007871

4-10-90

EPA No.: 68D80056
DYNAMAC No.: 244-B
TASK No.: 2-44B
April 10, 1990

CASWELL FILE
225

DATA EVALUATION RECORD

SAN 619F (Cyproconazole)

Chronic Toxicity/Oncogenicity Feeding Study in Rats

APPROVED BY:

Robert J. Weir, Ph.D.
Program Manager
Dynamac Corporation

Signature: Ronnen J Pierta

Date: April 10, 1990

(44)

EPA No.: 68D80056
DYNAMAC No.: 244-B
TASK No.: 2-44B
April 10, 1990

DATA EVALUATION RECORD

SAN 615 F (Cyproconazole)

Chronic Toxicity/Oncogenicity Feeding Study in Rats

REVIEWED BY:

William L. McLellan, Ph.D.
Principal Reviewer
Dynamac Corporation

Signature: William L. McLellan
Date: April 9, 1990

John Liccione, Ph.D.
Independent Reviewer
Dynamac Corporation

Signature: John J. Liccione
Date: April 9, 1990

APPROVED BY:

Roman J. Pienta, Ph.D.
Department Manager
Dynamac Corporation

Signature: Roman J. Pienta
Date: April 9, 1990

Linda L. Taylor, Ph.D.
EPA Reviewer, Section II
Toxicology Branch II
(H-7509C)

Signature: Linda L. Taylor
Date: 4-16-90

K. Clark Swentzel
EPA Section Head, Section II
Toxicology Branch II
(H-7509C)

Signature: K. Clark Swentzel
Date: 4/18/90

DATA EVALUATION RECORD

GUIDELINE §85-5

STUDY TYPE: Chronic toxicity/oncogenicity feeding study in rats.

MRID NUMBER: 411647-01.

TEST MATERIAL: SAN 619F.

SYNONYM: Cyproconazole.

STUDY NUMBER: 357-R.

SPONSOR: Sandoz Crop Protection Corporation.

TESTING FACILITY: Sandoz Ltd. Agro Development, Toxicological Department Basle/Switzerland.

TITLE OF REPORT: San 619F Chronic Toxicity/Oncogenicity Feeding Study in Rats.

AUTHORS: Warren, S.F.P., Carpy, S., and Müller, F.

REPORT ISSUED: April 22, 1988.

CONCLUSIONS:

Under the conditions of the study, SAN 619F was not carcinogenic when fed to KFM-Wistar rats for up to 118 (males)/121 (female) weeks at dietary levels of 0, 20, 50, or 350 ppm (corresponding to an intake of 1.0, 2.2, and 15.6 mg/kg/day for males and 0, 1.2, 2.7, and 21.8 mg/kg/day for females). At the 350-ppm dose, mean body weights were significantly depressed in females throughout most of the study, although the differences were generally less than 10% below control values. In males, slightly (<6% below control value) depressed body weights were observed in the high dose compared to control. Mean weight gains from initiation to week 13 or 79 were depressed 6 to 8% in high-dose males and 10 to 15% in high-dose females. Food consumption was not affected. An increase in the incidence of fatty change was observed in the liver of males receiving 350 ppm. Hepatocellular hypertrophy was observed in females receiving 350 ppm at the 78-week sacrifice (4/10 rats), but the lesion was not observed in the main group of animals or at the 52-week sacrifice. Liver-to-body weight ratios were significantly increased in females receiving 350 ppm at both 12 and 18 months and at terminal sacrifice. Serum alanine aminotransferase activity was significantly increased at week 118 and aspartic aminotransferase was significantly increased at 78 weeks in males at the 350 ppm dose level. Cholesterol levels were increased in mid- and high-dose females at 78, 105, and 121 weeks, although the increase was significant only at 105 weeks (not dose-related). No effects were observed on any parameter at the low dose level.

Based on the lack of: 1) any biologically significant body weight decrement; 2) any significant histopathological correlate accompanying the increased relative liver weight in the high-dose females; 3) any increase in the liver enzyme activities in the female; 4) any consistent change in the liver enzyme activities in the high-dose males, suggests that the dose levels chosen were not adequate to determine the carcinogenic potential of the test material.

Based on the decreased body weights in high-dose females and the increased incidences of fatty infiltration of the liver in high-dose males, the LOEL for systemic toxicity is 350 ppm and the NOEL is 50 ppm.

Classification: Core Supplementary under guideline 83-2, Oncogenicity Study.

Core Minimum under guideline 83-1, Chronic Toxicity Study.

4

A. MATERIALS:

1. Test Compound: SAN 619F; description: light brown powder; batch No.: 8507; purity: 95.6 ± 1%.

2. Test Animals: Species: Albino rat; strain: KFM Wistar (of HAN Wistar origin); age: 7 weeks at initiation; weight: males--172 g to 229 g; females--126 to 175 g; source: KFM Breeders, Switzerland.

