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CASWELL

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

C10879

MAR 30 1994

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Cyproconazole: 13-Week Feeding Study in Rats - Marked 6(a)(2)

TO: Carl Grable
PM Team Reviewer (21)
Registration Division (7509C)

FROM: Linda L. Taylor, Ph.D. Linda Lee Taylor 3/28/94
Toxicology Branch II, Section II
Health Effects Division (7509C)

THRU: K. Clark Swentzel K. Clark Swentzel 3/28/94
Section Head II, Toxicology Branch II
Health Effects Division (7509C)

and

Marcia van Gemert, Ph.D. Marcia Gemert 3/29/94
Chief, Toxicology Branch II, HED (7509C)

Registrant: Sandoz Crop Protection Corporation
Chemical: α-(chlorophenyl)-α-(1-cyclopropylethyl)-1H-1,2,4-triazole-1-ethanol
Synonyms: Cyproconazole Technical
Caswell No.: 272E
Case : 285399
Submission: S458256
Barcode: D199359
Identifying No.: 285399
Shaughnessy No.: 128993
MRID No.: 430786-01
Action Requested: Please review attached 6(a)(2) submission: Final report of 13-week rat feeding study. SWAT team priority: 180 days.

Comment: The Registrant has submitted the final report of a 13-week Wistar rat feeding study with histopathological evaluation that reports the presence of macrophages that were not previously identified. The study has been reviewed, and the DER is appended.

The current 90-day feeding study in rats was performed to characterize the subchronic toxicity of Cyproconazole and to justify dosage levels used in the rat carcinogenicity study [Project 357-R] found inadequate by the HED CPRC. The results do not affect the Agency's original assessment of the carcinogenicity

(1)

study; i.e., the highest dose utilized [350 ppm] in the rat carcinogenicity study was inadequate to assess the carcinogenic potential of Cyproconazole. Other than initially in males, body weight/gain were not affected by treatment at the 350 ppm dose level for either sex in the current 90-day study. Additionally, in females at the 700 and 1400 ppm dose levels, comparable body-weight gain deficits were observed and, although final body weights were decreased at both dose levels compared to the control, the decrease was inversely related to dose. Additionally, the magnitude of the decrease in body weight may have been affected by the "inadvertent fasting" that occurred during the study on several occasions.

The effects observed in the liver are those expected and as observed previously, result from adaptive processes and are not toxic effects per se. The occurrence of vascular macrophages is another normal process of the body's defensive mechanism against injury. Only one high-dose male displayed any signs of necrosis, and the only other rats displaying necrosis were two low-dose females with single cell necrosis. It is of interest that the increase in the incidence and severity of thyroid microfollicular epithelial hypertrophy was attributed by the Registrant to an adaptive response rather than a toxic change. Based on the results of this study and those of the rat carcinogenicity, the previous 13-week rat and 4-week rat studies, dose levels of Cyproconazole up to 1400 ppm appear appropriate for a repeat rat carcinogenicity study, as recommended by REngler [calculated via a simulated risk assessment; memo from Engler to Swentzel dated 9/21/92].

NOTE: It is not evident why the terminology of the microscopic findings differs between the Study Director's final report and that of the Pathologist's report [e.g., necrosis with centrilobular bridging vs centrilobular scarring (post necrotic scarring)] or why the text of the Pathologist's report uses different terminology than that used in the individual animal data sheets [e.g., foamy macrophages vs vascular macrophages; slightly pigmented foamy macrophages vs histiocytosis]. Although it will not affect the current assessment of the study, the Registrant should be requested to clarify the use of varying terminologies with respect to the microscopic findings. Additionally, a more detailed discussion of the "inadvertent fasting" should be requested [duration, whether all cages of each group were affected each fast, how close to terminal sacrifice was the last fast and what was its duration].

EXECUTIVE SUMMARY: Oral administration of Cyproconazole via the diet to Wistar rats [10 /sex/group] for 13 weeks at dose levels of 0, 20 ppm [1.5 ♂/2.0 ♀ mg/kg], 350 ppm [27.3 ♂/35.4 ♀ mg/kg], 700 ppm [55.6 ♂/74.6 ♀ mg/kg], and 1400 ppm [114.5 ♂/139.4 ♀ mg/kg] resulted in signs of toxicity [decreased body weight at 13 weeks {♂♂ 90%, and 81% of control and ♀♀ 90% and 93% of control at 700 and 1400 ppm, respectively}, decreased overall body-weight gain {♂♂ 84% (700 ppm) and 69% (1400 ppm) of control; ♀♀ 80% (700 ppm) and 79% (1400 ppm) of control}, decreased food efficiency, changes in

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several clinical chemistry parameters consistent with liver toxicity and/or fasting, decreased spleen {♂♂ & ♀♀} and pituitary {♂♂} weights and increased liver weight {♂♂ & ♂♂}, and microscopic, treatment-related changes in the liver {↑ incidence/severity of hepatocellular centrilobular hypertrophy and vacuolation} and thyroids {↑ incidence/severity of microfollicular cell hypertrophy}, an ↑ in the incidence of histiocytosis in the spleen, and an ↑ in the number of vascular macrophages of the liver, kidneys, and lungs].

The NOEL can be set at 20 ppm [1.5 ♂/2.0 ♀ mg/kg/day], the LEL at 350 ppm [♂♂ 27.3/♀♀ 35.4 mg/kg/day], based on decreased body-weight gain in males and increased liver weight in females.

This study is classified Core Minimum, and it satisfies the guideline requirement [82-1(a)] for a subchronic oral toxicity study in rodents.

Reviewed by: Linda L. Taylor, Ph.D. Linda Lee Taylor 3/28/94
Tox. Branch II, Section II (7509C)
Secondary Reviewer: K. Clark Swentzel K. Clark Swentzel 3/28/94
Tox. Branch II, Head Section II (7509C)

DATA EVALUATION REPORT

STUDY TYPE: 90-Day Oral - rat

TOX. CHEM. NO.: 272E

MRID NO.: 430786-01

Shaughnessey No.: 128993

TEST MATERIAL: Cyproconazole

SYNONYMS: α-(chlorophenyl)-α-(1-cyclopropylethyl)-1H- 1,2,4-triazole-1-ethanol; SAN 619 F

STUDY NUMBER: Project # 479R; TDS BS3401; Agro Tox Report 93/011

SPONSOR: Sandoz Agro, Inc.

