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Reg.83 258 - VINCLOZOLIN

STUDY TYPE: CHRONIC FEEDING - RAT (83-1a)

#12053

Prepared for

Health Effects Division,
Office of Pesticide Programs
U.S. Environmental Protection Agency
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Prepared by

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DATA EVALUATION REPORT

STUDY TYPE: Chronic Feeding - Rat (83-1a)

TOX. CHEM. NO: 323C

P.C. CODE: 113201

MRID NO.: 43254701

TEST MATERIAL: Reg. No. 83-258 (Vinclozolin)

SYNONYMS: 3-(3,5-Dichlorophenyl)-5-ethenyl-5-methyl-2,4-oxazolidinedione; 3-(3,5-dichlorophenyl)-5-methyl-5-vinyloxazolidine-2,4-dione; BAS 353F; Ronilan (Merck Index)

STUDY NUMBER: 71S0375/88026

SPONSOR: BASF Corporation, Agricultural Products, Research Triangle Park, NC

TESTING FACILITY: BASF Aktiengesellschaft, Department of Toxicology, Ludwigshafen/ Rhein, FRG

TITLE OF REPORT: Chronic Toxicity Study with Reg. No. 83 258 - Vinclozolin in Rats Administration in the Diet for 24 months.

AUTHOR: W. Mellert

REPORT ISSUED: May 3, 1994 (study completion date)

EXECUTIVE SUMMARY: In a chronic toxicity study (MRID# 43254701), groups of 20 male and 20 female Wistar rats were administered 0, 150, 500, 1500, or 4500 ppm (0, 7, 23, 71, or 221 mg/kg/day, respectively, for males and 0, 9, 29, 88, or 257 mg/kg/day, respectively, for females) of vinclozolin in their diets for 104 weeks.

Survival was not significantly affected in either male or female rats fed vinclozolin. Growth was markedly reduced in both sexes fed the test material. During the second year of treatment with 4500 ppm, male and female rats weighed 17-33% and 14-30%, respectively, less than controls. At termination body weight gain was reduced by about 45% in both sexes receiving 4500 ppm, due in part to reduced food consumption. At termination and 1500 ppm, body weight gain in females was 76% ($p<0.01$) and in males 82% ($p>0.05$, N.S.) of control values.

The overall relative efficiency of food utilization from week 52 to 102 in males and females showed a biologically significant dose related decrease at 1500 and 4500 ppm. In control, 1500 and 4500 ppm, respectively, the values for males are 4.1, -0.03 and -3.3 and for females the values are 6.9, 4.5 and -0.2. Water consumption for week 0-102 was increased at 4500 ppm and at 1500 ppm for week 0-13 in males and females.

Organ weight changes in male and female rats fed 4500 ppm of the test material included statistically significant increases in absolute and relative liver and adrenal weights. Increases in absolute and relative testes weights occurred at all doses, but relative weights were statistically significant only at 4500 ppm.

At various times during treatment bilateral lesions such as cataracts, bosselated structures in the lens, bulbiform thickening of the lens, and focal opacity were seen in both sexes receiving doses ≥ 500 ppm and in males at all dose levels. Serous fluid accumulation in the anterior chamber of the eyes was noted in males and females at 4500 ppm.

Microscopically in males, several lesions were statistically significantly increased in the testes, such as increased tubular calcification at all dose levels and diffuse tubular atrophy at ≥ 500 ppm, and in the epididymides and prostate at ≥ 500 ppm. There was a significant decrease in the incidence of focal Leydig cell hyperplasia at ≥ 1500, and statistically significant increase in hyperplastic rete testes at 4500 ppm. Microscopic lesions were noted in the epididymis (notably reduced size or atrophy and azoospermia/oligospermia at doses ≥500 ppm), seminal vesicle (reduced size or atrophy at doses ≥1500 ppm), coagulation gland (atrophy at doses ≥1500 ppm), and prostate (reduced size or reduced secretion at doses ≥500 ppm). In addition, interstitial fibrosis was a notable lesion in the prostate, showing a dose-related increase in incidence and severity at all dose levels (perhaps indicative of past inflammatory damage or cellular replacement). In female rats, a statistically significant increase in the incidence of interstitial lipidosis in the ovaries occurred at all doses. Atrophy of skeletal muscle fibers was significant in males and females at 4500 ppm. Vinclozolin inhibits androgen receptor binding and interferes with lipid metabolism and possibly steroidogenesis; therefore, the lesions in the testes, male accessory organs and ovaries may be related to a hormonal imbalance due to excessive stimulation by luteinizing hormone (LH) or by interference with aspects of lipid/cholesterol metabolism/storage. In addition, the muscle atrophy and body weight gain reduction seen may be due in part to the inhibition of androgen receptors on muscles.