B. STUDY DESIGN:

1. Animal Assignment: Animals were acclimated to laboratory conditions for 14 days, and were assigned randomly by sex to the following test groups using a computer-generated randomization procedure:

Test group	Dose in diet (ppm)	Number of Animals: Main study (24 months) Males	Main study (24 months) Females	Interim sacrifice (12 months) Males	Interim sacrifice (12 months) Females	Interim sacrifice (18 months) Males	Interim sacrifice (18 months) Females
1 (K) Control	0	50	50	10	10	10	10
2 (A) Low (LDT)	20	50	50	10	10	10	10
3 (B) Mid (MDT)	50	50	50	10	10	10	10
4 (C) High (HDT)	350	50	50	10	10	10	10

Rats were housed individually in a room with temperature and humidity set at 23°C and 50%, respectively, with a 12-hour light/dark cycle. Animals received ear punchmarks, allowing individual identification, plus color-coded and individually numbered cage labels.

2. Diet Preparation: Premixes were prepared by mixing SAN 619F with powdered diet (Kliba No. 21-343-4) for 1 hour to produce a premix with a concentration of 10 mg SAN 619F per gram (1%). This diet premix was renewed at least monthly. Final diets were prepared weekly by mixing diet premix with additional powdered diet to achieve the appropriate concentrations. The stability, homogeneity, and concentration of the premixes and diets were analyzed before the start of the study and at monthly intervals thereafter.

Results:

The analyzed concentrations of test material in the diets were reported as generally acceptable and within 10% of nominal concentrations. All diets were higher (22 to 50%) than nominal concentrations at 28 weeks. Deviations from nominal were greater than 15% at seven, six, and two intervals of analysis at nominal levels of 20, 50, and 350 ppm, respectively. Mean concentrations for the entire study, however, were within 5% of the nominal at all dose levels (Table 1). Data on homogeneity and stability were not provided but reference is made to indicate that both are documented elsewhere.

TABLE 1. Mean Concentrations of SAN 619F in Formulated Rodent Diet

Treatment Group	Nominal (ppm)	n (weeks)	Mean Analyzed Concentration (% of Nominal ± S.D.)
1% Premix	10,000	31	102.1 ± 6.35
A	20	31	105.3 ± 10.48
B	50	31	101.5 ± 13.47
C	350	31	99.1 ± 7.79

3. Food and Water Consumption: Animals received Kliba No. 21-343-4 powdered diet and tap water ad libitum.

4. Statistics: The following procedures were utilized in analyzing the numerical data: Levene's test was used to analyze homogeneity of variances. One-way analysis of variance (ANOVA) was used for data with homogeneous variance, and if significant differences between groups were found, pairwise comparison with controls using Dunnett's test was performed. Nonparametric data were analyzed by the Kruskal-Wallis test followed by multiple comparisons with the Dunn-Bonferroni test; the Mann-Whitney U-test was used if there was only one treatment group.

5. Quality Assurance: A quality assurance statement was signed and dated April 22, 1988.

C. METHODS AND RESULTS:

1. Observations: Animals were inspected twice daily for signs of morbidity and mortality. Detailed examinations and

palpation of masses were conducted biweekly. Cages were inspected for traces of blood or abnormal feces/urine.

Results: No compound-related signs of toxicity were seen. A small number of rats (nine in all groups) were observed with skin ulceration, which was considered to be due to bacterial infection. They were treated with an anti-bacterial aerosol spray.

Survival was not affected by dosing. Table 2 summarizes data for mortality and percent survival in the main groups. Animals in the satellite groups that died before the 12- and 18-month interim sacrifices were replaced by animals from the main groups.

2. Body Weight: Body weights were recorded weekly for the first 13 weeks of treatment; at weeks 15, 17, 19, and 21; and then weekly thereafter.

Results: Tables 3 and 4 present mean body weight data and weight gain data, respectively, for selected intervals during the study. Mean body weights in males receiving 20 and 50 ppm tended to be slightly increased when compared to controls throughout the study. Mean body weights in high-dose males were 4 to 6% lower than controls, but the differences were not consistently significant. In females receiving 350 ppm, the mean body weights were consistently lower ($p < 0.05$) than controls from weeks 1 to 104 although the increase did not exceed 10% except at week 104. Mean weight gains during the first 2 weeks of the study were 12 and 17% lower in high-dose males and females than in their respective controls, suggesting a palatability problem. However, this was not supported by the data on food consumption (see below). For the first 79 weeks, the respective gains were 8 and 15% lower than control gains in males and females receiving 350 ppm (Table 4); however, the mean weekly gains in both sexes were only 0.5 g lower in the high dose than in controls.

3. Food Consumption and Compound Intake: Consumption was measured, and mean daily dietary consumption was calculated on a weekly basis, up to 13 weeks of treatment. Consumption was then determined for weeks 15, 17, 19, 21 and weekly thereafter. Diet wastage was estimated and corrected for. Efficiency and compound intake were calculated from the consumption and body weight gain data.