TESTING FACILITY: Sandoz Agro, Inc., Department of Toxicology-Switzerland

TITLE OF REPORT: Cyproconazole - 13-Week Feeding Study in Rats

AUTHOR: SFP Warren, F Dorobek, and F Müller

REPORT ISSUED: May, 1993

QUALITY ASSURANCE: A quality assurance statement and a GLP Compliance Statement were provided.

EXECUTIVE SUMMARY: Oral administration of Cyproconazole via the diet to Wistar rats [10 /sex/group] for 13 weeks at dose levels of 0, 20 ppm [1.5 ♂/2.0 ♀ mg/kg], 350 ppm [27.3 ♂/35.4 ♀ mg/kg], 700 ppm [55.6 ♂/74.6 ♀ mg/kg], and 1400 ppm [114.5 ♂/139.4 ♀ mg/kg] resulted in signs of toxicity [decreased body weight at 13 weeks {♂♂ 90%, and 81% of control and ♀♀ 90% and 93% of control at 700 and 1400 ppm, respectively}, decreased overall body-weight gain {♂♂ 84% (700 ppm) and 69% (1400 ppm) of control; ♀♀ 80% (700 ppm) and 79% (1400 ppm) of control}, decreased food efficiency, changes in several clinical chemistry parameters consistent with liver toxicity and/or fasting, decreased spleen {♂♂ & ♀♀} and pituitary {♂♂} weights and increased liver weight {♂♂ & ♂♂}]. Additionally, microscopic, treatment-related, changes were observed in the liver {↑ incidence/severity of hepatocellular centrilobular hypertrophy and vacuolation}, thyroids {↑ incidence/severity of microfollicular cell hypertrophy}, and spleen {↑ incidence of histiocytosis}, and an ↑ in the number of vascular macrophages was observed in the liver, kidneys, and lungs. **The NOEL can be set at 20 ppm [1.5 ♂/2.0 ♀ mg/kg/day], the LEL at 350 ppm [♂♂ 27.3/♀♀ 35.4 mg/kg/day], based on decreased body-weight gain in males and increased liver weight in females.**

This study is classified Core Minimum, and it satisfies the guideline requirement [82-1(a)] for a subchronic oral toxicity study in rodents.

A. MATERIALS:

1. Test Compound: Cyproconazole; Description: pale brown colored powder; Batch #: Lot #: 8507; Purity: listed as ≈95% in final report, 94.8% in analytical report.

2. Test Animals: Species: rat; Strain: Wistar; Age: ≈28 days old on receipt; Weight: within a weight range of ± 10% of mean value per sex on receipt; a mean of 54.5 g ♂♂/53.4 g ♀♀ at week -2; Source: BRL Breeding Laboratories, CH-4414-Füllinsdorf, Switzerland.

3. Statistics: The following tests were used: parametric data: ANOVA followed by Dunnet's. If ANOVA failed, the following non-parametric methodology was used: non-parametric data: Kruskal-Wallis analysis of ranks followed by Mann-Whitney-U; count data: Chi-square, followed by Fisher's exact test. VAX-computer-based software package PSA [Scientific Computer Consultants INC., NJ/USA and METTLER/EPSON computer-based software package [Mettler Toledo (Schweiz) AG, Switzerland] were utilized. For pathology findings, a dose-related trend test [Armitage] was performed on selected findings, and Chi^2 tests with trends were performed on the distribution of selected liver lesions.

B. STUDY DESIGN

1. Methodology: Fifty males and 50 females [acclimated for 14 days; 2 rats/cage; sexes separate] were selected from the original 54 rats/sex. On arrival, 2 rats of the same sex were housed per cage using a computer-generated cage distribution plan, with rats being allocated to successive cages without discrimination in chronological order of unpacking. After one week, the rats were weighed, and the body weights were tested for inter-group homogeneity by computer. Final acceptance of rats in groups was decided according to the results of this homogeneity test. Four rats/sex [selection process not stated] were used for health check purposes: blood was drawn from each during acclimatization period for WBC counts and virus serology, and each was subjected to necropsy. The findings indicated that the batch of rats was suitable for testing. TB II notes that all rats that were received were used. It is not clear to this reviewer how the individual cages containing 2 rats of the same sex were allocated to the various dose groups. There were five groups, each composed of 10 rats/sex, and each was administered the test material [0, 20, 350, 700, or 1400 ppm] via the diet for 13 consecutive weeks. The controls received untreated diet. The rats were provided with feed [KLIBA powdered diet # 32-343-4] and water ad libitum, both offered freshly each week . This study was performed to "characterise the subchronic toxicity of Cyproconazole by

dietary admixture and to justify dosage levels used on a carcinogenicity study." The dose levels were selected on the basis of doses selected in a carcinogenicity study performed at this laboratory [Project 357-R].

Dose preparation: The powdered test material was mixed by direct dilution with powdered diet [1% premix] each month, and the premix was stored at room temperature until used. The final diets were prepared weekly by direct dilution of the premix. Test material stability in the diet has been demonstrated previously [Sandoz Agro Report CBK 6798/87]. Samples of the diets prepared during weeks 1, 3, 5, and 10 [12 in analytical report] were analyzed to determine the accuracy of the preparations and to verify the mixing procedure. Homogeneity of the premix and low dose was verified at week 1.

