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DATA EVALUATION RECORD

CYPROCONAZOLE (SAN 619F)

Oncogenicity Feeding Study in Mice

APPROVED BY:

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Date: 6/16/90

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DATA EVALUATION RECORD

GUIDELINE §83-2

STUDY TYPE: Oncogenicity feeding study in mice.

MRID NUMBER: 411472-01.

TEST MATERIAL: Cyproconazole (SAN 619F).

SYNONYM(S): Alto; cyproconazol; 2-(4-chlorophenyl-3-(1H-1,2,4-triazol-1-Yl)butan-2-01.

STUDY NUMBER(S): Project 388-M/398-M.

SPONSOR: Sandoz Crop Protection Corporation, Des Plaines, IL.

TESTING FACILITY: SANDOZ AG, Agro Division, Dept. of Toxicology, Basle, Switzerland.

TITLE OF REPORT: The Potential Oncogenicity of SAN 619F by Prolonged Dietary Administration to Mice.

AUTHOR(S): Warren, S., Morand de Jouffrey, S., Müller, F., and Karapaly, J. C.

REPORT ISSUED: April 24, 1989 (reissued May 31, 1989).

CONCLUSIONS:

Cyproconazole was fed to CD-1 mice at dietary levels of 5, 15, 100, or 200 ppm for 81 weeks (males) or 88 weeks (females). There were increased incidences of combined adenomas and carcinomas in the livers of males receiving 15, 100, and 200 ppm, and in females receiving 200 ppm. Male and female mice receiving 100 and 200 ppm had increased incidences of focal hepatocytic inflammation, single-cell necrosis, and diffuse hepatocytic hypertrophy; male mice were more severely affected. There was a decrease in the amount of testicular germinal epithelium in males receiving 200 ppm. The increased testicular germinal epithelial deficit corresponded to the increased incidence of flaccid testes in male mice of the 200-ppm dose group. Body weight gains were decreased by more than 10% in both sexes fed cyproconazole at dietary levels of 100 and 200 ppm for 26 weeks. Significant increases in liver weights were seen in both males and females treated with 200 ppm cyproconazole in the diet for 13 weeks. There was no effect of dosing on mortality, food consumption, or hematology parameters. Based on a significantly increased incidence of hepatocytic single cell necrosis and diffuse hepatocytic hypertrophy in male and female mice receiving 100 and 200 ppm, and a significantly increased incidence of periacinar hepatocytic vacuolation in female mice receiving 100 ppm, the NOEL for systemic toxicity is 15 ppm, corresponding to an intake of approximately 1.8 mg/kg/day in males and 2.6 mg/kg/day in females.

Classification: Core Guideline.

A. MATERIALS:

1. Test Compound: Cyproconazole technical (SAN 619-F); description: colorless crystals; batch No.: 8507; purity: 95.1%.

2. Test Animals: Species: mouse; strain: CD-1; age: 28 days; weight: mean body weights at initiation of the main study--30 g (males) and 22 g (females); source: Charles River (Wiga) Breeding Laboratories, D-8741 Sulzfeld, BRD.

B. STUDY DESIGN:

1. Animal Assignment: Animals were acclimated to laboratory conditions for 15 days and were assigned by sex to the following test groups using computer-generated randomization:

Test group	Dietary Level (ppm)	Main study (88 weeks)[a] Males	Females	Interim sacrifice (13 weeks) Males	Females
1	0[c] (Control-1)	50	50	10	10
2	5	50	50	-	-
3	15	50	50	-	-
4	100	50	50	-	-
5	200	50	50	10	9[b]
6	0[c] (Control-2)	50	50	-	-

[a]Females were treated for 88 weeks, males for 81 weeks.

[b]One female from group 5 was used to replace a mis-sexed main group animal at the start of the study, leaving only nine in the high-dose female group.

[c]Untreated diet.

Mice were housed individually in cages in a room with temperature and humidity controls set at 23°C and 50%, respectively, and with a 12-hour light/dark cycle. No formal health screening was performed prior to treatment. However, after animals died in a neighboring room during week 46, blood samples were drawn from the orbital sinus, under light ether anesthesia, from the first six mice in each room and assayed for virus antibody titres. In addition, blood samples were drawn immediately postmortem during weeks 71-74 from mice killed in extremis, and during week 82 from male mice killed at termination, and analyzed for antibody titres.

2. Diet Preparation: Diets (100- and 200-ppm) were prepared by diluting a 1% premix with the appropriate amount of untreated diet to give the desired concentration. The 1% premix was also used to prepare a premix of 1000 ppm, which was then used to prepare diets of 5 or 15 ppm by direct dilution. The homogeneity and stability of dietary samples were determined in a previous study. During the study, samples of each premix were analyzed for accuracy of mixing at monthly intervals, and samples of each final diet were analyzed at least each 2 months.

