21200 PR 178993 file 11 CYPROCONAZOLE

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HEALTH EFFECTS DIVISION
SCIENTIFIC DATA REVIEWS
EPA SERIES 361

OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

MEMORANDUM

SUBJECT: Peer Review of Cyproconazole

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Background

Cyproconazole is a triazole proposed for use as a turf fungicide. Increased incidences of adenomas and carcinomas were seen in the livers of male and female mice in a long-term feeding study. The Health Effects Division Peer Review Committee has not considered this pesticide. It is requested that this Committee conduct a peer review on this compound and make a recommendation for the classification of an oncogenicity category for cyproconazole. The Committee is also requested to recommend whether or not quantitative risk assessments need to be made for the uses of this pesticide.

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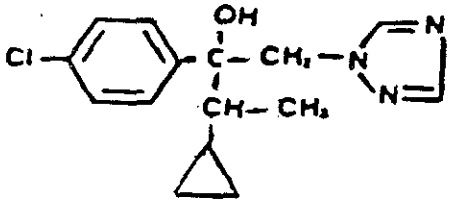
B. Material Reviewed

The material available included reviews of the mouse oncogenicity feeding study and the rat chronic toxicity/oncogenicity feeding study. There were also summaries of a one-year dog study, rat and rabbit teratology studies, a two-generation rat reproduction study, mutagenicity, and subchronic studies.

C. Background Information

Cyproconazole is also called SAN 619F. It is alpha-(4-chlorophenyl)-alpha-(1-cyclopropylethyl)-1H-1,2,4-triazole-1-ethanol, and its Caswell number is 272E. The structure is:



Cyproconazole is a turf fungicide for use on golf courses and sod farms. The registrant is Sandoz Crop Protection Corporation. Cyproconazole is a colorless to brownish powder that is stable and soluble in water.

D. Evaluation of Carcinogenicity Studies of Cyproconazole

1. Long-term Mouse Oncogenicity Study

Reference: Warren, S., Morand de Jouffrey, S., Muller, F., and Karapaly, J.C., May 31, 1989. The Potential Oncogenicity of SAN 619F by Prolonged Dietary Administration to Mice. Study no. 388-M/398-M, Sandoz AG, Agro Division, Basle, Switzerland. MRID No. 411472-01.

Cyproconazole (technical, 95.1% pure) was fed to Charles River CD-1 mice (50/sex/dose group) at concentrations of 5, 15, 100, and 200 ppm. Females were treated for 88 weeks and males for 81 weeks. Ten animals/sex were assigned to control and high dose groups for 13-week interim sacrifice. There were two control groups of 50 animals/sex for the entire study.

Table 1 shows the incidences of liver tumors in male and female mice. There were increased incidences of adenomas, of carcinomas, and of adenomas and carcinomas combined.

In male mice there were statistically significant trends for adenomas and for combined tumors. Males also had statistically significant incidences of carcinomas at the three highest doses, of adenomas at the 100 ppm level, and of combined tumors at the two highest doses.

In female mice there were statistically significant trends for adenomas, for carcinomas, and for combined tumors. Also, all three parameters were statistically significant by pair-wise comparison for the high dose females.

Table 1. Liver Tumor Incidence in Cyproconazole Study with CD-1 Mice

DOSES ppm:	0[a]	5	15	100	200
MALES:					
ADENOMA	6/92(7)**[b]	4/49(8)	5/48(10)	12/47(26)*	12/48(25)
CARCINOMA	0/74(0)	0/38(0)	3/46(7)*	3/43(7)**	1/41(2)**
COMBINED	6/92(7)**	4/49(8)	8/48(17)	15/47(32)**	13/48(27)*
FEMALES:					
ADENOMA	0/61(0)**	0/34(0)	0/28(0)	2/41(5)	6/39(15)**
CARCINOMA	0/69(0)**	0/41(0)	0/31(0)	0/43(0)	7/40(18)**
COMBINED	0/69(0)**	0/41(0)	0/31(0)	2/43(5)	13/40(33)**

[a]Composed of two control groups, 50 animals in each one.
[b]Number of tumor bearing animals/Number of animals examined, excluding those that died before observation of the first tumor.
( ) Percent.
Note: Significance of trend analysis denoted at column for controls; significance of pair-wise comparison with control denoted at dose level. * = $p < 0.05$ and ** = $p < 0.01$.