RESULTS

The procedures utilized in mixing the diets were determined to result in homogeneous diet formulations. For the two highest dose levels, the proposed concentrations were attained. For the two low-dose groups, it was stated that a consistent apparent underdosage of 12.1 to 16.2% occurred. Four sampling time points are provided in the report, and the first two indicate all diets were within ± 20% of the nominal concentration. For both of these sampling times, analyses of the samples occurred on the day the samples were prepared. For the next two sampling time points, analysis was performed one or three days after sample preparation, and the 350 ppm dose group for one time point and all groups for the last time point show a decrease of greater than 14% from the nominal concentration. NOTE: TB II notes that Table 1 of the analytical report [page 208] lists the concentrations as mg/kg, but the final report [methods' section, page 12] and other sections of the report list ppm.

Table 1. Dosage of Test Material Achieved [mg/kg/day]

Intake (mg/kg)/Dose (ppm)	20 ppm	350 ppm	700 ppm	1400 ppm
males	1.5	27.3	55.6	114.5
females	2.0	35.4	74.6	139.4

Table 2. Nominal Concentrations Achieved [% difference from target]

Time point♦/Dose (ppm)	20 ppm♣	350 ppm	700 ppm	1400 ppm	premix♥
5/6/92	-15	-15	3.3	-21.7	-
5/20/92	-7.4	-8.3	-25.0	-24.0	-7.4
6/10/92	-6.7	-2.3	-1.4	-19.3	-3.6
7/28/92	12.1	2.6	15.1	-14.4	-

♦ date of preparation; ♣ analytical report lists the doses as mg/kg; ♥ 10000 [listed as mg/kg]

The study started on May 7, 1992 and ended in August 6-12,

1992 [necropsy].

Clinical Observations: All rats were observed twice daily [once on weekends/holidays] for mortality and signs of systemic reaction to treatment or ill-health. A detailed inspection of each rat was performed each week, which included palpation. Individual body weights were determined one week prior to treatment, on the day of study initiation, and weekly thereafter. Food consumption was determined once a week on a cage basis [calculated as the difference in food provided and food remaining at week's end], and food efficiency was calculated. Test material intake was calculated for each cage using body weight data, food consumption data, and the nominal dietary concentration of test material.

RESULTS

Survival and Clinical Observations

All rats survived to study termination. The only observation attributed to treatment was a pale brown discoloration of the feces in the second half of the study [Table 3].

Table 3. Clinical Observations [# with finding out of 10]

Group/Dose [ppm]	0	20	350	700	1400
MALES					
pale brown feces discoloration					
week 0-4	0	0	0	0	0
week 5-6	0	0	0	0	2
week 7-13	0	0	0	6	10
FEMALES					
week 0-9	0	0	0	0	0
week 10-13	0	0	0	0	2

Body Weight

In males, body weight was decreased at the 1400 ppm dose level throughout the study and at the 700 ppm dose level during the first 3 weeks compared to the controls. In females, the 700 ppm and 1400 ppm dose groups displayed statistically significant decreases in body weight, but these were not dose-related. At termination, the 1400 ppm dose level males displayed a 19% decrease in body weight compared to the controls [Table 4]. Initially [weeks 0 to 1 (♂♂ & ♀♀) and 0 to 3 (♂♂)], there were dose-related decreases in body-weight gains in both sexes, which attained statistical significance in males at doses of 350 ppm and above and in females at doses of 700 ppm and above [Table 5].

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Table 4. Mean Body Weight (% of control)*

Week/Dose	20 ppm	350 ppm	700 ppm	1400 ppm
MALES				
-2	99	97	97	97
-1	99	99	99	100
0	98	99	98	101
1	96	93	88*	79**
2	103	97	92**	84**
3	98	94	88**	83**
4	101	97	93	88**
5	101	98	93	88**
6	102	98	92	87**
7	98	96	90	85**
8	98	96	89	84**
9	99	96	89	83**
10	99	97	90	84*
11	100	97	90	83*
12	100	97	90	82**
13	100	97	90	81**
FEMALES				
-2	98	104	98	101
-1	99	103	99	105
0	97	103	99	105
1	95	100	94	98
2	98	102	92	98
3	97	100	90*	96
4	97	100	92	95
5	98	99	91*	93
6	98	99	89**	91*
7	99	99	90*	91*
8	100	101	89*	92*
9	98	99	88**	90*
10	98	98	87**	91*
11	99	99	88**	91*
12	99	98	89*	91*
13	100	100	90*	93

* p <0.5; ** p<0.01

Table 5. Mean Body-Weight Change [grams (% of control value)♦]

Interval (wk)/Group	0 ppm	20 ppm	350 ppm	700 ppm	1400 ppm
MALES					
-2 to -1	42.7	42.3	43.6	43.4	44.2
-1 to 0	43.0	41.5	42.8	41.9	45.2
0 to 1	43.5	39.4	32.4(75)**	23.6(54)**	1.0(2)**
1 to 2	22.6	35.1	29.3	27.1	28.5
2 to 3	45.9	35.3	36.6	31.5(69)	37.2
3 to 4	-5.7	0.9	2.5	9.9	8.0
4 to 5	22.2	22.6	22.5	20.5	18.0
5 to 6	20.8	23.2	21.4	15.4	15.0
6 to 7	21.2	11.6	15.6	14.4(68)	12.5
7 to 8	16.7	16.0	15.4	11.2(67)	9.9(59)
8 to 9	13.9	17.1	11.5	12.1	8.3(60)
9 to 10	-1.5	-0.8	2.9	1.8	2.2
10 to 11	79.7	11.9	9.0	8.4	7.8
11 to 12	-0.4	0.4	0.6	-0.9	-5.9
12 to 13	31.0	28.8	31.4	27.2	23.7
0 to 3	112.0	109.8	98.3(88)**	82.2(73)**	66.6(60)**
0 to 7	170.5	168.1	160.3	142.4(84)**	120.2(70)**
7 to 13	69.4	73.4	70.8	59.8(86)	46.0(66)
0 to 13	239.9	241.5	231.1	202.2(84)*	166.2(69)**
FEMALES					
-2 to -1	34.4	34.3	34.9	34.2	38.3
-1 to 0	26.9	25.1	28.1	26.7	28.3
0 to 1	19.8	16.7	16.1	13.1(66)**	10.6(54)**
1 to 2	12.7	15.6	15.3	9.3	13.1
2 to 3	17.9	15.6	14.5	12.0	13.6
3 to 4	6.6	6.8	8.1	10.0	5.6
4 to 5	10.1	12.7	7.9	7.5	6.0
5 to 6	10.4	10.2	9.8	5.7	6.3
6 to 7	6.3	6.4	6.0	6.9	5.7
7 to 8	5.3	7.5	8.8	4.3	6.1
8 to 9	8.1	5.1	4.9	4.0	3.6(44)
9 to 10	1.9	2.0	0.7	0.5	2.8
10 to 11	3.0	5.5	3.6	4.2	2.5(83)
11 to 12	-8.2	-9.3	-9.0	-6.2	-7.4
12 to 13	10.0	12.5	12.9	11.9	13.6
0 to 3	50.4	47.8	46.0	34.4(68)**	37.2(74)**
0 to 7	83.8	84.0	77.7	64.5(77)**	60.9(73)**
7 to 13	20.1	23.3	21.9	18.7	21.2
0 to 13	103.9	107.3	99.6	83.3(80)**	82.1(79)**