Results: Food consumption, in general, was similar in dosed and control groups. It was increased (6%) in high-dose females but not males during the first 13 weeks of the study as compared to controls; however, the study authors considered that the increased consumption was caused by

7

TABLE 2. Cumulative Mortality and Percent Survival in Rats Fed SAN 619F for 121 Weeks[a]

Dose Group (ppm)	Mortality (Percent Survival) at Week: 52	80	92	104	Termination[b]
			Males		
0	2 (96)	11 (78)	12 (76)	20 (60)	36 (28)
20	2 (96)	9 (82)	14 (72)	25 (50)	34 (32)
50	3 (94)	12 (76)	19 (62)	22 (56)	30 (40)
350	0 (100)	4 (92)	12 (76)	17 (66)	28 (44)
			Females		
0	2 (96)	10 (80)	15 (70)	21 (58)	32 (36)
20	2 (96)	8 (84)	11 (78)	16 (68)	31 (38)
50	1 (98)	7 (86)	11 (78)	13 (74)	29 (42)
350	1 (98)	5 (90)	7 (86)	18 (64)	33 (34)

[a]Mortality and percent survival were based on 50 rats/sex/dose of the main group. An additional 20 rats/sex/dose (10/sex/dose at 52 weeks and 10/sex/dose at 78 weeks) survived until their respective scheduled-sacrifice dates and are not included in this table.

[b]Males were terminated at week 118 and females at week 121.

TABLE 3. Mean Body Weights at Selected Intervals for Rats Fed SAN 619F for 121 Weeks

Dietary Level (ppm)	Mean Body Weight (g ± S.D.) at Study Weeks: 0	13	26	52	78	104	118 (males) 121 (females)
			Males				
0	196 ± 11	429 ± 36	480 ± 42	573 ± 58	613 ± 67	605 ± 83	586 ± 133
20	198 ± 11	440 ± 34	493 ± 35	587 ± 48	621 ± 56	628 ± 70	571 ± 72
50	195 ± 10	437 ± 35	491 ± 41	584 ± 62	636 ± 64	642 ± 81	588 ± 70
350	195 ± 13	417 ± 41	461 ± 43*	547 ± 56*	583 ± 68	574 ± 85	551 ± 42
			Females				
0	144 ± 9	246 ± 22	269 ± 25	330 ± 41	379 ± 59	407 ± 60	397 ± 75
20	145 ± 7	246 ± 19	267 ± 22	327 ± 44	368 ± 51	398 ± 67	397 ± 66
50	146 ± 8	249 ± 20	270 ± 22	328 ± 34	376 ± 47	406 ± 72	410 ± 63
350	145 ± 7	237 ± 17*	254 ± 20**	298 ± 34**	343 ± 51**	353 ± 51**	362 ± 68

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

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

TABLE 4. Mean Weight Gains (g ± S.D.) at Selected Intervals for Rats Fed SAN 619F

Dietary Level (ppm)	Weight Gain (g/rat/week) During Weeks: 0-2	0-13	0-79
		Males	
0	38.8 ± 4.6	18.1 ± 2.3	5.9 ± 0.8
20	40.0 ± 3.8	18.7 ± 2.2	5.9 ± 0.7
50	39.6 ± 4.9	18.6 ± 2.3	6.1 ± 0.8
350	34.3 ± 6.5**	17.1 ± 2.7	5.4 ± 0.9
		Females	
0	17.3 ± 2.9	7.9 ± 1.3	3.3 ± 0.6
20	17.1 ± 2.7	7.8 ± 1.2	3.1 ± 0.7
50	17.5 ± 3.2	8.0 ± 1.2	3.1 ± 0.7
350	14.4 ± 2.7**	7.1 ± 1.0**	2.8 ± 0.6**

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

food scattering. Food consumption for weeks 1 to 79 was similar in control (122 ± 11 g/rat/week) and high-dose females (123 ± 11 g/rat/week). A slight decrease in food efficiency was observed in high-dose males and females during the first 13 weeks. The food conversion ratio (mean food consumption divided by mean weight gain) was 9.9 in high-dose males as compared to 9.4 for controls, and in high-dose females the ratio was 19.0 as compared to 16.1 for controls. The calculated intakes of SAN 619F were 1.01, 2.22, and 15.59 mg/kg/day for males at dietary levels of 20, 50, or 350 ppm and 1.24, 2.73, and 21.76 mg/kg/day for females at the same doses.

4. Ophthalmological Examinations: Ophthalmologic examinations were performed on one occasion only, during weeks 98 and 99, on all surviving rats of the control and high-dose groups, respectively.

   Results: No treatment-related ophthalmic findings were noted.