Table 2. Historical Control Data from Different Sources for Liver Tumors in CR CD-1 Mice

Sex and Tumor Type	24-Month Data Mean %	24-Month Data Range %	18-Month Data Mean %	18-Month Data Range %
Males:				
Adenomas	8.3	0-28	8.2	0-16.3
Carcinomas	6.5	0-21	1.4	0-6
Combined	14.8	5-34		
Females:				
Adenomas	1.7	0-8	1.4	0-2.7
Carcinomas	1.3	0-4	0.2	0-0.7
Combined	3.0	0-10		

The registrant did not provide historical control data for this strain of mouse from this laboratory. The historical control data available from other sources are shown in Table 2. These data for liver tumors in CD-1 mice are from two sources. The 24-month data are from a series of 36 separate studies at International Research and Development Corporation, where either one or two control groups were used. The second source was a pamphlet published by the Charles River Company (Patricia L. Lang, Spontaneous Neoplastic Lesions in the CRL:CD-1 (ICR)BR Mouse). These 18-month data were from eight groups of control animals in studies that were conducted in different laboratories and completed between 1978 and 1984.

Comparison of Tables 1 and 2 shows that the percent tumors in the following groups in the cyproconazole study exceeded the ranges of percent tumors in the 18-month historical controls: adenomas in 100 ppm males, adenomas in 200 ppm males, carcinomas in 100 ppm males, adenomas in 200 ppm females, and carcinomas in 200 ppm females. The combined tumors in the female mice fed 200 ppm cyproconazole were higher than the range of combined tumors for females in the 24-month historical data. The combined tumor percentages in the male mice fed 100 or 200 ppm cyproconazole were above the mean for combined tumors in 24-month studies.

Male and female mice receiving 100 and 200 ppm cyproconazole had increased incidences of focal hepatocytic inflammation, single-cell necrosis, and diffuse hepatocytic hypertrophy; male mice were more severely affected. Increases in centriacinar hepatocytic vacuolation were seen in high dose males and in mid and high dose females; the mid dose females also had increased periacinar hepatocytic vacuolation. Significant increases in liver weights were seen in both males and females treated with 200 ppm for 13 weeks. At termination, the liver weights of males and females in the two highest doses showed statistically significant increases compared to controls.

There was a decrease in the amount of testicular germinal epithelium in males receiving 200 ppm, which corresponded to the incidence of flaccid testes in this group. Body weight gains were decreased by more than 10% in both sexes fed at 100 and 200 ppm levels for 26 weeks. Mean body weights were significantly decreased in males and females receiving 100 and 200 ppm at 26 and at 52 weeks. In these two groups at termination, the males had 13% lower body weights and the females had 6% lower body weights than the controls. There was no increase in mortality with dose, and no effect of dosing on food consumption or hematology parameters.

Based on the significantly increased incidences of hepatocytic single cell necrosis and diffuse hepatocytic hypertrophy in males and females at 100 and 200 ppm, and a significantly increased incidence of periacinar hepatocytic vacuolation in females at 100 ppm, the NOEL for systemic toxicity is 15 ppm. Fifteen ppm corresponds to an intake of approximately 1.8 mg/kg/day in males and 2.6 mg/kg/day in females.

2. Two-year Rat Chronic/Oncogenicity Study

Reference: Warren, S.F.P., Carpy, S., and Muller, F., April 22, 1988. San 619F Chronic Toxicity/Oncogenicity Feeding Study in Rats. Study No. 357-R, Sandoz Ltd. Agro Development, Basle, Switzerland. MRID No. 411647-01.

Cyproconazole (technical, 94.6-96.6 % purity) was fed to 70 KFM-Wistar rats per sex per group at 20, 50, and 350 ppm. These levels correspond to intakes of 1.0, 2.2, and 15.6 mg/kg/day for males and 1.2, 2.7, and 21.8 mg/kg/day for females. Ten rats/sex/dose were sacrificed at 12 and at 18 months.

There were decreased body weights in high dose females, although generally less than 10 percent different from control values, and an increased incidence of fatty infiltration of the liver in the high dose males. The liver findings are listed in Table 3. Based on these effects, the LOEL for systemic toxicity was 350 ppm and the NOEL was 50 ppm.

The study did not show carcinogenicity. However, the lack of: 1) any biologically significant body weight decrement; 2) any significant histopathological correlate accompanying the increased relative liver weight; 3) any increase in the liver enzyme activities in the females; and 4) any consistent change in the liver enzyme activities in the high dose males, all suggest that the dose levels chosen were not adequate to determine the carcinogenic potential of the test material.