♦ statistics provided for week intervals: 0-1, 0-3, 0-7, and 0-13 only

Food Consumption: It is stated that the rate of growth of some groups may have been affected by "inadvertent fasting". This inadvertent fasting was described as: "a result of inadequate amounts of food supplied in the hoppers", which apparently occurred in weeks 3-4 and 9-12 among cages of all groups. Although the author states that this [fasting] affected cages of each group equally, it is stated that the possibility exists that "particularly at 350 ppm an effect on bodyweight gain retardation may be partially masked because of a possible slightly retarded weight gain in controls." The logic here escapes this reviewer. Food consumption values were provided for week intervals 0-1, 2-3, 6-7, and 12-13 only, since these are the time points during which adequate food was available, apparently [Table 6]. Food consumption was decreased in both sexes initially [weeks 0-1], although statistical significance

was attained only in males. Females at the three highest dose levels displayed increased overall food consumption compared to the control values, and the increase was inversely related to dose. It is not evident how long the rats were without food at any of these "fasting" incidents [hours, days ?] or if all groups "ran out of food" at each occurrence; all rats were to be observed at least once each day. It is also unclear how these episodes of fasting might have affected the decreases in body-weight gains at the two highest dose levels; it is possible that the magnitude of the decreases at the 700 and 1400 ppm dose levels may have been exaggerated over what it might have been had the fastings not occurred.

Table 6. Mean Food Consumption [grams/rat (% of control value)]

Interval/Group	0 ppm	20 ppm	350 ppm	700 ppm	1400 ppm
MALES					
0 to 1	21.4	20.1	18.7	18.7	14.6**(68)
2 to 3	24.3	21.5	24.4	23.1	22.9
6 to 7	21.0	20.6	21.2	19.8	18.8(90)
12 to 13	21.8	20.8	20.7	19.8	21.0
FEMALES					
0 to 1	16.1	15.9	15.5	15.1	13.7(85)
2 to 3	20.2	23.2	22.8	21.5	23.1
6 to 7	18.1	19.1	18.5	18.0	17.3
12 to 13	15.0	15.6	19.5**(130)	18.3**(122)	17.7**(118)

* p <0.05; ** p<0.01

Food Efficiency: Food conversion ratios [weight of food consumed per unit gain in body weight] were provided, and the authors concluded that efficiency of food utilization for both sexes at the 700 and 1400 ppm dose levels was "clearly impaired by treatment; and that by females receiving 350 ppm minimally so." TB II notes that there is no dose response in the females [Table 7].

Dose (ppm) Interval (weeks)	Table 7. Food Utilization	
	MALES	FEMALES
0	7.6	14.4
20	7.4	14.5
350	7.8	16.0 (111)
700	8.6 (113)♦	18.4 (128)
1400	10.1 (133)	17.8 (124)

♦ (% of control); no statistics performed

3. Clinical Pathology

Blood was collected (following an overnight fast and deprivation of water; via superficial venesection of the sublingual vein, without anaesthetic) from all rats a few days prior to study termination. Hematology investigations were performed using an E-2500 automated hematology analyzer, and

blood chemistry investigations were performed using a Beckman Synchron CX-5 automated analyzer. Blood smears were prepared from all rats [stains: Brilliant Cresyl Blue and modified Wright's]. The CHECKED (X) parameters were evaluated.

Hematology

X	Hematocrit (HCT)	X	Leukocyte differential count
X	Hemoglobin (HGB)	X	Mean corpuscular HGB (MCH)
X	Leukocyte count (WBC)	X	Mean corpusc. HGB conc. (MCHC)
X	Erythrocyte count (RBC)	X	Mean corpusc. volume (MCV)
X	Platelet count	X	Reticulocyte count
	Blood clotting measurements	X	Red cell morphology
	(Thromboplastin time)		
	(Activated partial thromboplastin time)		
	(Prothrombin time)		

RESULTS

There was an increase in both the erythrocyte and leucocyte counts in both sexes at study termination at the highest dose level and slightly decreased mean corpuscular volume and mean corpuscular hemoglobin. The increased total leucocyte count was due mainly to an increase in lymphocytes, with the 700 ppm dose level females displaying a slight trend.