Results: Table 1 summarizes data on nominal and analyzed dietary levels of cyproconazole. Values for test compound were generally in agreement with nominal values. The percentage deviation from nominal values, determined at various intervals throughout the treatment period, were -20% to +36%, -13% to +27%, -16% to +23%, and -22% to +6% at dose levels of 5, 15, 100, and 200 ppm, respectively.

TABLE 1. Dietary Levels of Cyproconazole

Nominal Level (ppm)	Analyzed Level	
	ppm	Range
5	5.2 ± 0.8	(4.0 - 6.3)
15	15.9 ± 1.7	(13.0 - 19.0)
100	100.6 ± 10.4	(84.0 - 123.0)
200	192.4 ± 14.5	(174.0 - 206.0)

3. Food and Water Consumption: Animals received KLIBA powdered diet No. 32-343-4 (designated 21-343-4 until week 40) and water ad libitum.

4. Statistics: The following procedures were utilized in analyzing the numerical data. One-way analysis of variance, followed by a multiple comparison to controls using Dunnett's test, was used for comparison of controls and dosed groups when variance was homogeneous. The Kruskal-Wallis test was used when variance was not homogeneous and when more than one treated group was present. The Dunn-Bonferroni test was used to perform a multiple comparison to the control group if a significant difference existed between the groups. The Mann-Whitney U test was performed for samples of four or more animals per group when only one treated group was present; with three or fewer animals per group, the Student's t-test was used.

   Adjusted mortality rate was calculated by the method of Kaplan and Meier, and intergroup differences in mortality were analyzed with Cox's method using the Tarone test for linear trend. Age-adjusted tumor analysis was essentially performed by the method of Peto, analyzing fatal tumors with lifetable analysis, incidental tumors by prevalence analysis and all tumors by combined analysis.

5. Quality Assurance: A quality assurance statement was signed and dated May 26, 1989; an updated GLP compliance statement was also present. A separate quality assurance statement for the final pathology report was dated May 30, 1989.

C. METHODS AND RESULTS:

1. Observations: Animals were inspected twice daily for signs of mortality and for gross signs of ill-health or reaction to treatment. Detailed examinations were performed biweekly from week 66 and weekly from week 78.

   Results: Cumulative mortality and survival data are summarized in Table 2. Mortality of mice receiving 100 or 200 ppm was less than that of the controls or mice receiving 5 or 15 ppm. Statistical analysis of mortality (Tarone's one-tailed test) indicated a negative trend for males ($p < 0.001$) and females ($p = 0.004$). By pairwise comparison, there was a significantly increased survival compared to controls in males receiving 15 and 100 ppm ($p < 0.05$) and 200 ppm ($p < 0.01$); for females, a significant effect ($p < 0.05$) was noted at 100 ppm but not

TABLE 2. Cumulative Mortality and Percent Survival in Mice Fed Cyproconazole for 88 Weeks[a,b]

Dose Group (ppm)	Mortality (Percent Survival) at Week: Satellite Groups 13	Main Groups 52	80	Termination[a]
		Males		
0	10(100)	4(92)	30(40)	31(38)
5	--	1(98)	29(42)	30(40)
15	--	1(98)	19(62)	25(50)
100	--	2(96)	21(58)	21(58)
200	10(100)	1(98)	11(78)	14(72)
0	--	3(94)	32(36)	34(32)
		Females		
0	10(100)	2(96)	25(50)	33(34)
5	--	0(100)	18(64)	26(48)
15	--	2(96)	24(52)	30(40)
100	--	2(96)	9(82)	14(72)
200	9(100)[c]	5(90)	12(76)	23(54)
0	--	2(96)	14(72)	27(46)

[a]Female mice were treated for 88 weeks, males for 81 weeks; terminal sacrifices were performed during week 90 for females and week 82 for males.

[b]Percent survival was based on 50 mice/sex/dose in the main group and 10 mice/sex/dose in the satellite group.

[c]One group 5 female mouse was used to replace a mis-sexed main group mouse at the start of the study, leaving only nine in the high-dose female group.

at 200 ppm. There were no deaths among the 39 mice allocated to the satellite group.