Table 3. Liver Effects in the Chronic Rat Study

	Males				Females			
Doses ppm:	0	20	50	350	0	20	50	350
No. examined[a]	50	49	50	50	50	50	50	50
Fatty change	23	19	29	38	23	15	15	10
Hyperplastic nodule	3	3	0	2	3	1	1	2
Bile duct proliferation	22	23	19	26	27	35	32	38
Sinusoidal cell pigmentation	1	1	4	5	5	7	6	13
Vacuolated focus	14	18	19	16	2	9	9	9
No. examined[b]	50	50	50	50	50	50	50	50
Adenoma	1	0	1	1	0	0	1	1
Carcinoma	0	0	1	1	0	1	0	0

[a]Includes animals in the main study that died or were sacrificed moribund as well as those sacrificed at study termination.
[b]Includes all rats in the main groups.

E. Additional Toxicology Information

1. Mutagenicity

Cyproconazole has been tested in several mutagenicity studies considered acceptable by the agency. Ames tests in S. typhimurium were negative with and without activation. There was no in vitro cell transformation with Syrian hamster embryo cells, with and without activation. HGPRT gene mutation tests in Chinese hamster ovary cells were negative with and without activation. A micronucleus assay in mice was negative.

An assay for potential to induce chromosome aberrations in Chinese hamster ovary cell was positive in nonactivated and activated conditions. These results indicate that cyproconazole can be clastogenic without metabolic activation.

2. Metabolism

No metabolism data are available for cyproconazole. However, the liver effects, such as were seen in the one-year dog study and the chronic rat study, are consistent with the metabolism of cyproconazole being like that of structurally similar compounds (see part 5 below).

3. Acute, Subchronic, and Chronic Toxicity

The acute oral LD50 in rats was over 1000 mg/kg.

Effects associated with treatment in a thirteen-week rat feeding study (20, 80, 320 ppm, with a recovery period) were inhibited body weight gain, increased blood sodium, and increased liver weights at the high dose. The high dose also had histological changes in the liver, that is, vacuolated hepatocytes and a distinct lobular pattern associated with enlarged hepatocytes. Blood creatinine was increased and calcium was decreased at the high and low doses, but not at the mid dose; thus this study did not attain a NOEL.

Changes associated with treatment in a thirteen-week dog feeding study included, at the high dose of 500 ppm, slack muscle tone, inhibited body weight gain, and decreases in bilirubin, total cholesterol, HDL-cholesterol, triglycerides, total protein, and albumin. There were increases in platelet counts, alkaline phosphatase, gamma glutamyl transferase, absolute and relative liver weights, relative kidney weights, and relative brain weights. Liver toxicity was shown by hepatocytomegaly, degeneration of single hepatocytes, and cytoplasmic inclusions. The mid-dose (100 ppm) dogs had increased absolute liver weights and hepatocytomegaly. The LEL was 100 ppm and the NOEL was 20 ppm.

Effects on the liver in a one-year dog feeding study were indicated by elevated alkaline phosphatase and ALAT levels; decreased total protein, albumin, and cholesterol levels. Absolute and relative liver weights were increased. Relative kidney weights were increased in low and high dose females and cytochrome P450 was increased in mid and high dose animals. Laminar eosinophilic intrahepatocytic bodies were observed in high dose animals. The NOEL was set at 30 ppm and the LEL at 100 ppm based on liver effects.

4. Developmental and Reproductive effects

A teratogenicity study in rats (6, 12, 24, and 48 mg/kg/day on gestation days 6-15) showed dose related increases in the number of fetuses with supernumerary ribs at all doses. The two highest doses showed decreased total number of fetuses/dam, decreased number of live fetuses/dam, increased fetal resorptions, decreased body weight, and incomplete or absent ossification in some foot bones. There were instances of hydrocephaly and cleft palate at the two highest doses. There were maternal body weight differences, but the influence of treatment-related intrauterine effects on maternal weight make the evidence for maternal toxicity, and the maternal NOEL of 6 mg/kg, equivocal. The developmental NOEL was 6 mg/kg and the LEL 12 mg/kg.

In a rabbit teratology study (2, 10, 50 mg/kg/day on gestation days 6-18) a developmental NOEL was not attained. Hydrocephalus internus was found at all doses and agenesis of the kidney and ureter occurred at the high dose. Also at the high dose there was a decreased number of live fetuses/dam and an increased incidence of non-ossification in some digits. Resorptions increased at the mid and high dose. The maternal NOEL of 10 mg/kg and LEL of 50 mg/kg, based on inhibited body weight gain and decreased food consumption, are equivocal. This study was classified as supplementary.

These studies will be assessed by the HED Developmental Toxicity Peer Review Committee.