Table 8. Hematology Parameters

Interval/Group	0 ppm	20 ppm	350 ppm	700 ppm	1400 ppm
MALES					
RBC [MI/CMM]	9.92	9.809	10.06	9.93	10.61**(107)
MCV [CMU]	52.8	52.5	52.2	51.2	48.7**(88)
MCH [PG]	17.39	17.25	16.92	16.66*(96)	15.78**(91)
WBC [TH/CMM]	5.47	5.96	6.65(122)♦	6.14	9.90**(181)
SEGS [% WBC]	21.3	18.7	20.4	19.6(92)	15.5(73)
LYMP [% WBC]	76.0	79.3	77.2	78.7	81.2(107)
MONO [% WBC]	1.4	1.2	1.5	1.3	2.6(186)
SEGS [TH/CMM]	1.16	1.06	1.35	1.17	1.53(131)
LYMP [TH/CMM]	4.15	4.79	5.15(124)	4.88(117)	8.03**(193)
MONO [TH/CMM]	0.087	0.067	0.091	0.071(82)	0.276(317)
FEMALES					
RBC [MI/CMM]	9.25	9.05	9.00	9.28	10.0**(108)
MCV [CMU]	54.6	56.9**(104)	55.45	53.5	49.5**(91)
MCH [PG]	17.97	18.77*(104)	18.26	17.4	15.9(((88)
WBC [TH/CMM]	4.28	4.41(103)	4.77(111)	5.62(131)	7.23**(169)
SEGS [% WBC]	21.5	19.9(93)	18.2(85)	13.3*(62)	10.3**(48)
LYMP [% WBC]	75.4	77.7(103)	78.2(104)	83.9*(111)	86.0**(114)
MONO [% WBC]	1.7	1.4(82)	2.5(147)	2.4(141)	2.6(153)
SEGS [TH/CMM]	0.95	0.87(91)	0.87(91)	0.76(80)	0.71(75)
LYMP [TH/CMM]	3.19	3.44(108)	3.73(117)	4.69**(147)	6.26**(196)
MONO [TH/CMM]	0.073	0.061(84)	0.118(162)	0.143(196)	0.174(238)

♦ (% of control); * p <0.05; ** p<0.01

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Blood Chemistry

Electrolytes:

X	Calcium
X	Chloride
	Magnesium
X	Phosphorous
X	Potassium
X	Sodium
	Iron

Enzymes

X	Alkaline phosphatase (ALK)
	Cholinesterase (ChE)
	Creatine kinase (CK)
X	Lactate dehydrogenase (LAD)
X	Serum alanine aminotransferase
X	Serum aspartate aminotransferase
X	Gamma glutamyl transferase (GGT)
	Glutamate dehydrogenase (GLDH)
	Ornithine carbamyltransferase (OCT)

Other:

X	Albumin
X	Blood creatinine
X	Blood urea nitrogen
X	Cholesterol
X	Globulin
X	Glucose
	Phospholipids
X	Total bilirubin
X	Total Protein (TP)
X	Triglycerides
	A/G ratio
	Triiodothyronine [T3]
	Thyroxine [T4]

RESULTS

Several statistically significant differences were observed in the monitored parameters, mainly those signifying possible changes in liver function. However, TB II notes that decreased glucose and elevated ALT are also observed following fasting, which occurred inadvertently from weeks 9 to 12 of the study.

Table 9. Biochemistry Parameters

Interval/Group	0 ppm	20 ppm	350 ppm	700 ppm	1400 ppm
MALES					
TP	71.9	72.7	75.6	75.9(106)	77.96(108)
GLOB	39.7	40.8	42.8(108)	43.97(111)	45.97*(116)
ALP	45.7	47.4	50.7(111)	50.6(111)	61.4**(134)
ALT	13.2	12.2	16.4(124)	20.7**(156)	28.4**(214)
AST	50.8	49.0	58.1(115)	56.7(112)	72.97**(144)
GGT	3.1	2.6(85)♦	3.5(112)	2.7(86)	3.5(111)
LD-P	237.7	197.2(83)	221.3(93)	275.9(116)	442.9**(186)
CK	112.6	107.4(95)	110.8	111.9	130.6(116)
TRIG	1.2	1.2	1.2	1.0(89)	0.7**(65)
GLU	4.6	4.99	4.77	4.43	3.76*(81)
FEMALES					
TP	76.8	74.7	75.3	76.1	78.8(103)
GLOB	39.1	38.5	40.4	42.1(108)	45.6*(117)
ALP	19.8	23.2(117)	19.2	24.3(123)	26.1(132)
ALT	14.4	18.6(129)	15.9(110)	15.4(107)	18.5**(129)
AST	59.1	60.4	56.7	63.8(108)	68.8(116)
GGT	2.5	2.5	2.8(112)	3.5(138)	7.05**(282)
LD-P	332.4	265.5(80)	336.8	353.4(106)	468.9(141)
CK	109.9	123.4(112)	152.0(138)	110.7	127.7(116)
TRIG	0.77	0.66(86)	0.88(115)	0.85(111)	1.03*(134)
GLU	5.1	5.4(106)	4.96(98)	4.89(96)	4.73(93)
CHOL	1.07	0.95(89)	1.18(110)	1.39(129)	1.70**(158)

♦ (% of control); * p <0.05; ** p<0.01

4. Urinalysis: Urine samples were collected from all rats [fasted and deprived of water overnight] a few days prior to study termination. Semi-qualitative analyses were performed on the parameters marked with ♦ using Ames Multistix SG test strips evaluated with a Clinitek 200+ autoanalyzer. The CHECKED (X) parameters were examined.

X	Appearance (transparency)	X	Glucose♦
X	Volume	X	Ketones♦
X	Specific gravity	X	Bilirubin♦
X	Ph	X	Blood♦
X	Sediment (microscopic)	X	Nitrite♦
X	Protein♦	X	Urobilinogen♦
X	Leukocytes♦	X	Color

RESULTS

Ketones were elevated at the 700 ppm [118 % of control] and 1400 ppm [181 % of control] dose levels in males, and Ph was elevated at the high-dose level [106% of control] in females. TB II notes that an increase in ketone bodies can result from prolonged fasting. No other findings were reported.

Ophthalmoscopy: Prior to study initiation and at termination [control and high-dose groups], the eyes were examined for pathological changes [eyes dilated prior to examination using 'Mydriaticum", 0.5% Tropicamide] by a trained ophthalmologist using a ophthalmoscope.

RESULTS

There were no treatment-related effects.

