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

OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

SEP 26 1990

MEMORANDUM

SUBJECT: Peer Review of Cyproconazole

FROM: Patricia McLaughlin, Ph.D. P M Laughlin 8/14/90
Toxicology Branch II - Herbicide, Fungicide and
Antimicrobial Support
Health Effects Division (H7509C)

and

George Z. Ghali, Ph.D. G. Ghali 8.2.90
Science Analysis and Coordination Branch
Health Effects Division (H7509C)

TO: Susan Lewis, Acting PM 21
Fungicide-Herbicide Branch
Registration Division (H7505C)

The Health Effects Divison (HED) Peer Review Committee met on June 20, 1990 to discuss and evaluate the weight-of-the-evidence on cyproconazole with special reference to its carcinogenic potential. Based on all information available, the Committee concluded that cyproconazole should be placed in Group "C", possible human carcinogen. This classification was based upon increased incidence of hepatocelluar adenomas and carcinomas in male and female CD-1 mice. Quantification of potential human cancer risk, using the low dose extrapolation model (Q1*), was also recommended.

A. Individuals in Attendence

1. Peer Review Committee (Signature indicates concurrence with the peer review unless otherwise stated.)

William Burman William J Burman

Reto Engler

Karl Baetcke

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Marcia van Gemert

Kerry Dearfield

Marion Copley

Esther Rinde

William Sette

Yin-Tak Woo

Hugh Pettigrew

George Ghali

2. Peer Review Members in Absentia (Those unable to attend the discussions; signature indicates concurrence with overall conclusions of the Committee.)

Penelope A. Fenner-Crisp

Richard Hill

John Quest

Robert Beliles for

Julie Du

3. Scientific Reviewers (Noncommittee members responsible for presentation of data; signature indicates technical accuracy of panel report.)

Clark Swentzel

Linda Taylor

Patricia McLaughlin

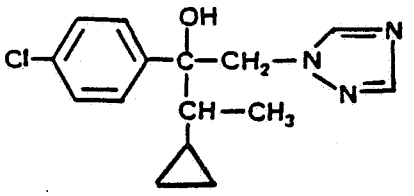
Bernice Fisher

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 1-year dog study, rat and rabbit teratology studies, a two-generation rat reproduction study, mutagenicity, and subchronic studies.

C. Background Information

Cyproconazole* [∝-(4-chlorophenyl)-∝-(1-cyclopropyl-ethyl)-1H-1,2,4-triazole-1-ethanol] is a turf fungicide manufactured by Sandoz Crop Protection Corporation proposed for use on golf courses and sod farms.



Cyproconazole
(SAN 619F)

D. Evaluation of Carcinogenic Evidence

1. 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.

Experimental Design - 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.

Non-Neoplastic Lesions - 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.

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*Has been investigated under the code name SAN 619F.

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 percent in both sexes fed at 100 and 200 ppm at 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 percent lower body weights and the females had 6 percent lower body weights than the controls. There was no increase in mortality with dose, and no effects of dosing on food consumption or hematology parameters.

Considerations of Adequacy of Dose Selection - The committee concluded that the high dose tested was adequate for the assessment of carcinogenic potential of this chemicals in this strain of mice, based upon statistically significant increase in liver weights and the significantly increased incidences of hepatocytic single-cell necrosis and diffuse hepatocytic hypertrophy in males and females, and a significantly increased incidence of periacinar hepatocytic vacuolation in females.

Neoplastic Lesions - 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 positive trends for adenomas and for combined tumors. Males also had statistically significant increased 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 positive 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.

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.

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.
[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.
* = $p < 0.05$.
** = $p < 0.01$.

2. 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.

Experimental Design - Cyproconazole (technical, 94.6 to 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 18 months.

Non-Neoplastic Lesions - 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 2.

Table 2. 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.

Neoplastic Lesions - The treatment did not alter the spontaneous tumor profile in this strain of rats.

Consideration of the Adequacy of Dose Selection - There is evidence that the high-dose level tested was inadequate for the carcinogenicity bioassay. This was apparent by the lack of any biologically significant body weight decrease, the absence of any histopathological changes accompanying the increase in relative liver weight, and finally the lack of any increase in the liver enzyme activity in females, and the inconsistency of such change in the high-dose males. All of these suggest that the high-dose level was not adequate to determine the carcinogenic potential of cyproconazole. The Committee recommended a repeat of the carcinogenicity phase of the rat study.

E. Other Relevant Toxicology Information

1. Genotoxicity - Cyproconazole has been tested in several mutagenicity studies considered acceptable by the Agency. Testing in S. typhimurium was negative with and without metabolic activation. There was no in vitro cell transformation with Syrian hamster embryo cells, with and without activation. A micronucleus assay in mice was negative.