Serology samples were drawn during week 46 as a response to unexpected, sudden mortality in an adjacent room. Results showed the presence of antibodies to mouse hepatitis virus (MHV) in an unspecified number of six mice tested from each room; however, no symptoms of ill health were observed in the study, and the putative infection could be shown to be present only as a result of seroconversion. Blood samples were also drawn from mice killed in extremis during weeks 71 to 74; these were compared with samples from male mice killed at termination at week 82 to determine whether virus antibody titres had increased in mice that had died spontaneously, which might have indicated an adverse effect on survival due to the infection. These samples again revealed seroconversion to MHV (19/29 of mice tested). Seroconversion of 16/29 mice to Mycoplasma pulmonis, and of 1/29 mice to Sendai virus, was also noted. However, no pattern of incidence was seen of seroconversion between mice killed in extremis or killed at termination, or between mice in the two different rooms in which the study was housed.

The study authors concluded that the presence of seroconversion to the agents detected did not correlate with an adverse effect on the study due to these pathogens.

2. Body Weight: Mice were weighed before treatment and once each week thereafter.

Results: Table 3 summarizes data on mean body weights at selected intervals. Mean body weights were significantly decreased ($p < 0.01$) in males and females receiving 100 and 200 ppm at weeks 26 and 52. At termination, mean body weights in males receiving 100 and 200 ppm were 13% lower ($p < 0.01$) than controls; in females receiving the same doses, the body weights were decreased ($p < 0.05$) by 6%. Table 4 summarizes data on mean body weight gains between 0 and 26 weeks. Body weight gains were significantly decreased by more than 10% in both sexes receiving 100 and 200 ppm for 26 weeks.

3. Food Consumption and Compound Intake: Food consumption was determined weekly throughout the study duration. Compound intake was calculated from the consumption and body weight gain data and from nominal dietary concentrations.

Results: No treatment-related effects on food consumption were seen. A statistically greater food consumption (4.4% greater than control) among male mice receiving 5 ppm during the first 26 weeks of treatment was not considered to be of toxicological significance. Mean compound intake

TABLE 3. Mean Body Weight at Selected Intervals in Mice Fed Cyproconazole for 88 Weeks[a]

Dietary Level (ppm)	Mean Body Weights (g ± S.D.) at Week: 0	26	52	81
		Males		
0	30 ± 2	42 ± 5	44 ± 5	46 ± 8
5	30 ± 2	40 ± 5	42 ± 5	44 ± 5
15	29 ± 1	40 ± 4	43 ± 5	44 ± 6
100	30 ± 2	38 ± 4**	40 ± 4**	40 ± 5**
200	30 ± 1	38 ± 4**	41 ± 5**	40 ± 4**
0	30 ± 2	42 ± 5	44 ± 5	44 ± 7
		Females		
0	22 ± 1	29 ± 2	32 ± 3	33 ± 4
5	22 ± 1	29 ± 2	31 ± 3	31 ± 3*
15	22 ± 2	30 ± 3	32 ± 3	33 ± 5
100	22 ± 2	28 ± 2*	30 ± 2**	31 ± 3*
200	22 ± 2	28 ± 2**	30 ± 3**	31 ± 3*
0	22 ± 2	29 ± 3	32 ± 4	32 ± 3

[a]Females were treated for 88 weeks, males for 81 weeks.

*Significantly different from control value ($p < 0.05$).

**Significantly different from control value ($p < 0.01$).

TABLE 4. Mean Body Weight Gains in Mice Fed Cyproconazole for 26 Weeks

Dietary Level (ppm)	Mean Weight Gain (gram gain/mouse/week) Between 0 and 26 Weeks	
	Males	Females
0 (control-1)	0.44	0.27
5	0.41 (-7.8)[a]	0.25 (-5.7)
15	0.41 (-8.0)	0.30 (+10.8)
100	0.32** (-28.3)	0.22** (-19.2)
200	0.32** (-28.7)	0.22* (-17.7)
0 (control-2)	0.44 (-1.3)	0.27 (-0.7)

[a]The values in parentheses are the percent decreases/increases in mean weight gain compared to controls.

*Significantly different from control value ($p \leq 0.05$).

**Significantly different from control value ($p \leq 0.01$).

values for 81 weeks (males) or 88 weeks (females) of the study were 0.69, 1.84, 13.17, and 27.85 mg/kg/day for males and 1.03, 2.56, 17.65, and 36.30 mg/kg/day for females receiving 5, 15, 100, or 200 ppm, respectively.

4. Ophthalmological Examinations: Ophthalmological examinations were not performed.

5. Hematology and Clinical Chemistry: Blood smears were prepared after 52 weeks, after 79 weeks, and prior to female terminal sacrifice (90 weeks) from mice of the control group and from the 200-ppm group for differential white blood cell count. The following cell types were evaluated by light microscopy: banded neutrophils, segmented neutrophils, lymphocytes, monocytes, eosinophils, and basophils. Clinical chemistry parameters were not examined.

   Results: No effects of dosing on leukocyte differential count were seen.