7. Gross Pathology: All animals were subjected to a full macroscopic examination at sacrifice (following 13 weeks of treatment), which included an examination of the external surfaces, all orifices, the cranial cavity and brain, thoracic, abdominal, and pelvic cavities with associated organs and tissues, and the neck with its tissues. The following organs were weighed: kidneys, liver, testes, adrenal glands, brain, heart, ovaries, pituitary [after fixation], and spleen.

RESULTS

Macroscopically, an accentuated lobular pattern was observed more frequently in the livers of the two highest dose groups [both sexes] than in control and lower dose groups [Table 10]. The spleen in one rat per sex at the 1400 ppm dose level was diminished in size. Enlarged thyroids were observed in males at the three highest dose levels and in high-dose females only.

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Table 10. Macroscopic Findings [# with finding; n=10]

Lesion	MALES					FEMALES				
Group / Dose	0	20	350	700	1400	0	20	350	700	1400
LIVER										
enlarged	0	0	0	2	5	0	0	0	0	1
accentuated lobular pattern	0	1	2	6	9	2	2	2	5	6
discoloration	0	1	3	2	7	1	2	1	2	4
pale	0	1	2	2	6	1	2	1	2	2
SPLEEN										
diminished in size	0	0	0	0	1	0	0	0	0	1
foci	0	0	0	0	0	0	0	0	0	1
THYROID										
enlarged	0	0	1	2	2	0	0	0	0	1

Organ weights: Increased liver weights were observed in both sexes at the three highest dose levels. In males, the increases in absolute [Table 11] and relative-to-body weight liver weight [Table 12] were dose-related, but the relative-to-brain weight liver weight [Table 13] was not. In females, only the increase in relative-to-body weight liver weights was dose-related. Absolute spleen weights were decreased in both sexes at the highest dose level and in females at the next lower dose level [dose-related]. Relative-to-body weight spleen weight was also decreased in females at these dose levels, but statistical significance was attained only at the highest dose level. Relative-to-brain weight [Table 13] spleen weights were decreased in both sexes at the two highest dose levels, but a statistical assessment apparently was not performed [summary tables of absolute and relative-to-body weight organ weights were provided; individual relative-to-brain weight values provided]. Decreased pituitary weight was observed in males at the two highest dose levels, but only the highest dose group attained statistical significance. Increased relative brain weight was displayed in females at the two highest dose levels, but the increase was not dose-related.

Group Dose (ppm) Organ	Table 11. ABSOLUTE ORGAN WEIGHT (grams) 0	20	350	700	1400
MALES					
spleen	0.736	0.716	0.746	0.662(90)♦	0.590*(80)
liver	15.29	14.49	17.07(112)	17.22(113)	20.28*(133)
adrenals	56.2	48.1	55.0	46.5(83)	54.4
heart	1.042	0.999	1.007	0.923(89)	1.019
kidneys	2.62	2.49	2.52	2.213(84)	2.355(90)
pituitary	12.7	11.9	12.8	11.2(88)	10.4**(82)
testes	3.77	3.80	3.71	3.691	3.644
brain	2.050	2.074	2.030	2.072	2.030
BODY WEIGHT	**393.2**	**386.1**	**382.2**	**354.0(90)**	**325.7**(83)**
FEMALES					
spleen	0.535	0.542	0.534	0.391*(73)	0.361**(67)
liver	7.82	8.29	9.93**(127)	9.48*(121)	10.81**(138)
adrenals	71.2	79.4	80.4	79.7	71.3
heart	0.705	0.706	0.728	0.687	0.682
kidneys	1.575	1.611	1.651	1.518	1.555
pituitary	16.6	18.5	16.4	15.1	16.3
ovaries	0.146	0.145	0.149	0.138	0.144
brain	1.873	1.870	1.923	1.885	1.932
BODY WEIGHT	**231.3**	**220.8**	**219.5(95)**	**198.0*(86)**	**203.8*(88)**

* p<0.05; ** p<0.01; ♦ (% of control)

Group Dose (ppm) Organ	Table 12. RELATIVE-TO-BODY ORGAN WEIGHT (%) 0	20	350	700	1400
MALES					
spleen	0.188	0.187	0.195	0.187	0.181
liver	3.90	3.76	4.45**(114)♦	4.87**(125)	6.17**(158)
adrenals	0.014	0.013	0.014	0.013	0.016(113)
heart	0.266	0.259	0.264	0.261	0.317*(119)
kidneys	0.671	0.647	0.661	0.626	0.725
pituitary	0.0033	0.0031	0.0034	0.0032	0.0032
testes	0.966	0.987	0.977	1.04(108)	1.13*(117)
brain	0.525	0.539	0.533	0.587*(112)	0.630**(120)
FEMALES					
spleen	0.235	0.246	0.245	0.198(84)	0.177*(75)
liver	3.43	3.73	4.53**(132)	4.79**(140)	5.31**(155)
adrenals	0.031	0.036	0.037	0.040*(129)	0.035(112)
heart	0.309	0.322	0.333	0.348	0.336
kidneys	0.691	0.730	0.753	0.769(111)	0.765(111)
pituitary	0.0073	0.0085	0.0075	0.0077	0.0080
ovaries	0.064	0.067	0.069	0.070	0.071
brain	0.820	0.855	0.884	0.960*(117)	0.950**(116)

♦ (% of control value); * p<0.05; ** p<0.01

Group Dose (ppm) Organ	Table 13. RELATIVE-TO-BRAIN ORGAN WEIGHT (%)				
	0	20	350	700	1400
MALES					
spleen	35.9	34.7	36.7	32.0(89)	29.0(81)♦
liver	746	699	841(113)	832(112)	997(134)
adrenals	2.74	2.32	2.71	2.24	2.65
heart	50.8	48.1	49.7	44.6	50.2
kidneys	128	120	124	107	116
pituitary	0.62	0.574	0.629	0.54	0.512(83)
testes	184	183	183	178	180
FEMALES					
spleen	28.6	29.0	27.7	20.9(73)	18.8(66)
liver	418	443	516	504	561(134)
adrenals	3.79	4.25	4.18	4.23	3.69
heart	37.7	37.8	37.9	36.4	35.3
kidneys	84.1	86.1	86.0	80.6	80.6
pituitary	0.888	0.993	0.852	0.800	0.844
ovaries	7.83	7.76	7.74	7.31	7.46