Increased incidences of lesions were seen at one or more dose levels in the liver of both sexes (cellular hypertrophy, single cell necrosis, and eosinophilic foci (1500 and 4500 ppm), kidney of male rats (urothelial hyperplasia, ≥500 ppm; renal pelvis calcification, 4500 ppm), lungs of both sexes (foam cell aggregates, 4500 ppm), pancreas of both sexes (vacuolated acinar cells, ≥500 ppm), skeletal muscle of both sexes (focal fiber atrophy, 4500 ppm), and adrenal gland of both sexes (lipidosis, ≥500 ppm for males and ≥1500 ppm for females, extracortical nodules, 4500 ppm).

Incidences of lesions showing statistically significant decreases in males

and/or females, which may be secondary to the antiandrogenicity and/or the interference with lipid metabolism/storage of vinclozolin, were noted in the liver, adrenal, myocardium, pituitary, prostate and mammary gland.

Several clinical chemistry values were biologically and statistically significantly changed (increased liver SGGT and other parameters)(serum triglycerides were consistently reduced in males and cholesterol was increased in females) in males and females mostly at 4500 ppm.

The LOEL for systemic toxicity is 150 ppm (7 mg/kg/day for males and 9 mg/kg/day for females) based on bilateral lenticular degeneration of the eyes, seminiferous tubular calcification in the testes, and interstitial fibrosis in the prostate of male rats and interstitial cell lipidosis in the ovaries of female rats. There is no corresponding NOEL, because the lowest dose tested is the LOEL.

This study showed some evidence of carcinogenicity probably involving a hormonal imbalance. Leydig cell tumors (mostly benign) occurred in male rats at ≥ 500 ppm. Hepatocellular carcinomas at incidences of 0/20, 0/20, 1/20, 1/20, 9/20*[1] occurred in controls, 150, 500, 1500, and 4500 ppm, respectively. In addition, the total number of 4500-ppm male rats with malignant tumors at any site (13/20 vs. 3/20) was significantly increased. Females had significantly increased incidences of adrenal cortical tumors (0/20, 0/20, 0/20, 1/20, 6/20*), benign sex cord tumors in the ovaries (0/20, 0/20, 2/20, 4/20*, 10/20*), and all ovarian tumors combined (4/20, 3/20, 4/20, 7/20, 11/20*). However, there was a dose related decrease in the incidence of pituitary adenomas in females rats (14/20, 12/20, 12/20, 6/18*, 5/20**) and mammary fibroadenomas (5/20, 5/9, 3/10, 6/9, 1/18). The development of the Leydig cell tumors may be due indirectly to the antiandrogenic activity of vinclozolin, which disrupts the feedback mechanism for luteinizing hormone (LH), resulting in over stimulation of the Leydig cells by LH and not to a direct effect of the test material on the testes. The development of adrenal cortical and ovarian tumors may also be related to a hormonal imbalance or interference with aspects of lipid metabolism/storage. The maximum tolerated dose was clearly exceeded in animals receiving 4500 ppm and possibly at 1500 ppm as evidenced by marked reduction in growth and induction of numerous nonneoplastic lesions and the decreased efficiency seen in males and females at dose levels ≥ 1500 ppm. However, the development of Leydig cell, adrenal cortical, and ovarian tumors is probably not due to the excessive toxicity, but to a hormonal imbalance or interference with aspects of lipid metabolism/storage. Except for one male rat in the 1500-ppm group, hepatocellular carcinomas developed only in male rats fed 4500 ppm. The lack of adenomas either accompanying or preceding the hepatocellular carcinomas suggests that the development of hepatocellular carcinomas is not an indirect effect of hepatocellular toxicity. However, the induction of hepatocellular carcinomas was not confirmed in the carcinogenicity study (MRID No. 43254703) where male rats fed 3000 ppm

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[1] * & ** = statistical significance at $p \leq 0.05$ & 0.01, respectively.

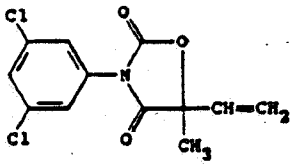
for 104 weeks did not develop hepatocellular carcinomas at a significantly increased incidence.

Core Classification: Acceptable. This study (MRID# 43254701) and the supplementary study using lower doses (MRID# 43254702) combined are acceptable for a Guideline (83-1) study for chronic toxicity in the rat. Toxic effects and a NOEL are shown only when the two studies are combined.

A. MATERIAL

1. Test material: Reg. No. 83 258 (Vinclozolin)

   Description: solid crystal (Merck Index)
   Lot/Batch No.: N 183
   Purity: 99.2% a.i.
   Stability of compound: at least 2 years
   CAS No.: 50471-44-8 (Merck Index)
   Structure: Merck Index



2. Vehicle and/or positive control

   The test material was mixed directly with food; no other vehicle was used. A positive control was not included in this study.

3. Test animals

   Species: rat
   Strain: Wistar (Chbb:THOM (SPF))
   Age at the start of study: 42 days at study initiation
   Weight at the start of study: males, mean weight was 181 g (range = 161 to 198g), females, mean weight was 148 g (range = 135 to 166 g)
   Source: Dr. Karl Thomae GmbH, Biberach/Riss, FRG
   Housing: single housing in stainless steel wire mesh cages
   Environmental conditions:
   Temperature: 20 to 24°C
   Humidity: 30 to 70%
   Air Changes: not reported

Photoperiod: 12 h day/12 h dark
Acclimation period: 10 days

B. STUDY DESIGN

1. Animal assignment

Animals were assigned randomly by weight to the test groups in Table 1. No scheduled interim sacrifice was included in this study.