6. Urinalysis: Not performed.

7. Sacrifice and Pathology: All animals that died and that were sacrificed on schedule were subject to gross pathological examination; the CHECKED (X) tissues were collected for histological examination. In addition, the (XX) organs were weighed:

	Digestive System		Cardiovasc./Hemat.		Neurologic
X	Tongue		Aorta†	XX	Brain
X	Salivary glands†	XX	Heart†	X	Peripheral nerve (sciatic nerve)†
X	Esophagus†	X	Bone marrow†	X	Spinal cord (3 levels)
X	Stomach†	X	Lymph nodes†	X	Pituitary†
X	Duodenum†	XX	Spleen	X	Eyes (optic nerve)†
X	Jejunum†	X	Thymus		
X	Ileum†				
X	Cecum†				
X	Colon†				
X	Rectum†		Urogenital		Glandular
XX	Liver†	XX	Kidneys†	XX	Adrenals†
X	Gallbladder†	X	Urinary bladder†		Lacrimal gland
X	Pancreas†	XX	Testes†	X	Mammary gland†
		X	Epididymes	X	Thyroids†
		X	Prostate	X	Parathyroids†
		X	Seminal vesicle		Harderian glands
	Respiratory	X	Ovaries		
X	Trachea†	X	Uterus		
X	Lung†	X	Cervix		Other
				X	Bone (sternum and femur)†
				X	Skeletal muscle†
				X	Skin
				X	All gross lesions and masses

†Recommended by Subdivision F (October 1982) Guidelines.

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Results:

a. Organ Weights: After 13 weeks of treatment, the liver weights of male and female mice receiving 200 ppm were significantly increased ($p < 0.01$) when compared to controls for both the absolute weight and the weight relative to body weight. At termination, the liver weights of males and females receiving 100 or 200 ppm were again significantly increased ($p < 0.01$) when compared to controls. Following an analysis in which the weights of livers with a tumor greater than 10 mm diameter were excluded, the study authors concluded that the increase in liver weight was not a consequence of neoplasia. There were no other organ weight changes that could be considered a result of treatment. Table 5 presents liver weight data.

b. Gross Pathology: Table 6 summarizes the incidence of frequently observed gross lesions in mice sacrificed or dying during the treatment period of the main study. A significantly increased incidence of hepatic accentuated lobular pattern was found among males treated with 15, 100, and 200 ppm cyproconazole, and among females treated with 100 and 200 ppm. The incidence of hepatic masses was also increased in male mice of the 100-, and 200-ppm dose group, and in female mice treated with 200 ppm. The hepatic masses corresponded to the treatment-related increases in hepatic adenomas and carcinomas in both sexes. There were also increased incidences of nonspecified areas of hepatic change and enlargement in female mice of the 200-ppm dose group, and of "granular" livers in the corresponding male group. Significantly increased incidences of flaccid testes were noted in male mice of the 15- and 200-ppm dose groups; however, these increased incidences corresponded to an increased testicular germinal epithelial deficit in male mice of the 200-ppm dose group only. Increased incidences of skin ulceration observed in all treated groups of both sexes were significantly different only in female mice of the 200-ppm dose group. Significant reductions in the incidences of edema of the subcutis were reported in male mice treated with 100 and 200 ppm. Renal findings that may relate to treatment were restricted to a reduced incidence of granular kidneys in females receiving 200 ppm, and to an apparent reduction in kidney size of the corresponding male group. A number of other gross findings not considered related to dosing were reported. These findings included increased incidences of apparent heart auricle enlargement in males, reduced incidence of cystic

TABLE 5. Mean Liver Weights (g ± S.D.) and Liver Weights as a Percentage of Body Weight in Mice Fed Cyproconazole for 88 Weeks[a]

Dietary Level (ppm)	Liver Weights at Time of Sacrifice			
	Interim Sacrifice (Week 14)			
	Males		Females	
	(g)	(%)	(g)	(%)
0	1.355 ± .283	3.988 ± .372	1.045 ± .136	4.410 ± .582
200	1.913 ± .314**	6.097 ± .547**	1.511 ± .467**	6.425 ± 1.51**
	Terminal Sacrifice			
	Males		Females	
	(g)	(%)	(g)	(%)
0	2.554 ± 1.350	5.488 ± 2.634	1.730 ± 0.251	4.963 ± 0.479
5	2.193 ± .248	4.885 ± 0.630	1.536 ± 0.221	4.878 ± 0.560
15	2.426 ± .446	5.322 ± 1.028	1.828 ± 0.669	5.257 ± 2.077
100	2.770 ± .884	6.682 ± 1.769**	2.145 ± 0.384**	6.407 ± 0.932**
200	3.228 ± 1.242**	7.789 ± 2.602**	2.570 ± 0.621**	7.897 ± 1.692**
0	2.252 ± 0.500	5.108 ± 1.144	1.648 ± 0.194	4.895 ± 0.544

[a]Females were treated for 88 weeks, males for 81 weeks.