5. Hematology and Clinical Chemistry: Blood was collected by superficial venesection of the sublingual vein at weeks 14, 26, 52, 78, 105, and termination for hematology and clinical analysis from 10 male and 10 female animals of each dose group. Blood was collected from the same animals for the first three intervals (those scheduled for the 52-week sacrifice), from the animals sacrificed at 78 weeks, and from 10/sex/group at termination. The CHECKED (X) parameters were examined:

   a. Hematology:

X	Hematocrit (HCT)†	X	Leukocyte differential count
X	Hemoglobin (HGB)†	X	Mean corpuscular HGB (MCH)
X	Leukocyte count (WBC)†	X	Mean corpuscular HGB concentration (MCHC)
X	Erythrocyte count (RBC)†	X	Mean corpuscular volume (MCV)
X	Platelet count†		Coagulation:thromboplastin time (PT)
X	Reticulocyte count (RETIC)		
	Red cell morphology		

   Results: No effects of toxicological importance were observed on any hematologic parameter. Reticulocyte counts were slightly, and nonsignificantly, increased in high-dose females at 14 weeks but not at other intervals. Total WBC were slightly decreased in high-dose males at 14 weeks.

†Recommended by Subdivision F (November 1984) Guidelines.

b. Clinical Chemistry

	Electrolytes		Other
X	Calcium†	X	Albumin†
X	Chloride†		Albumin/globulin ratio
	Magnesium†	X	Blood creatinine†
X	Phosphorus†	X	Blood urea nitrogen†
X	Potassium†	X	Cholesterol†
X	Sodium†	X	Globulins
		X	Glucose†
	Enzymes	X	Total bilirubin†
X	Alkaline phosphatase (ALP)		Direct bilirubin
	Cholinesterase	X	Total protein†
X	Creatine phosphokinase†[a] (CPK)	X	Triglycerides
	Lactic acid dehydrogenase	X	Hemolytic score
X	Serum alanine aminotransferase (ALAT)†		
X	Serum aspartate aminotransferase (ASAT)†		
X	Gamma glutamyltransferase[a] (GGT)		

Results: Total bilirubin levels were reported to be lower in high-dose males and females than in controls throughout the study. The decreases did not reach a level of significance and were most obvious at weeks 14, 26, and 52 in males and weeks 14 and 26 in females. Although levels were slightly decreased in high-dose rats of both sexes at weeks 78, 105, and at termination, there were no dose-related trends. These changes were not considered to be of toxicologic importance or to be related to dosing.

Cholesterol levels were significantly ($p < 0.05$) increased compared to controls at 105 weeks in mid- and high-dose females but this increase was not dose related. Similar increases were seen at 78 and 121 weeks, but the increases did not reach a level of significance. There were no effects on cholesterol levels in males. It is to be noted that increased cholesterol values have been observed in other studies on the test material. Triglyceride levels tended to be decreased in high-dose females except at week 121; slight decreases were also seen in mid- and low-dose females at 14, 26, and 52 weeks, but there was no clear dose-related trend; a level of significance was not

†Recommended by Subdivision F (October 1982) Guidelines.

[a]CPK and GGT were not analyzed after week 78. In addition, serum corticosterone levels were measured at 52 weeks, and liver samples from rats sacrificed at 52 weeks were frozen and analyzed for glycogen content and glucose-6-phosphatase and fructose-1,6-diphosphatase activities.

12

reached. Triglyceride levels were somewhat decreased in males receiving 350 ppm at weeks 14, 26, and 52, but the decrease was only significant ($p < 0.05$) at 26 weeks. Table 5 presents mean data for cholesterol and triglycerides for females.

Table 6 presents mean data for ASAT, ALAT, and GGT in male rats. At week 78, ASAT, ALAT, and GGT activities were increased in males receiving 350 ppm, but the increase was significant only for ASAT ($p \leq 0.01$). The increases in the mean activity of these enzymes in high-dose males at week 78 were a reflection of the high levels of the activities of all enzymes detected in 3 of the 10 animals. These same three males were not sampled at either earlier or later bleeding intervals. Increases were also observed in mean ASAT and mean ALAT activity in high-dose males at 118 weeks, but the increase was significant for only ALAT.

Urea levels in females receiving 350 ppm were consistently 8 to 10% higher than in controls, but these changes were considered marginal and no effects were seen in males. Total protein and globulin levels were slightly increased in high-dose females at 78, 105, and 121 weeks. The increases were significant at week 78 ($p < 0.05$) and 121 ($p < 0.01$). No other clinical chemistry finding could be correlated with dosing.

Analysis of livers of rats sacrificed after 52 weeks for glycogen content, glucose-6-phosphatase, and fructose-1,6-diphosphatase activities did not indicate any effects of dosing; serum levels of corticosterone were unaffected at the same interval.

6. Urinalysis: Urine was collected from 10 fasted rats per sex per group during weeks 14, 26, 52, 78, and 105, and prior to termination. The CHECKED (X) parameters were examined:

X	Appearance†	X	Glucose†
X	Volume†	X	Ketones
X	Specific Gravity†	X	Bilirubin†
X	pH	X	Blood†
X	Sediment (microscopic)†		Nitrate
X	Protein†	X	Urobilinogen

Results: No compound-related effects on urinary parameters were observed.