A two generation rat reproduction study (4, 20, and 120 ppm cyproconazole in the diet) showed liver toxicity by increased lipid storage and relative liver weight. Longer gestation (at high and mid dose) and fewer implantation sites (at high dose) were seen. There were decreases in litter sizes (in high and mid-dose), live birth index (high dose), and viability index (high dose). The LEL was 20 ppm and the NOEL 4 ppm.

5. Structure-Activity Relationships

Cyproconazole is structurally similar to other triazole pesticides such as hexaconazole, triadimefon (Bayleton), triadimenol (Baytan), propiconazole (Tilt), uniconazole (Prunit), terbuconazole (Folicur), etaconazole (Sonax, Vanguard), bitertanol (Baycor), and azaconazole. These compound structures are in Figure 1.

Bayleton was associated with increased incidences of hepatocellular adenomas in male and female mice when administered in diet at a concentration of 1800 ppm. No increase in liver carcinomas was reported.

Triadimenol, a primary and major metabolite of Bayleton, was classified by the HED Peer Review Committee in their meeting of October 1, 1987 as group C, a possible human carcinogen, based upon increased incidences of liver adenomas in female CF1-W74 mice (HED Report dated January 29, 1988). This classification was upheld by the FIFRA Scientific Advisory Panel in their meeting of December 15, 1987 (report dated December 23, 1987).

Propiconazole was associated with increased incidences of hepatocellular adenomas and carcinomas in female CD-1 mice, and was classified by the HED Peer Review Committee in group C, possible human carcinogen (HED Reports dated April 29, 1987, July 21, 1988, April 28, 1989, and January 22, 1990).

Etaconazole was associated with increased incidence of liver adenomas and carcinomas in both male and female Albino Swiss mice. However, the registration application was voluntarily withdrawn by the registrant and therefore no further action was taken regarding its cancer classification.

Uniconazole, a new pesticide currently under evaluation, was associated with statistically significant increases in the incidence of liver adenomas and adenomas/carcinomas combined in male CD-1 mice.

Bitertanol and azaconazole were reported to be negative for carcinogenicity in mice when administered in the diet up to 180 and 200 ppm, respectively. With the exception of cyproconazole and hexaconazole, all other triazole fungicides were tested at dietary concentrations of 1500 ppm or higher before inducing any carcinogenic response in mice.

Hexaconazole was associated with increased incidence of benign Leydig cell tumors in the testes of rats, a tumor type not previously reported in response to compounds of this type. In addition, no treatment related liver tumors were noted in mice; however, the highest dietary concentration used, 300 ppm, was well below the concentration where hepatocellular tumors occurred in response to other compounds.

Most of these triazole pesticides were associated with some developmental toxicity, particularly skeletal variations and malformations. For example, bitertanol, bayleton and cyproconazole were reported to induce cleft palates, a rare effect in rats. Triadimenol, terbuconazole and hexaconazole, along with cyproconazole, were associated with increased incidence of supernumerary ribs in rats.

F. Weight of Evidence Considerations

The committee considered the following facts regarding the toxicology data on cyproconazole to be of importance in a weight-of-the-evidence determination of carcinogenic potential.

1. Administration of cyproconazole was associated with an increased incidence of hepatocellular tumors in male and female CD-1 mice. At a dietary dose level of 200 ppm, incidences of carcinomas and of combined adenomas and carcinomas were significantly increased in both sexes. Also at this dose, females had a significantly increased incidence of adenomas. Males had significantly increased incidences of adenomas, of carcinomas, and of both tumor types combined at 100 ppm dose of cyproconazole, as well as of carcinomas at 15 ppm dose.

2. In both the female and male mice, there were statistically significant trends for adenomas and for combined tumors. Female mice showed a statistically significant trend for carcinomas, also.

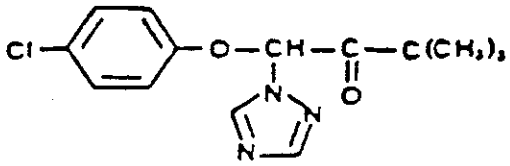
3. The increased incidences of hepatocellular tumors noted at 100 and 200 ppm in male CD-1 mice and at 200 ppm in female CD-1 mice generally exceeded the available historical control incidences for these same tumor types in mice of this strain. However, the available historical control data were generated at other laboratories under different conditions and at different time periods. Some of these studies were of longer duration than the cyproconazole study. In these circumstances, the historical control data are considered ancillary information and only minimally confirmatory in nature.

4. There was no compound-related increase in tumors observed in male or female rats. However, it appears that the dose levels fed in the long term rat study were not adequate to determine the carcinogenic potential of the test material.