**Significantly different from control value ($p < 0.01$).

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TABLE 6. Representative Gross Findings in Mice Fed Cyproconazole for 88 Weeks[a,b]

Organ/Finding	Males						Females					
	Control Group 1: 0	5	15	100	200	Control Group 2: 0	Control Group 1: 0	5	15	100	200	Control Group 2: 0
Heart, auricle												
Enlarged	11	9	19**	6	8	7	2	2	5	2	5	2
Kidneys												
Granular	14	16	17	13	12	13	21	21	17	17	10*	18
Cystic	14	14	14	5*	9	11	7	7	4	9	3	8
Liver												
Accentuated lobular pattern	3	5	10*	13***	23***	3	3	4	3	12**	21***	4
Masses	5	5	8	17***	20***	5	1	1	3	5	18***	2
Granular	0	0	0	1	4*	1	1	0	1	0	3	1
Enlarged	1	0	1	2	5	3	2	1	1	6	10**	2
Skin												
Ulceration	6	7	7	7	10	3	2	6	4	6	9*	4
Subcutis Edematous	8	12	11	3*	2*	12	6	3	6	0*	5	4
Testes												
Flaccid	1	4	9**	6	9**	3	0	0	0	0	0	0

[a]Females were treated for 88 weeks, males for 81 weeks.

[b]Includes all animals in main groups (50/sex/group), but not interim sacrifice animals.

*Significantly different from control value ($p < 0.05$).

**Significantly different from control value ($p < 0.01$).

***Significantly different from control value ($p < 0.001$).

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kidneys in males, increased incidence of seminal vesicle enlargement, increased skin pallor in males, and a reduction in perineal staining in females. No significant pathological changes were detected among mice in the satellite study.

c. Microscopic Pathology:

1) Nonneoplastic: The only nonneoplastic effects observed at the interim sacrifice, and that were attributed to dosing, were in the liver. The incidence of periacinar hepatic hypertrophy was increased in the high-dose males (10/10) and females (8/10) when compared to controls (males--4/10, females--0/10). In addition, periacinar hepatic vacuolation was seen in five males and non-zonar hepatocytic vacuolation in four males and eight females. Hepatocytic vacuolation was not seen in any of the controls at the interim sacrifice (week 14). Table 7 summarizes the incidences of frequently occurring nonneoplastic lesions in mice in the main group, combining data for those that died, were sacrificed moribund, or were sacrificed at termination. The primary treatment-related alterations following oral treatment with cyproconazole were seen in the liver. Increased incidences of focal hepatocytic inflammation, single-cell necrosis, and diffuse hepatocytic hypertrophy were noted in both male and female mice receiving 100 and 200 ppm. An increased incidence of centriacinar hepatocytic vacuolation was seen in males receiving 200 ppm and in females receiving 100 and 200 ppm. An increased incidence of periacinar hepatocytic vacuolation was seen in females treated with 100 ppm and 200 ppm, and in males treated at 5 ppm. A number of significant but trivial changes were also reported. In male mice receiving 100 and/or 200 ppm, these changes included increased incidences of epididymal aspermia, optic nerve gliosis, skin ulceration, cellulitis, and testicular epithelial deficit. Decreased incidences of pancreatic edema and subcutaneous edema, and interstitial degeneration in the salivary glands, were also seen in these males. In female mice receiving 100 and/or 200 ppm, increased incidences of aortic arteritis and lymphoid hyperplasia in the mesenteric lymph nodes, and decreased incidences of subcutaneous edema and spinal cord compression, were reported. Slight to

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TABLE 7. Representative Nonneoplastic Finding in Mice Fed Cyproconazole for 88 Weeks[a,b]