†Recommended by Subdivision F (October 1982) Guidelines.

13

TABLE 5. Mean Cholesterol and Triglyceride Levels in Female Rats Fed SAN 619F

Dietary Level (ppm)	Mean Value (± S.D.) at Week: 14	26	52	78	105	121
			Cholesterol (mmol/L)			
0	1.03 ± 0.30	1.17 ± 0.39	2.15 ± 0.93	1.64 ± 0.49	1.66 ± 0.34	1.83 ± 0.40
20	0.81 ± 0.43	0.97 ± 0.58	1.98 ± 0.51	1.65 ± 0.47	1.99 ± 0.43	2.38 ± 1.07
50	0.98 ± 0.39	1.21 ± 0.40	1.96 ± 0.64	2.19 ± 1.68	2.21 ± 0.37*	2.47 ± 0.68
350	0.92 ± 0.38	1.07 ± 0.43	1.78 ± 0.29	2.21 ± 0.42	2.14 ± 0.54*	2.85 ± 0.94
			Triglycerides (mmol/L)			
0	0.65 ± 0.27	0.70 ± 0.31	1.16 ± 0.86	1.01 ± 0.58	1.29 ± 0.58	1.11 ± 0.59
20	0.47 ± 0.13	0.48 ± 0.13	0.73 ± 0.30	0.84 ± 0.41	1.41 ± 0.87	1.54 ± 0.99
50	0.55 ± 0.19	0.58 ± 0.14	0.81 ± 0.51	1.44 ± 2.16[a]	1.21 ± 0.56	1.32 ± 0.58
350	0.51 ± 0.17	0.51 ± 0.14	0.70 ± 0.36	0.64 ± 0.16	0.93 ± 0.23	1.58 ± 0.77

[a]The mean calculated excluding one outlier value is 0.85 mmol/L.

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

TABLE 6. Mean Activity of Aspartic Aminotransferase, Alanine Aminotransferase, and Gamma Glutamyl Transferase in Male Rats Fed SAN 619F

Parameter/ Dietary Level (ppm)	Mean Activity (U/L ± S.D.) at Week: 14	26	52	78	105	118
ASAT						
0	41.7 ± 7.3	42.8 ± 15.7	39.8 ± 7.4	36.0 ± 7.6	39.5 ± 8.9	49.1 ± 10.6
20	43.8 ± 4.9	42.1 ± 14.0	33.8 ± 2.8	39.8 ± 10.0	43.8 ± 15.8	51.0 ± 25.6
50	43.9 ± 3.3	42.5 ± 7.7	43.2 ± 12.9	44.2 ± 15.5	43.4 ± 7.9	48.6 ± 14.1
350	42.9 ± 4.4	39.4 ± 5.8	42.2 ± 7.9	62.5 ± 34.0**	42.6 ± 13.1	63.5 ± 30.0
ALAT						
0	20.6 ± 3.5	24.7 ± 13.9	19.5 ± 3.3	19.5 ± 3.8	23.2 ± 10.6	21.0 ± 6.0
20	21.6 ± 3.9	26.9 ± 25.0	18.4 ± 5.9	20.9 ± 4.7	26.8 ± 14.7	37.7 ± 43.5[a]
50	22.5 ± 3.6	23.6 ± 8.3	22.9 ± 11.3	26.6 ± 9.6	19.5 ± 5.0	21.0 ± 6.8
350	23.2 ± 4.0	23.0 ± 3.8	22.0 ± 5.6	58.1 ± 71.6	24.0 ± 13.5	31.8 ± 10.2**
GGT						
0	0.7 ± 1.0[b]	0.7 ± 0.7	0.1 ± 0.2	0.9 ± 2.0[c]		
20	0.5 ± 0.6	0.8 ± 0.7	0.2 ± 0.2	0.3 ± 0.3		
50	0.6 ± 0.6	0.9 ± 0.7	0.3 ± 0.4	0.3 ± 0.3		
350	0.7 ± 0.6	1.1 ± 0.3	0.3 ± 0.5	4.1 ± 8.6[d]		

[a]If an outlier value of 151 is not included, the mean value is 23.5.

[b]If an outlier value of 3.2 is not included, the mean value is 0.4 ± 0.4.

[c]If an outlier of 6.4 is not included, the mean value is 0.3 ± 0.5.

[d]If outliers of 3.4, 27.1, and 9.4 are not included, the mean value is 0.1 ± 0.3.

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

15

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

Digestive System	Cardiovasc./Hemat.	Neurologic
X Tongue	X 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† (cervical & mesenteric)	XX 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†
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	XX Ovaries	
X Trachea†	X Uterus†	
X Lung†		

Other
X Bone (sternum and femur)†
X Skeletal muscle†
X Skin
X All gross lesions and masses

Results:

a. Organ Weights: Table 7 presents data on liver weights. The liver-to-body weight ratios in high-dose females were significantly increased ($p < 0.01$) when compared to controls at the 52-, 78-, and 121-week sacrifices. There were only slight increases in the absolute liver weights in these females at 78 and 121 weeks. The mean body weights at necropsy were 20, 15, and 16% lower than controls in high-dose females at 52, 78, and 121 weeks. There were no corresponding effects on liver

†Recommended by Subdivision F (October 1982) Guidelines.