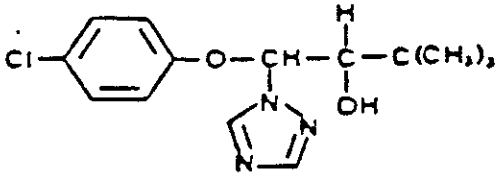
5. Although several genetic toxicity tests were negative, an assay on induction of chromosome aberrations in Chinese hamster ovary cells was positive in nonactivated and activated conditions. These results indicate that cyproconazole can be clastogenic without metabolic activation.

6. Cyproconazole dosing was associated with teratogenicity in the form of hydrocephaly and cleft palate in rats and of hydrocephalus internus and agenesis of the kidney and ureter in rabbits. There were also supernumerary ribs in rats and incomplete or absent ossification in some foot bones in both species.

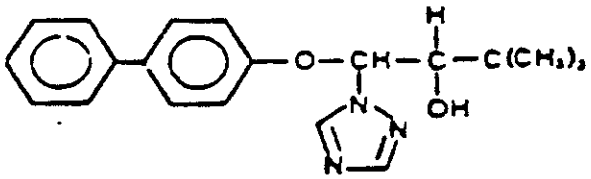
7. Cyproconazole is structurally related to several triazole pesticide compounds, most of which have been associated with the formation of liver adenomas or carcinomas. Also, most of these triazoles were associated with developmental toxicity, particularly of the skeleton.



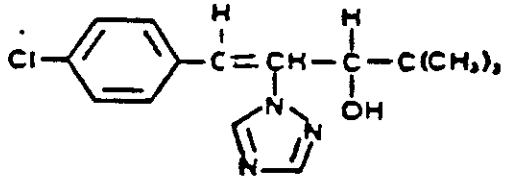
Triadimefon
(Bayleton)



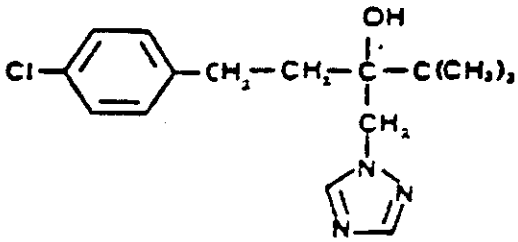
Triademenol
(Baytan)



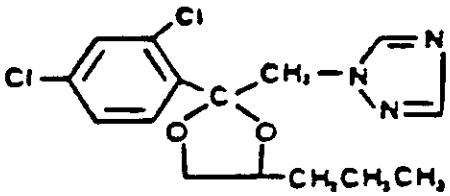
Bitertanol
(Baycor)



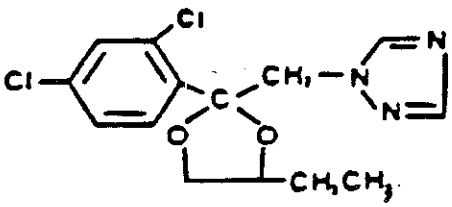
Uniconazole
(Prunit)



Terbuconazol
(Folicur)

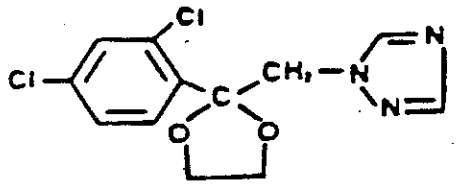


Propiconazole
(Tilt)

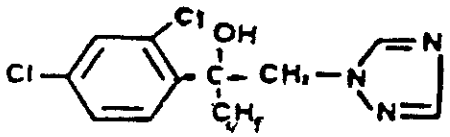


Etaconazole
(Vangard)

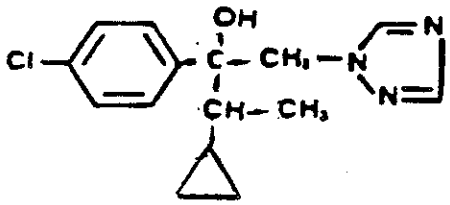
Figure 1. Structurally Related Compounds



Azaconazole



Hexaconazole
(Anvil)



Cyproconazole
(SAN 619F)

Figure 1. Structurally Related Compounds

13544

R055354

Chemical:	**Cyproconazole**
PC Code:	**128993**
HED File Code	**14100 Other Risk Documents**
Memo Date:	**05/30/90 12:00:00 AM**
File ID:	**00000000**
Accession Number:	**412-04-0044**

HED Records Reference Center
12/12/2003