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

It was therefore determined that an additional mutagenicity study would be necessary to address a mutagenicity concern. A rodent dominant lethal study needs to be performed with this compound. For the weight-of-the-evidence discussed at the Peer Review Committee meeting that supports this request, see Kerry Dearfield memorandum of June 20, 1990.

2. Metabolism - No metabolism data are available for cyproconazole.

3. Acute, Subchronic, and Chronic Toxicity - The acute oral LD_{50} in rats was over 1000 mg/kg. Effects associated with treatment in the 13-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.

   Changes associated with treatment in a 13-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 1-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 developmental toxicity 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 the mid- and high-dose levels. 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 makes the evidence for maternal toxicity, and the maternal NOEL of 6 mg/kg, equivocal. The developmental NOEL was 6 mg/kg/day, and the LEL 12 mg/kg/day.

   In a rabbit developmental toxicity 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.

   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. The chemical was classified by the HED Peer Review Committee on June 6, 1990 as group C, possible human carcinogen.

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, 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 male CD-1 mice, and was classified by the HED Peer Review Committee as 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; 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 500 ppm. Terbuconazole and hexaconazole were reported to be negative for carcinogenicity in mice when administered at dietary concentrations up to 180 and 200 ppm, respectively. With the exception of

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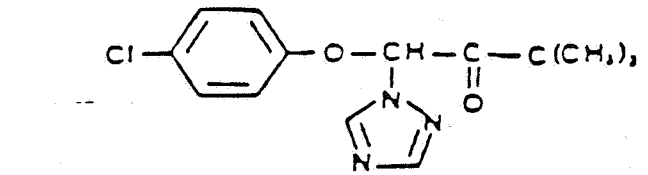
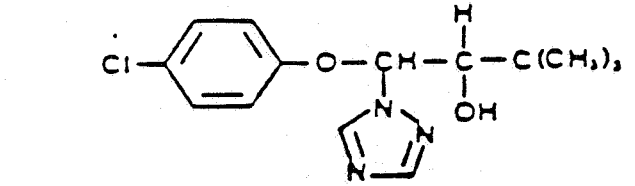
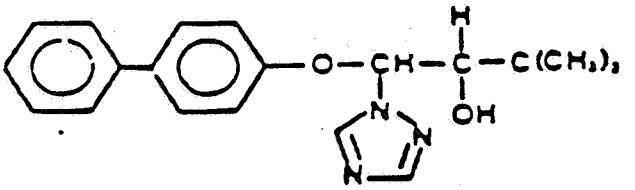


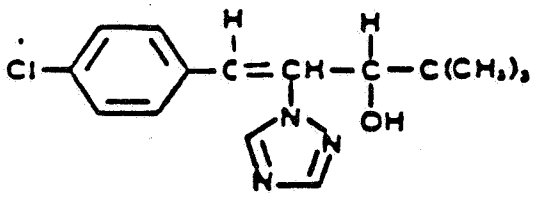
Triadimefon
(Bayleton)



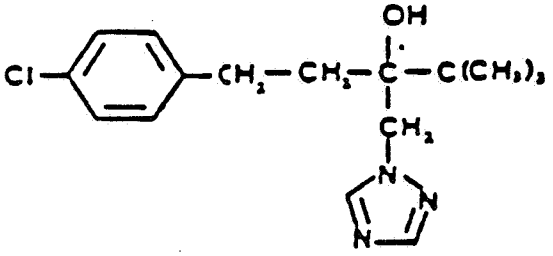
Triademenol
(Baytan)



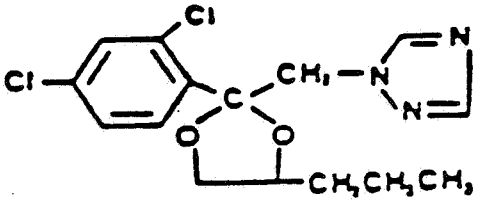
Bitertanol
(Baycor)



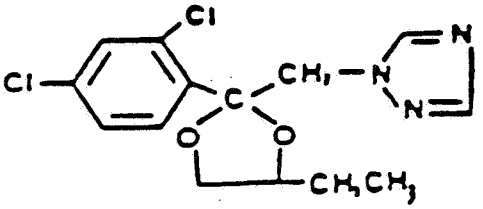
Uniconazole
(Prunit)



Terbuconazol
(Folicur)

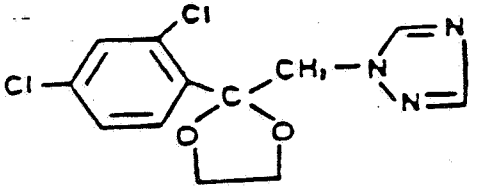


Propiconazole
(Tilt)