16

TABLE 7. Mean Liver Weights (g ± S.D.) and Liver-to-Body Weight Ratios (% S.D.) in Rats Fed SAN 619F

Dietary Level (ppm)	Week 52 (g)	Week 52 (%)	Week 78 (g)	Week 78 (%)	Termination[a] (g)	Termination[a] (%)
			Males			
0	14.44 ± 1.59	2.51 ± 0.23	14.94 ± 2.15	2.52 ± 0.23	17.0 ± 3.3	3.26 ± 0.92
20	14.54 ± 1.46	2.51 ± 0.15	15.68 ± 2.21	2.58 ± 0.30	16.3 ± 2.3	3.02 ± 0.43
50	13.83 ± 1.86	2.46 ± 0.25	16.23 ± 2.51	2.56 ± 0.30	17.6 ± 2.8	3.06 ± 0.39
350	13.90 ± 1.30	2.61 ± 0.20	16.24 ± 2.45	2.77 ± 0.34	18.2 ± 3.6	3.55 ± 0.60
			Females			
0	8.71 ± 1.77	2.62 ± 0.18	8.58 ± 0.84	2.46 ± 0.31	13.3 ± 3.7	3.43 ± 0.78
20	8.06 ± 0.99	2.70 ± 0.23	8.81 ± 1.10	2.50 ± 0.22	13.0 ± 2.8	3.58 ± 1.04
50	8.86 ± 1.12	2.82 ± 0.34	9.86 ± 2.17	2.69 ± 0.58	13.1 ± 2.6	3.44 ± 0.79
350	8.13 ± 1.01	3.06 ± 0.25**	9.55 ± 0.80	3.19 ± 0.23**	13.9 ± 2.7	4.31 ± 0.60**

[a]Males were sacrificed at 118 weeks and females at 121 weeks. Values at termination are means for 14, 16, 20, and 22 (males) and 18, 19, 21, and 17 (females) at dietary levels of 0, 20, 50, and 350 ppm, respectively.

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

weights in males, and necropsy body weights did not differ significantly in the control and high-dose groups.

Kidney-to-body weight ratios in high-dose females were slightly increased at 78 weeks ($p < 0.05$) and 121 weeks ($p < 0.01$), but there were no effects on absolute kidney weights. Moderate but significant increases in relative heart and brain weight were also observed in females receiving 350 ppm ($p < 0.05$ for brain at 78 and 121 weeks and for heart at 52 weeks, and $p < 0.01$ for brain at 52 weeks and for heart at 78 and 121 weeks); no effects on absolute weights of heart and brain were observed, nor were corresponding effects seen in males.

b. Gross Pathology: It was reported that the recorded gross findings were those commonly found in rats of this strain and that their incidence was similar in treated and control groups. A summary tabulation of gross findings was not presented. A scan of the individual pathology sheets (for high-dose animals) did not reveal any unusual findings.

c. Microscopic Pathology:

1) Nonneoplastic: An increase of fatty changes of the liver (hepatocyte vacuolization) was observed in high-dose males. Table 8 summarizes the incidence and severity of fatty changes in the liver at the 52- and 78-week sacrifices and for animals in the main study (includes deaths, moribund sacrifices, and terminal-sacrifice rats). Both the incidence and severity of the changes were increased. There were significant positive trends ($p = 0.0026$ at 52 weeks and $p < 0.0005$ at 78 weeks and in the main study group). The incidence was not increased in dosed females; in fact, it was decreased in the high-dose group (main group). Hepatocellular hypertrophy (mild) was observed in four females receiving 350 ppm at 78 weeks; it was not seen in female rats in the main groups or in those sacrificed at week 52. One mid-dose male and one high-dose male (main group) displayed this lesion also. The authors considered that all other nonneoplastic lesions noted in the study were incidental and commonly found in rats of this age and strain. Table 9 summarizes frequent non-neoplastic findings in the main study. A similar pattern of nonneoplastic findings was seen at the 52- and 78-week sacrifices.

2) Neoplastic: Table 10 presents selected neoplastic lesions in the main groups. There were no compound- or dose-related increases in neoplasms.