Organ/Finding	Dietary Level (ppm)											
	Males						Females					
	0	5	15	100	200	0	0	5	15	100	200	0
Liver	(50)[c]	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)
Focal inflammation	1	1	4	5*	8**	1	1	5	9	5	4	6
Single cell necrosis	0	2	3	14***	25***	2	0	3*	2	4*	9***	0
Diffuse hepatocytic hypertrophy	4	4	6	26***	36***	10	5	6	6	7	20***	8
Centriacinar hepatocytic vacuolation	0	0	0	1	3*	0	0	0	0	4*	3*	0
Periacinar hepatocystic vacuolation	0	4*	3	1	1	1	0	0	1	17***	6**	1
Periacinar hepatocytic hypertrophy	1	2	5*	4*	2	0	0	1	0	0	3*	0
Focal hepatocytic hyperplasia	1	0	2	2	2	1	0	0	1	0	5**	0
Testes	(50)	(50)	(50)	(50)	(50)	(50)	(0)	(0)	(0)	(0)	(0)	(0)
Germinal epithelium deficit	22	31	29	34**	33*	23	0	0	0	0	0	0
Optic nerve	(45)	(45)	(44)	(45)	(46)	(41)	(40)	(40)	(43)	(38)	(37)	(39)
Gliosis	0	2	2	2	3*	0	0	2	0	1	2	1
Salivary gland	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)
Interstitial degeneration	8	6	1	0*	0*	2	3	4	0	0	0	0
Skin	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)
Ulceration	4	5	4	6	9*	2	1	4	3	5	4	3
Cellulitis	4	5	6	8*	8*	1	1	4	3	5	6	3
Subcutaneous edema	7	5	12	3	2*	11	6	4	6	1*	1*	11
Epididymides	(50)	(50)	(50)	(50)	(50)	(50)	(0)	(0)	(0)	(0)	(0)	(0)
Aspermia	10	20	15	26**	21*	15	0	0	0	0	0	0
Heart, ventricle	(50)	(50)	(50)	(50)	(50)	(50)	(49)	(50)	(50)	(50)	(50)	(50)
Aortic arteritis	0	0	0	0	0	1	1	0	0	2	4*	0
Mesenteric lymph node	(48)	(45)	(49)	(49)	(47)	(50)	(47)	(49)	(46)	(50)	(50)	(50)
Lymphoid hyperplasia	0	0	2	2	0	1	3	2	1	2	13*	7

(continued)

TABLE 7. (continued)

Organ/Finding	Dietary Level (ppm)											
	Males						Females					
	0	5	15	100	200	0	0	5	15	100	200	0
Pancreas	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)
Edema	3	12	8	1	0*	9	6	6	7	2	2	4
Caecum	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)
Submucosal edema	5	7	11	4	6	12	7	7	8	3*	0**	12
Spinal cord (cervical)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(49)	(50)	(50)	(50)
Cord compression	6	3	5	2	4	2	5	1	5	2	0*	6

[a]Female mice were treated for 88 weeks, males for 81 weeks.

[b]Fisher's two-tailed Exact test comparing pooled controls vs. the treated groups was used for statistical evaluation of the data.

[c]The numbers in parentheses are the numbers of animals with specific organ examined microscopically.

*Significantly different from control value ($p < 0.05$).

**Significantly different from control value ($p < 0.01$).

***Significantly different from control value ($p < 0.001$).

moderate amyloidosis was observed in both sexes, with the incidences in different organs varying greatly. Also, the incidence of amyloidosis varied between the sexes. Table 8 summarizes data on amyloid deposition. An increase in amyloid deposition was seen in the gallbladder, ileum, salivary gland, and testes of male mice. A decrease in splenic amyloid deposition was noted in both male and female mice. A decrease in amyloid deposition was seen in the heart, kidney, and liver of female mice. The study authors noted that a probable "sparing" effect of amyloid deposition may have contributed to an increased survival among mice receiving 100 or 200 ppm.

2) Neoplastic: Table 9 summarizes the incidence of neoplastic findings in mice in the main group, combining data for those that died, were sacrificed moribund, or were sacrificed at termination. There were no increases in neoplasms at any site except the liver. A significant ($p < 0.01$) increase in the incidence of hepatocytic adenoma in males receiving 100 and 200 ppm was noted. An increased incidence of hepatocytic carcinoma in males receiving 15 and 100 ppm and in females receiving 200 ppm was reported. An increased incidence of hepatocellular tumors (adenoma and carcinoma) was seen in males receiving 15, 100, and 200 ppm and in females receiving 200 ppm. However, the increased incidence of hepatocytic tumors in males receiving 15 ppm was not considered to be treatment-related by the study authors, but rather was considered to be a consequence of increased survival. Age-adjusted tumor incidence was analyzed by the authors by the method of Peto combining fatal and incidental tumors. For hepatocellular tumors (adenomas and carcinomas combined), the adjusted rates in males were 16.1, 17.2, 39.0, 52.2, and 32.7%, and in females the rates were 0, 0, 0, 6.1, and 33.6% at dose levels of 0, 5, 15, 100, or 200 ppm, respectively. The increase in males was significant at 100 ppm ($p = 0.003$) but not at 15 ppm ($p = 0.188$) or at 200 ppm ($p = 0.052$). In females, the increase was significant at 200 ppm ($p = 0.001$) but not at 10 ppm ($p = 0.135$). There was a significant dose trend in both males ($p < 0.01$) and females ($p < 0.001$). The authors' interpretation of the hepatocarcinogenic no-effect level of 15 ppm for males was based on a consideration of the greater longevity of male mice receiving 15, 100, and 200 ppm, and of females receiving 200 ppm, and the results of the Peto statistical analysis using

