights were influenced by treatment-related intrauterine effects (e.g., increased number of resorptions, decreased fetal weight etc.), the evidence for maternal toxicity is equivocal.

Evidence of fetal toxicity was apparent from observed dose-related increases in the number of fetuses with supernumerary ribs at dosages of 6 mg/kg and above. Embryo/fetal toxicity was apparent at 24 and 48 mg/kg from the following observations: decreased total number of fetuses/dam, decreased number of live fetuses/dam, increased percentage and number of fetal resorptions, decreased body weight and incomplete ossification in phalangeal nuclei and the absence of ossification in calcanea.

There was evidence of teratogenicity in the 24 and 48 mg/kg groups. Hydrocephaly was observed in 1 fetus in the 24 mg/kg and 2 fetuses in the 48 mg/kg groups. Cleft palate was observed in 2 fetuses in the 48 mg/kg group.

The NOEL for developmental toxicity was not determined, based on induced fetotoxicity (supernumerary ribs) at 6 mg/kg. The NOEL for maternal toxicity was 6 mg/kg (equivocal).

The registrant should submit data which show the litter incidence of supernumerary ribs (number of litters/group with the noted change) with appropriate statistical analyses to aid in the determination of a possible NOEL for developmental toxicity in this study.

Core classification - supplemental (can possibly be upgraded to minimum by submitting requested data)

#### 2) Teratogenicity Study in Rabbits

A suspension of Cyproconazole in distilled water mixed with carboxymethylcellulose sodium salt (CMC, 4%) was administered daily to pregnant Chinchilla rabbits (16/group) via oral gavage from day 6 through 18 of gestation at dosage levels of 2, 10 and 50 mg/kg.

Evidence of maternal toxicity, which was not remarkable, included inhibited body weight gain during treatment and decreased food consumption during the initial phase of treatment, both at 50 mg/kg. However, since corrected body weight changes between groups were comparable, the evidence of compound-induced maternal toxicity in this study is not convincing.

Embryo/fetal toxicity, observed at 50 mg/kg, was evident from the decreased number of live fetuses/dam and an increased incidence of non-ossification in certain forelimb and hind limb digits. Evidence of embryo/fetal toxicity at dosages of 10 and 50 mg/kg was indicated by an increased incidence of embryonic and fetal resorptions.

Evidence of teratogenicity included hydrocephalus internus, observed in 1 fetus at



each dosage level, and agenesis of the left kidney and ureter in 1 high-dose fetus. Hydrocephaly was also seen at 2 dosage levels in a developmental toxicity study in rats with this test material, however, this anomaly did not occur in the control group of either study.

Since a teratogenic response to the test material was observed at the lowest dose tested, a NOEL for developmental toxicity was not attained in this study. Although evidence of maternal toxicity at 50 mg/kg was not remarkable, the 10 mg/kg dosage level is clearly a no-effect level for maternal toxicity.

This study is not acceptable for regulatory purposes because: 1) a NOEL for developmental toxicity apparently was not attained and 2) the concentrations of test material were not within the acceptable  $\pm 15\%$  of nominal concentration for the mid- and high-dose suspensions immediately after preparation.

Developmental toxicity NOEL: not attained ;  $< 2$  mg/kg/day (LDT)  
Maternal toxicity NOEL: 10 mg/kg (equivocal)

Core classification: supplementary

### Reproductive Toxicity

#### Two-Generation Study in Rats

Four groups of KFM-Wistar rats were administered technical Cyproconazole at dietary levels of 0 (control), 4, 20 and 120 ppm during the pre-mating (10 weeks and 12 weeks, respectively, for the  $F_0$  and  $F_1$  generations), mating, pregnancy and lactation periods to assess the potential reproductive toxicity of the test compound.

Two of the reproductive parameters investigated in parental animals were affected by treatment in  $F_0$  rats only: the duration of gestation at the mid- and high doses was increased and a lower number of implantation sites was seen in high-dose females, both in comparison to respective concurrent control values. Evidence of liver toxicity was seen in high-dose  $F_0$  males (increased lipid storage and relative weight) and females (increased relative weight).

Parameters examined among the offspring which showed treatment-related effects included decreased litter sizes in both the  $F_1$  and  $F_2$  high-dose groups and the  $F_1$  mid-dose group during the early phase of lactation (litters were standardized at day 4 post partum), decreased live birth index in the high-dose  $F_1$  offspring and decreased viability index in the high-dose  $F_1$  and  $F_2$  offspring.

Based on the increased duration of gestation in  $F_0$  dams and the decreased litter sizes observed in  $F_1$  offspring, the LEL in this study was 20 ppm and the NOEL was 4 ppm, which correspond to approximate average dosage levels of 1.7 and 0.4 mg/kg/day, respectively.

Core-classification: minimum (provided test compound stability data and a description of the sampling technique used for the analyses of dietary levels of test compound are submitted)

## Mutagenicity Studies

### Gene Mutation

#### 1) Gene Mutation - Ames Salmonella Microsome Reverse Mutation Assay

No evidence of a mutagenic effect at the histidine locus in any of the S. typhimurium strains (TA98, TA100, TA1535, TA1537, TA1538) used at dose levels of 1, 5, 10, 100, 500 or 1000 ug/plate either with or without rat S9 mix.