18

TABLE 8. Incidence and Severity of Fatty Changes in the Livers of Rats Fed SAN 619F

Incidence/ Grade[a]	Dietary Level (ppm)							
	Males				Females			
	0	20	50	350	0	20	50	350
52-Week Sacrifice (10/sex/group)								
Total incidence	4	5	7	10	1	0	0	1
Minimal/slight	4	5	7	4	1	0	0	1
Moderate	0	0	0	6	0	0	0	0
78-Week Sacrifice (10/sex/group)								
Total incidence	3	1	4	10	0	0	0	0
Slight	3	1	4	5	0	0	0	0
Moderate	0	0	0	5	0	0	0	0
Main Study (50/sex/group)								
Total incidence	23	19	29	38	23	15	15	10
Minimum/slight	9	5	19	18	13	5	12	3
Moderate/marked	14	14	9	20	4	1	0	3
Massive	0	0	1	0	0	0	0	0

[a]The lesions were graded on the basis of 1 to 5:
1 = minimum
2 = slight
3 = moderate
4 = marked
5 = massive.

TABLE 9. Selected Nonneoplastic Findings in Rats Fed SAN 619F for up to 121 Weeks[a]

Organ/ Finding	Dietary Level (ppm)							
	Males				Females			
	0	20	50	350	0	20	50	350
Adrenal	(50)[b]	(50)	(50)	(50)	(50)	(50)	(50)	(50)
Altered cell focus	15	23	26	28	15	21	17	16
Cystic cortical degeneration	0	2	2	1	31	33	37	39
Heart	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)
Necrosis/fibrosis	35	37	39	35	23	28	31	37
Kidneys	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)
Chronic nephropathy	34	34	36	38	15	13	8	25
Tubular dilatation	13	9	10	7	25	14	23	17
Tubular pigment	1	3	7	7	8	5	5	8
Mineralization	3	2	1	3	33	34	32	34
Lymphoid infiltration	40	41	31	36	12	18	16	24
Liver	(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
Lungs	(50)	(49)	(50)	(50)	(50)	(50)	(50)	(50)
Lipoproteinosis	24	21	19	26	6	14	13	13
Thyroid	(50)	(48)	(49)	(50)	(50)	(50)	(49)	(50)
C-cell hyperplasia	4	0	3	7	5	8	5	6
Lymph nodes (Mesenteric)	(47)	(48)	(48)	(49)	(48)	(49)	(47)	(49)
Pigmented macrophages	21	31	33	39	30	34	37	40
Pancreas	(49)	(49)	(50)	(50)	(50)	(50)	(50)	(49)
Islet cell hyperplasia	16	9	21	20	8	7	8	4

[a]Includes animals in the main study that died or were sacrificed moribund as well as those sacrificed at study termination (week 118 for males and week 121 for females).

[b]The numbers in parentheses represent the number of rats with the specific tissue/organ examined microscopically.

TABLE 10. Representative Neoplasms in Rats Fed SAN 691F for up to 121 Weeks[a]

Organ/ Finding	Dietary Level (ppm)							
	Males				Females			
	0	20	50	350	0	20	50	350
Adrenal	(48)	(50)	(50)	(50)	(48)	(49)	(47)	(49)
Medullary tumor (B)	0	1	2	1	0	2	1	1
Hemolymphoreticular	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)
Malignant lymphoma	2	1	1	0	2	4	2	2
Fibrous histiocytoma	1	3	1	1	1	1	0	1
Liver	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)
Hepatocellular adenoma	1	0	1	1	0	0	1	1
Hepatocellular carcinoma	0	0	1	1	0	1	0	0
Mammary glands					(49)	(50)	(49)	(49)
Fibroadenoma					17	23	18	9
Adenocarcinoma					4	7	5	5
Adenoma					0	0	0	2
Ovaries					(50)	(50)	(50)	(50)
Theca cell tumor					0	3	3	1
Pancreas	(49)	(49)	(50)	(50)	(50)	(50)	(50)	(49)
Islet cell adenoma	7	7	8	0	9	3	3	0
Pituitary	(50)	(49)	(50)	(49)	(50)	(50)	(50)	(50)
Adenoma	25	29	29	31	41	39	45	47
Thyroid glands	(50)	(48)	(50)	(49)	(50)	(50)	(49)	(50)
Follicular adenoma	0	2	4	1	1	1	1	2
C-cell adenoma	4	2	3	2	3	8	4	3
Lymph node (mesenteric)	(47)	(48)	(48)	(49)	(48)	(49)	(47)	(49)
Hemangioma	5	0	1	5	0	1	1	0
Skin	(50)	(50)	(49)	(49)	(49)	(50)	(49)	(50)
Squamous carcinoma	0	1	2	1	0	0	0	0
Squamous papilloma	0	3	1	1	0	0	0	1
Fibroma	0	3	1	1	0	1	0	0

[a]Includes all rats in the main groups. Incidental neoplasms (2% in any group) have not been included for all tissues.

21

The incidence of neoplasms was comparable to that of concurrent controls at the 52- and 78-week sacrifices. One liver adenoma was observed in a high-dose male at 12 months; no other liver neoplasms were seen at interim sacrifices.

D. STUDY AUTHORS' CONCLUSIONS:

The authors concluded that SAN 619F was not carcinogenic in KFM-Wistar rats under the conditions of the study. The study clearly meets "published" MTD requirements, since a 6 and 10% retardation in body weight gain was seen at 13 weeks in males and females, respectively, receiving 350 ppm.