TABLE 8. Amyloid Deposition in Mice Fed Cyproconazole for 88 Weeks[a,b]

Organ/Finding	Dietary Level (ppm)									
	Males					Females				
	0	5	15	100	200	0	5	15	100	200
Liver	(100)[c]	(50)	(50)	(50)	(50)	(100)	(50)	(50)	(50)	(50)
	23	4*	12	4*	8	32	19	16	6**	3***
Heart, ventricle	(100)	(50)	(50)	(50)	(50)	(99)	(50)	(50)	(50)	(50)
	21	3	10	5	10	27	8	5*	2***	4**
Testes	(100)	(50)	(50)	(50)	(50)	(0)	(0)	(0)	(0)	(0)
	35	20	22	25	27*	0	0	0	0	0
Gallbladder	(97)	(49)	(49)	(48)	(48)	(99)	(49)	(48)	(49)	(49)
	0	1	0	0	5**	2	2	1	1	0
Ileum	(100)	(50)	(50)	(50)	(50)	(100)	(50)	(50)	(50)	(49)
	44	24	32	28	34**	44	29	30	35**	27
Salivary gland	(100)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)
	10	4	13*	11	16**	31	11	14	20	16
Spleen	(100)	(50)	(50)	(50)	(50)	(99)	(50)	(50)	(50)	(50)
	26	7	10	3**	1***	29	13	14	1***	1***
Adrenal gland	(100)	(50)	(47)	(49)	(50)	(100)	(50)	(49)	(50)	(50)
	54	29	27	26	25	44	22	28	18	18
Kidney	(100)	(50)	(50)	(50)	(50)	(100)	(50)	(50)	(50)	(50)
	57	28	31	25	28	54	28	31	28	21

[a]Female mice were treated for 88 weeks, males for 81 weeks.

[b]Fischer's two-tailed Exact test comparing pooled controls vs. the treated groups was used for statistical evaluation of the data.

[c]The numbers in parentheses are the numbers of animals with specific organ examined microscopically.

*Significantly different from control value ($p < 0.05$).

**Significantly different from control value ($p < 0.01$).

***Significantly different from control value ($p < 0.001$).

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TABLE 9. Neoplastic Lesions in Mice Fed Cyproconazole for 88 Weeks[a,b]

Organ/Finding	Dietary Level (ppm)									
	Males					Females				
	0	5	15	100	200	0	5	15	100	200
Liver	(100)[c]	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)
Hepatocytic Adenoma	6[d]	4	5	12**	12**	0	0	0	2	6**
Hepatocytic Carcinoma	0	0	3*	3*	1	0	0	0	0	7**
Hepatocellular Adenoma/Carcinoma	6[e]	4	8*	15***	13***	0	0	0	2	13***
Lungs	(100)	(50)	(50)	(50)	(50)	(99)	(50)	(50)	(50)	(50)
Pulmonary adenoma	10	3	7	4	2	9	3	5	1	3
Pulmonary carcinoma	3	0	0	1	1	0	0	0	1	0
Mammary gland	(0)	(0)	(0)	(0)	(0)	(46)	(21)	(25)	(12)	(15)
Adenocarcinoma (Type B)	0	0	0	0	0	1	0	2	1	0
Spleen	(100)	(50)	(50)	(50)	(50)	(99)	(50)	(50)	(50)	(50)
Hemangiosarcoma	0	0	0	0	0	2	1	0	1	0
Uterus + Cervix	(0)	(0)	(0)	(0)	(0)	(100)	(50)	(50)	(50)	(50)
Leiomyosarcoma	0	0	0	0	0	7	2	3	3	4
Leiomyona	0	0	0	0	0	3	5	4	4	5
Fallopian tube adenoma	0	0	0	0	0	1	0	0	0	0
Endometrial carcinoma	0	0	0	0	0	2	0	0	0	1

[a]Female mice were treated for 88 weeks, males for 81 weeks.

[b]Includes animals in main study that died, were sacrificed moribund, or were sacrificed at study termination.

[c]The numbers in parentheses are the numbers of animals with specific tissue examined histologically.

[d]Fischer's one-tailed Exact test comparing pooled controls vs. the treated groups was used for statistical evaluation of the data.

[e]Significant trend by the Cochran-Armitage test ($p < 0.001$).

*Significantly different from control incidence ($p < 0.05$).