♦ (% of control value); * p<0.05; ** p<0.01

7. Histopathology: The following organs/tissues (CHECKED (X)) were preserved from all rats. Microscopic examinations were restricted to macroscopic abnormalities, lungs, liver, and kidneys for all rats; all organs from all control and high-dose groups; tissues identified as target tissues [spleen and thyroid] from the low- and mid-dose groups; sections with the following additional staining: Perl's Prussian Blue staining of the spleen [all groups], Sudan III staining of liver [frozen formalin-fixed sections (all groups)], PAS and Perl's Prussian Blue staining of lungs [high-dose males]; Sudan III staining of lungs [formalin-fixed sections (high-dose/ both sexes)]. Immunostaining of selected slides for Factor VII and for macrophage antigen was used to assist in diagnosis of cell type, but results were not reported further.

Digestive system		**Cardiovasc./Hemat.**		**Neurologic**	
X	Tongue	X	Aorta	X	Brain
X	Salivary glands	X	Heart	X	Sciatic nerve
X	Esophagus	X	Bone marrow♥	X	Spinal cord/3 levels
X	Stomach	X	Lymph nodes	X	Pituitary
X	Duodenum	X	Spleen	X	Eyes/optic nerve
X	Jejunum	X	Thymus	**Glandular**	
X	Ileum	**Urogenital**		X	Adrenal gland
X	Cecum	X	Kidneys	X	Lacrimal gland
X	Colon	X	Urinary bladder	X	Mammary gland
X	Rectum	X	Testes	X	Parathyroids
X	Liver	X	Epididymides	X	Thyroids
	Gall bladder	X	Prostate	**Other**	
X	Pancreas	X	Seminal vesicle	X	Bone/joint/sternum/femur
Respiratory		X	Ovaries/Cervix	X	Skeletal muscle
X	Trachea	X	Uterus	X	Skin
X	Lung	X	Vagina	X	All gross lesions
	Nasal cavity		Harderian gland		Zymbal's gland
	Pharynx	X	Skull & ears		
	Larynx				

♥ sternum

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RESULTS

Microscopically, treatment-related changes were observed in the liver, blood vessels, lungs, kidneys, and thyroids. [Table 14]. The incidence and severity of hepatocellular

centrilobular hypertrophy and vacuolation were reported to be increased at the three highest dose levels in both sexes, but TB II notes that all 10 males of each group [including control] are listed with "HYPERTROPHY/VACUOLES" on page 239 of the Pathologist's report. Only the severity of the finding [Table 15] in males was increased at the 3 highest dose levels [page 244 of Pathologist's report]. One high-dose male was reported [in the final report summary and Discussion of Results] with inflammation and necrosis with centrilobular bridging; however, the Pathologist's summary table of microscopic findings does not list necrosis per se as a finding in males, and only a 350 ppm male was listed with inflammation [pathologist's report table on page 239]; single cell necrosis was listed for 2 low-dose females, and a high-dose male is listed in the table of individual findings in the Pathologist's report with centrilobular scarring (post-necrotic scarring). Increased numbers of foamy macrophages were reported [final report] in blood vessels of the liver, lungs, and kidneys mainly at the highest dose level, although in the Pathologist's summary and individual tables macrophages/vascular macrophages (not foamy) were listed. The narrative part of the Pathologist's report does list foamy macrophages in these organs, however. Additionally, increased numbers of pigmented foamy macrophages were reported [in both the final report and Pathologist's text] in the spleen at 700 and 1400 ppm, both sexes; however, pigmented foamy macrophages were not listed in the pathologist's summary tables or in the individual findings' tables for the spleen. Histiocytosis was reported in the Pathologist's summary report and tables in the spleen of all high-dose rats [both sexes] and in one male and 5 females at the next lower dose level. In the liver, vascular macrophages were observed in all high-dose males and in five high-dose females and 1 female at the next lower dose level. In the kidneys, vascular macrophages were observed only in females at 700 ppm and both sexes at 1400 ppm. Females at the two highest dose levels also displayed a dose-related increase in tubular pigment in the kidneys compared to the controls. In the thyroid glands, females displayed a dose-related increase in follicular hypertrophy, and one high-dose female displayed epithelial hypertrophy. In males, there was a slight increase in the incidence of follicular hypertrophy at the three highest dose levels, but a dose response was not shown. In the lungs, vascular macrophages were observed at the high dose in both sexes and in females at the next lower dose level. TB II notes that the final report summary of the findings in the blood vessels incorrectly lists 750 ppm as a dose in the study.

Table 15. Microscopic Findings [# with finding; n=10]

Lesion / Group / Dose	MALES					FEMALES				
	0	20	350	700	1400	0	20	350	700	1400
LIVER										
hypertrophy/vacuoles	10	10	10	10	10	0	0	9	10	10
Grade♦ 1	6	4	-	-	-	-	-	2	2	4
Grade 2	4	4	-	-	-	-	-	6	8	5
Grade 3	-	2	6	3	1	-	-	1	-	1
Grade 4	-	-	4	7	9	-	-	-	-	-
vascular macrophages	0	0	0	0	10	0	0	0	1	5
single cell necrosis	0	0	0	0	0	0	2	0	0	0
inflammation	0	0	1	0	0	0	0	0	0	0
pigment	0	0	0	0	1	0	0	0	7	6
mitotic activity	0	0	0	1	2	1	0	1	0	1
centrilobular scarring	0	0	0	0	1	0	0	0	0	0
SPLEEN										
histiocytosis	0	0	0	1	10	0	0	0	5	10
THYROID										
hypertrophy, microfollicular	2	3	8	5	9	0	0	2	3	5
hypertrophy, epithelial	1	0	0	1	0	0	0	0	0	1
LUNGS										
vascular macrophages	0	0	0	0	10	0	0	0	3	8
foam cell focus	1	1	1	3	1	1	1	3	3	4
KIDNEYS										
vascular macrophages	0	0	0	0	6	0	0	0	1	1
tubular vacuolation	0	0	0	0	1	0	0	1	0	0
tubular pigment	1	0	0	0	0	0	0	0	2	7