The principal effects seen were confined to the liver and included increased incidences of fatty changes and hepatocellular hypertrophy. The authors considered these changes to be a consequence of induction of hepatic enzymes and to be of limited toxicological significance. Increased liver weights, increases in the activities of serum gamma glutamyl transferase, ASAT, and ALAT, and possibly a decrease in bilirubin levels reflect these changes. There was no evidence of an effect of SAN 619F on the endocrine system. Increases in kidney- and heart-to-body weight ratios in high-dose females did not correlate with histologic findings, nor were they found in males; these changes are probably related to body weight decreases and are of doubtful toxicologic importance. The reduced triglyceride levels in dosed females were not considered of toxicologic importance, and the histologic findings (except for the liver) were considered normal for the strain and age of the rats. The NOEL was 20 ppm, which corresponds to a daily intake of 1 mg/kg/day.

E. REVIEWERS' DISCUSSION AND INTERPRETATION OF RESULTS:

The study design was complete (except as discussed below), and the reporting was adequate. It is noted that summary tabulations of gross pathology findings were not provided, and the final (necropsy) body weight of each animal was not recorded on the pathology sheets. Summary tables of all other parameters were accurate (except as noted below) and were supported by individual animal data.

- Discrepancies: In Appendix 3, page 278 (Listing of Animal by Week of Death) - control male No. 68 died at week 109 and 111; in Individual Symptomology Table, No. 68 is listed as dying at week 111 (page 288). Animal No. 469 is listed twice; once as a high-dose male (page 279 lists terminal sacrifice for this animal), and once as a high-dose female (78-weeks interim sacrifice on page 277, probably a typo-should be No. 496); animal No. 496 is not listed in the

22

Listings of Animal by Week of Death Table, but is listed as a high-dose female sacrificed at the 78-week sacrifice.

On page 27 of the study report, regarding hepatocellular hypertrophy, it is stated that male rats of the high dose displayed this lesion after 78 weeks, but not after 121 weeks. The males did not receive treatment for 121 weeks, but were sacrificed at 118 weeks. High-dose females displayed this lesion at 78 weeks.

We agree with the study authors' assessment that SAN 691F was not oncogenic under the conditions of the study. Neoplasms were not increased at any site, and the incidence of some neoplasms in high-dose groups was lower than in controls (mammary fibroadenomas in females and islet cell adenomas in both sexes). Hepatocellular adenomas and carcinomas were not increased compared to controls.

However, we do not agree with the study authors conclusions that a satisfactory maximum tolerated dose was achieved. In a 90-day feeding study in the same rat strain there was no effect on weight gain at 320 ppm. Since no definitive effects were observed in that study other than increased relative liver weight at the high-dose (320 ppm) with 1/15 mid dose males and 6/15 high-dose displaying vacuolated hepatocytes, it is suggested that the study may not have been rigorous enough to adequately predict dose levels for a chronic study.

In the chronic study, the decrease in body weight in high-dose animals compared to controls was not of great magnitude throughout most of the study. Body weight gain over the first 13 weeks was comparable to control (222 g for high-dose males versus 235 for controls and 92 g for high dose females versus 103 g for controls) although the decrement in females showed statistical significance ($p < 0.01$).

The reviewers did not consider the changes in clinical chemistry parameters that were observed and reported to be of toxicologic importance, primarily because of lack of correlations with histologic findings or organ weight changes. Increases in serum cholesterol were observed in females at weeks 78, 105, and 121 (significant only at week 78 in mid- and high doses), but no changes were seen in dosed males. However, the increase in the incidence and severity of fatty liver changes were seen in the males but not the females receiving 350 ppm. Increases in ASAT ($p < 0.05$, week 78) and ALAT ($p < 0.01$, week 118) in males were not accompanied by significant liver weight changes. In females, there were no definitive changes in serum liver enzymes that correlated with observed liver weight changes. Interpretation of clinical chemistry data was complicated since different sets of animals were sampled at week 78 and thereafter than were studied

through week 52; also, a large range of values for serum enzymes of individual animals was observed.

The relative liver weight increase in high-dose females at each scheduled sacrifice was not accompanied by any significant histopathological correlates. Conversely, the histologic liver changes in males were for the most part minimal and were not accompanied by weight changes or consistent liver enzyme effects. In light of the fact that the test material has been shown to be a liver enzyme inducer, it does not appear the dose levels chosen were adequate for a carcinogenicity study.

A LEL for chronic toxicity can be set at 350 ppm in females (21.8 mg/kg/day) based on decreased mean body weights and weight gains and a decrease in the liver-to-body weight ratio and set at 350 ppm in males (15.6 mg/kg/day) based on an increase in fatty liver changes; the corresponding NOEL is 50 ppm (2.2 mg/kg/day in males and 2.7 mg/kg/day in females) cyproconazole fed in the diet for two years.