Acceptable

#### 2) In Vitro HGPRT Gene Mutation Test using Chinese Hamster Ovary Cell Line V79

No indication of mutagenic activity either with or without S9 activation at dose levels of 0, 20, 50, 100 or 200 ug/ml. Test material was soluble only up to 200 ug/ml at which there was little or no evidence of cytotoxicity.

Acceptable

### Structural Abberation

#### Mutagenicity Evaluation of SAN 619F in the In Vivo Mouse Micronucleus Assay

No indication of a mutagenic response (a significantly increased incidence of micronucleated polychromatic erythrocytes) at any of the SAN F dose levels (16.7, 55.7 and 167 mg/kg) for any of the scheduled sacrifice times (24, 48 and 72 hrs).

Not acceptable (additional information regarding the purity of the test material is required).

### Other Genotoxic Effects

#### 1) Unscheduled DNA Synthesis in Rat Primary Hepatocytes with SAN 619F

No indication of increased incorporation of 3H-TdR from exposure to SAN 619F either at a single dose level or as part of a dose-related trend. Dose levels: 0.25, 3.3, 6.6, 10 and 25 ug/ml. However, highest dose level should have been greater than 25 ug/ml as there was no indication of a decreased incorporation of 3H-TdR at this level. Also, negative control values were 208.9 dpm, rather than in range of 50-150 dpm.

Not acceptable

#### 2) In Vitro Cell Transformation with Syrian Hamster Embryo (SHE) Cells

No transformation of SHE cells from exposure to SAN 619F for 6 or 48 hrs without S9 activation or as a result of 6-hr exposure to SAN 619F with S9 activation. Dose levels: 20, 50, 100 and 200 ug/ml. No evidence for cytotoxicity at 200 ug/ml but test material precipitated out at concentrations above 200 ug/ml.

Acceptable

3) Unscheduled DNA Synthesis (UDS) Test In vitro in Rat Hepatocytes

No indication of an increased level of incorporation of 3H-TdR in rat hepatocytes exposed to SAN 619 F at 0.15, 0.5, 1.5, 5, or 15 ug/ml with 18-20 hr exposure. Insufficient reporting as to levels of 50, 100 and 150 ug/ml were "Too toxic to be evaluated for UDS," particularly as LDH activities indicated toxicities below 100% (16%, 61% and 68% respectively).

Not Acceptable (additional information required)

4) Mutagenicity Evaluation of SAN 619F in the Mitotic Non-Disjunction Assay with Saccharomyces Cerevisiae Strain D6

No increased absolute number of cycloheximide-resistant colonies or of an increased incidence of aneuploids among these colonies following overnight exposure to SAN 619 F at 10, 100, 250, 400, 500 or 550 ug/ml in presence and absence of S9. Range of doses resulted in no, moderate and nearly complete cytotoxicity. No positive control with S9; no information as to how long "overnight" exposure was.

Not Acceptable (S9 activation plus additional information required)

End Use Product (SAN 619F 40 WDG)

Acute Toxicity Studies

- 1) Acute Oral Toxicity to Rats of SAN 619F 40 WDG  
LD50 = 780 mg/kg in males and 1340 mg/kg in females; Tox. category = III  
Core-classification: minimum
- 2) Acute Dermal Toxicity to Rats of SAN 619F 40 WDG  
Acute lethal dose > 2,000 mg/kg (only dose tested); Tox. category = III  
Core-classification: minimum
- 3) Irritant Effects on the Rabbit Eye of SAN 619F 40 WDG  
No primary irritation reaction; Tox. category = IV  
Core-classification: minimum
- 4) Irritant Effects on the Skin of SAN 619F 40 WDG  
Only a slight, transient irritation reaction was observed; Tox. category = IV  
Core-classification: minimum
- 5) Acute Inhalation Toxicity (study not performed) The registrant submitted a letter to the Agency (S. Janousky to L. Rossi, May 19, 1988) which indicated that acute inhalation testing with the 40 WG formulation is not applicable since the bulk of the material consists of large particles that are not inhalable by man. The following data were included to support this contention:

SAN 619F 40 WDG Particle Characterization

<u>Particle size (micrometers)</u>	<u>Percent</u>
1700-2000	1.5
1180-1700	32.2
850-1180	42.6
600-850	15.9
425-600	1.8
< 425	6.4

A description of methodology and examples of findings from tests performed to derive the submitted particle size data must be provided before a decision on the possible requirement of this test can be made. Data which show the proportion of particles in the range inhalable by man (< 10u) should also be submitted, if they are available.

Dermal Sensitization

Delayed Contact Hypersensitivity in the Guinea-pig with SAN 619F 40 WDG

Repeated topical applications of SAN 619F 40 WDG (50% w/w in distilled water) in guinea pigs did not induce delayed contact hypersensitivity under the conditions of this study.

The procedure used in this study was a modification of the method described in "Delayed Contact Hypersensitivity in the Guinea-pig" Buehler, E.V. (1965), Arch. Dermatol. 91, 171.

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The material not included contains the following type of information:

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  - Description of the product manufacturing process.
  - Description of quality control procedures.
  - Identity of the source of product ingredients.
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