♦ Grade 1=minimal; Grade 2=mild; Grade 3=moderate; Grade 4=marked

DISCUSSION

The results of this study are consistent with previous studies on Cyproconazole, in that the liver is the main target organ. Various parameters [liver weight, liver enlargement, enzyme levels, microscopic findings] indicative of liver involvement were affected by treatment in a dose-related manner in both sexes. Body-weight gains were decreased initially [weeks 0 to 1 and 0 to 3] in males at the three highest dose levels and in females at the two highest dose levels, and during weeks 0 to 7 and overall in both sexes at the two highest dose levels. Food consumption was adversely affected initially, and overall food efficiency was decreased in both sexes. Several clinical chemistry parameters [↓ glucose, ↑ ALT, ↑ AST, ↑ GGT, ↑ LDH] indicative of liver toxicity and/or fasting were affected by treatment at the 700 ppm and/or 1400 ppm dose levels in one or both sexes. Decreases in spleen weight were observed in females at the 700 ppm dose level and in both sexes at the 1400 ppm dose level. Liver weights were increased at the three highest dose levels in both sexes. The 1400 ppm group of males also displayed decreased absolute pituitary weights, but relative-to-body weights were comparable to controls. There were increases in relative-to-body weight organ weights for several organs, which may be attributed to decreases in body weight rather than to a compound-related effect. TB II notes

that the spleen and pituitary weights relative-to-**brain** and **body** weights are decreased compared to the control and may be compound-related effects. The changes observed on the thyroid [microfollicular hypertrophy], spleen [decreased weight and ↑ incidence of histiocytosis] and lung [↑ incidence of foam cell focus], and the ↑ incidence of vascular macrophages in several organs have not been reported previously for Cyproconazole.

With respect to the dose levels chosen for this study, these were said to have been selected "on the basis of doses selected in a carcinogenicity study performed at this laboratory (Project 357-R)." Additionally, it was stated that the study was performed in order to characterize the subchronic toxicity of Cyproconazole and to justify dosage levels used in the rat carcinogenicity study [Project 357-R] found inadequate by the HED CPRC. TB II notes that the two high doses used that are greater than the highest dose in the inadequate carcinogenicity study [350 ppm] are those calculated via a simulated risk assessment [memo from Engler to Swentzel dated 9/21/92]. The results obtained in the current study do not affect the Agency's original conclusion that the highest dose utilized in the rat carcinogenicity was inadequate to assess the carcinogenic potential of Cyproconazole. Other than initially in males, body weight/gain were not affected by treatment at the 350 ppm dose level for either sex. Additionally, in females at the 700 and 1400 ppm dose levels, comparable body-weight gain deficits were observed and, although final body weights were decreased at both dose levels compared to the control, the decrease was inversely related to dose. Additionally, the magnitude of the decrease in body weight may have been affected by the "inadvertent fasting" that occurred during the study on several occasions. The effects observed in the liver are those expected and as observed previously, result from adaptive processes and are not toxic effects per se. The occurrence of vascular macrophages is another normal process of the body's defensive mechanism against injury. Only one high-dose male displayed any signs of necrosis, and the only other rats displaying necrosis were two low-dose females with single cell necrosis. Based on the results of this study and those of the rat carcinogenicity, previous 13-week rat, and 4-week rat studies, dose levels of Cyproconazole up to 1400 ppm appear appropriate for a repeat rat carcinogenicity study, as recommended by REngler [memo dated 9/21/92].

CONCLUSION

Under the conditions of the study, oral administration of Cyproconazole via the diet to Wistar rats [10/sex/group] for 13 weeks at dose levels of 0, 20 ppm [1.5 ♂/2.0 ♀ mg/kg], 350 ppm [27.3 ♂/35.4 ♀ mg/kg], 700 ppm [55.6 ♂/74.6 ♀ mg/kg], and 1400 ppm [114.5 ♂/139.4 ♀ mg/kg] resulted in signs of toxicity [decreased body weight at 13 weeks {♂♂ 90% and 81% of control and ♀♀ 90% and 93% of control at 700 and 1400 ppm, respectively}, decreased overall body-weight gain {♂♂ 84% (700 ppm) and 69% (1400 ppm) of control; ♀♀ 80% (700 ppm) and 79% (1400 ppm) of control}, decreased food efficiency, changes in several clinical chemistry parameters consistent with liver toxicity and/or fasting, decreased spleen {♂♂ & ♀♀} and pituitary {♂♂} weights and increased liver weight {♂♂ & ♂♂}, and microscopic, treatment-related changes in the liver {↑ incidence/severity of hepatocellular centrilobular hypertrophy and vacuolation} and thyroids {↑ incidence/severity of microfollicular cell hypertrophy}, an ↑ in the incidence of histiocytosis in the spleen, and an ↑ in the number of vascular macrophages of the liver, kidneys, and lungs]. The NOEL can be set at 20 ppm [1.5 ♂/2.0 ♀ mg/kg/day], the LEL at 350 ppm [♂♂ 27.3/♀♀ 35.4 mg/kg/day], based on decreased body-weight gain in males and increased liver weight in females. This study is classified Core Minimum, and it satisfies the guideline requirement [82-1(a)] for a subchronic oral toxicity study in rodents.

NOTE: It is not evident why the terminology of the microscopic findings differs between the Study Director's final report and that of the Pathologist's report [e.g., necrosis with centrilobular bridging vs centrilobular scarring (post necrotic scarring)] or why the text of the Pathologist's report uses different terminology than that used in the individual animal data sheets [e.g., foamy macrophages vs vascular macrophages; slightly pigmented foamy macrophages vs histiocytosis].