**Significantly different from control incidence ($p < 0.01$).

***Significantly different from control incidence ($p < 0.001$).

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lifetable methodology to compensate for this extended longevity. Non-treatment-related neoplasms that occurred with an incidence of 2% or less in all groups included, in female mice, medullary adenomas, malignant lymphoma of the ileum, pituitary adenomas, osteogenic sarcoma, and skin basal cell tumor. In males, incidental neoplasms included oligodendroglioma, leiomyoma, skin merkel cell tumor, splenic hemangioma, testicular interstitial cell adenoma, urinary bladder papilloma, and hemangiosarcoma of the diaphragm.

D. STUDY AUTHORS' CONCLUSIONS:

The primary treatment-related effects of cyproconazole were observed in the liver, and included both toxic and neoplastic changes. There were increased incidences of combined adenomas and carcinomas in males fed cyproconazole at dietary levels of 15, 100, and 200 ppm, and in females fed 200 ppm. Appropriate statistical analysis (the method of Peto) revealed that the apparent increase in hepatocytic neoplasia seen at 15 ppm was due to increased survival among these mice. Male and female mice receiving 100 and 200 ppm had increased incidences of focal hepatocytic inflammation, single-cell necrosis, and diffuse hepatocytic hypertrophy; male mice were more severely affected. An increased incidence of periacinar hepatocytic vacuolation was seen in females treated with 100 ppm. Other changes that were associated with treatment included a decrease in the amount of testicular germinal epithelium in males receiving 200 ppm, and possibly an increase in skin ulcerations in all treated groups of both sexes, although statistically significantly different only in female mice of the 200-ppm dose group. The increased testicular germinal epithelial deficit corresponded to the increased incidence of flaccid testes in male mice of the 200-ppm dose group. Body weight gains were reduced by more than 10% in males and females fed cyproconazole at dietary levels of 100 and 200 ppm. At these dose levels, a "sparing" effect of amyloid deposition may have contributed to an increased survival among these mice. An increase in the liver weights of both sexes were seen following treatment with 200 ppm for 13 weeks when compared to controls for both the absolute weight and the weight relative to body weight. Increases in liver weights were also seen in females of the 100-ppm group at termination. The increase in liver weights was not a consequence of neoplasia. No effects on other organ weights were seen in either sex receiving 5, 15, 100, or 200 ppm. There was no effect of dosing on mortality, food consumption, or hematology parameters. The no-effect level (NOEL) for toxicity was established at 15 ppm.

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E. REVIEWERS' DISCUSSION AND INTERPRETATION OF RESULTS:

The study design was complete and adequate, and the data were well reported. Summary data were supported by individual animal data, and mean values that were validated agreed with the authors' values.

Actual dietary values were generally in agreement with nominal values; a deviation greater than ±20% from the normal limits was encountered on several occasions, especially among the low-level diets. However, the variations were not sufficient in degree or frequency to affect the interpretation of the study. The findings of this study suggest that the liver is a target organ for cyproconazole toxicity. The primary treatment-related alterations observed in the livers of male and female mice following oral treatment with cyproconazole (100 and 200 ppm) in the diet included focal hepatocytic inflammation, single-cell necrosis, and diffuse hepatocytic hypertrophy. An increased incidence of centriacinar hepatocytic vacuolation was seen in male mice receiving 200 ppm and in females receiving 100 and 200 ppm. Periacinar hepatocytic vacuolation was noted in females treated at 100 ppm and 200 ppm, and in males treated at 5 ppm; this alteration was not dose-related. Male mice were more sensitive to the toxic effects of cyproconazole than female mice. Histological changes in the liver correlated with gross pathological changes (accentuated lobular pattern) and increased organ weight. Liver weights in males were increased by 41% at week 14 and by 34% at termination. Liver weights in females were increased by 44% at week 14 and 52% at termination. The incidence of amyloid deposition varied greatly among organs and between male and female mice. Increased incidences of amyloid deposition were seen in the gallbladder, ileum, salivary gland, and testes of male mice. Decreased incidences of amyloid deposition were observed in the spleens of both sexes and in the heart, kidney, and liver of females. Although the study authors concluded that a "sparing" effect of amyloid deposition may have contributed to increased survival among the high-dose male and female groups, the data are insufficient to support the conclusion. No data were provided that showed a correlation between amyloid deposition and increased survival among mice. In addition, the changes in amyloid deposition were minimally to moderately severe and included increases as well as decreases in incidence. Increases in amyloid deposition are usually associated with cellular infiltration and degeneration of organs. The results of this study also indicate that cyproconazole is a possible carcinogen in male CD-1 mice. Increased incidences of combined liver adenomas and carcinomas were detected in females receiving 200 ppm, and in males receiving 15, 10[illegible], and 200 ppm.

We agree with the study authors' assessment of a NOEL of 15 ppm for systemic toxicity.