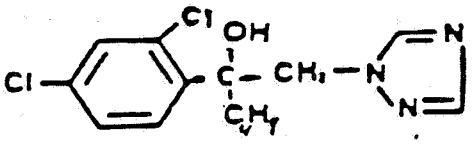


Etaconazole
(Vangard)

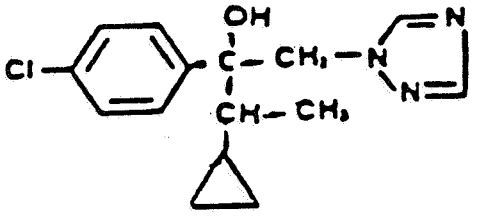
Figure 1. Structurally Related Compounds



Azaconazole



Hexaconazole
(Anvil)



Cyproconazole
(SAN 619F)

Figure 1.a. Structurally Related Compounds

cyproconazole and terbuconazole, 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 triazole compounds.

Among all triazole pesticides reviewed by the HED Peer Review Committee, Hexaconazole was the only chemical to induce this type of carcinogenic response in rats. Other triazoles were either negative or were not tested at adequate dose levels in the rat studies.

Among all triazole pesticides reviewed by the HED Peer Review Committee, only two chemicals, i. e. Uniconazole and Cyproconazole were reported to have potential to induce genotoxic response.

Uniconazole was slightly positive in the mouse micronucleus assay when tested in male mice. Uniconazole was also positive for chromosomal aberrations when tested up to cytotoxic levels with metabolic activation, but was negative when tested without metabolic activation. Uniconazole was also negative for bacterial gene mutation in Salmonella typhimurium when tested up to cytotoxic levels with metabolic activation, and up to solubility levels without metabolic activation. Uniconazole gave negative responses in E. coli with and without metabolic activation. Uniconazole did not appear to induce unscheduled DNA synthesis in primary mouse hepatocytes when tested in vivo/in vitro.

Cyproconazole was positive for potential to induce chromosomal aberrations in CHO cells with and without metabolic activation. The chemical was negative in micronucleus assay in mice, for gene mutation in the Salmonella assay, and for cell transformation with Syrian hamster embryo cells with and without metabolic activation. However, Cyproconazole has somewhat slightly different structure than other triazole pesticides, in that it has a cyclopropane moiety that is unique to Cyproconazole.

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-the-Evidence Considerations

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

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 the 100 ppm dose of cyproconazole, as well as of carcinomas at the 15 ppm dose.

2. In both female and male mice, there were statistically significant positive trends for adenomas and for combined tumors. Female mice showed a statistically significant positive 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 upto dietary concentration of 200 ppm. The dose levels fed in the long-term rat study, however, were not adequate to determine the carcinogenic potential of the test material. The Committee recommended that a new rat study should be submitted.

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

6. The Committee recommended that a rodent dominant lethal study be performed (K. Dearfield, memorandum dated June 20, 1990). This recommendation was based on the fact that administration of cyproconazole was associated with clastogenic activity; degeneration of testicular germinal epithelium in mice and dogs; fewer implantation sites, decreased litter sizes and decreased live births in a rat two-generation reproduction study; and decreased number of fetuses and live fetuses/dam, and increased fetal resorptions in developmental toxicity studies in rats and rabbits. These results suggest a potential for clastogenic effects and interaction in the germinal tissues.

7. Cyproconazole is structurally related to several triazole pesticide compounds, most of which have been associated with the formation of liver cell carcinomas and/or adenomas in either or both sexes in mice. It should be noted also that Cyproconazole induced this effect at relatively very low doses compared to other triazole peticides. Also, most of these triazoles were associated with developmental toxicity, particularly of the skeleton.

G. Classification

Considering the criteria contained in the Agency's Guideline for the Classification of Carcinogens [FR 51:33992-34003, 1986], the Committee concluded that cyproconazole should be placed in Group C, possible human carcinogen.

This conclusion was based upon statistically significant increases in the incidence of hepatocellular adenomas and carcinomas in male and female CD-1 mice, with a significantly positive trend.

This classification is further supported by structural similarity of cyproconazole to other triazole pesticides such as triadimefon, triadimenol, propiconazole, etaconazole, and uniconazole, all of which were associated with increased incidences of hepatocellular carcinomas and/or adenomas in one or more sex of mice.

Quantification of potential human cancer risk, using the low dose extrapolation model ($Q1^*$), was also recommended for both sexes. This recommendation was based on the fact that the tumor induced by cyproconazole was of a malignant nature, considered rare in females, and was also induced in two sexes of one species at a relatively low-dose level compared to other triazole pesticides. This recommendation was also supported by the structural similarity of cyproconazole to other carcinogenic triazole pesticides and some indication that cyproconazole has clastogenic activity.

The Committee recommended that the carcinogenic phase of the rat study be repeated. A range finding study (90 days) should be performed, and the Agency should be consulted on dose selection prior to the commencement of the carcinogenic study.