TABLE 1. STUDY DESIGN

Dose Group	Conc. in diet (ppm)	Dose (mg/kg/day)[a]		No. of Animals	
		Male	Female	Male	Female
0	0	0	0	20	20
1	150	7	9	20	20
2	500	23	29	20	20
3	1500	71	88	20	20
4	4500	221	257	20	20

[a]Time-weighted average daily compound intake was reported by the authors (MRID No. 432547-01, pp. 0050').

Dose selection rationale: Dose selection was based on four studies. (1) A 3-month feeding study in male and female Sprague-Dawley rats administered 150 or 450 ppm of the test material in the diet: no effects were observed on the clinical, hematological, clinical chemistry, urinalysis, and pathologic parameters evaluated. (2) A 3-month feeding study with 6-week recovery period in male and female Sprague-Dawley rats administered, 100, 300, 1000, or 2000 ppm of the test material in the diet: decreased erythrocyte count and hematocrit, increased absolute and relative liver weights, increased absolute adrenal weights, and histopathologic effects of the adrenal cortex at 1000 and 2000 ppm were observed. (3) A 4-week feeding study in rats (strain not reported) administered 900, 1800, 3000, or 15,000 ppm of test material: dose-related effects on the adrenal gland (gross pathologic effects and increased absolute and relative adrenal gland weights), increased absolute and relative liver weights, decreased erythrocyte values (females only, all doses), increased ascorbic acid content in adrenal gland and glycogen content in the liver (females all doses and males at the highest dose), and increased relative testes weight were observed at the highest dose. (4) Long-term (duration not reported) study in Sprague-Dawley rats administered 162, 486, 1458, or 4374 ppm of test material in the diet: effects included reduced body weight and food consumption, transient increases in

ascorbic acid excretion, and increased urinary 17-ketosteroids at two highest doses; no other effects were noted.

2. Diet preparation and analysis

The diet was prepared at 1- to 4-weeks intervals by mixing the test material with a small amount of food in a Bosch household mixer followed by adding an appropriate amount of food to the mixture (to obtain the target concentration) and mixing the preparation in a GEBR. LÖDIGE laboratory mixer. The conditions of storage were not reported. Dietary concentrations of the test material were verified at the start of the study (six samples per concentration) and at 3-month intervals thereafter (triplicate samples per concentration). The stability of the test material in the diet (50 ppm) was determined on samples stored for 10 and 32 days; homogeneity was determined at the beginning of the study on six samples taken from the 150- and 4500-ppm mixtures and two samples from 500- and 1500-ppm mixtures.

Results -

a. Homogeneity analysis - All samples were within 10% of the target concentrations.

b. Stability analysis - The mean concentration of the 50-ppm sample was 45.6 ppm at 0 time, 42.7 ppm (93.6%) at day 10, and 43.1 ppm (94.5%) at day 32.

c. Concentration analysis - All individual measurements (33) of all four analyzed concentrations were within $\pm 10\%$ of the target concentrations, except three individual samples from the 150-ppm mixture (-11 or -13%).

3. Diet

Animals received food (Kliba 343 Mehl) and water *ad libitum*.

4. Statistics

The statistical tests used to analyze these data are presented in the Appendix of this Data Evaluation Report (DER) (taken from MRID No. 432547-01; pages 44, 45, and 1083).

5. Signed and dated quality assurance and GLP statements were present.

C. METHODS AND RESULTS

1. Observations

Animals were inspected twice daily on week days and once a day on weekends and holidays for signs of toxicity and mortality.

Results - In male rats, clinical examination revealed cataracts and palpable

enlargement of the testes, both of which showed dose-related increases. In females, clinical examination revealed cataracts, particularly in groups fed ≥500 ppm, and reduced general health particularly in the 4500-ppm group. Terminal survival rates were similar in treated groups and controls and showed no treatment- or dose-related effects (Table 2).

TABLE 2. SURVIVAL RATES (%) OF MALE AND FEMALE RATS FED VINCLOZOLIN FOR 104 WEEKS

Week	0 ppm	150 ppm	500 ppm	1500 ppm	4500 ppm
Males					
Week 26	100[a]	90	95	100	100
Week 52	100	90	95	95	100
Week 78	90	85	95	95	100
Week 91	75	85	95	80	100
Week 104	55	60	70	70	60
Females					
Week 26	100	100	100	95	100
Week 52	100	100	100	95	95
Week 78	100	95	95	95	95
Week 91	90	95	85	95	85
Week 104	85	80	75	85	60

Data were taken from Tables 49 and 50, pages 124-125, MRID No. 432547-01.
[a]Percent surviving; 20 animals per group at study initiation.

2. Body weight

Individual animals were weighed once weekly up to week 14 and once every 4 weeks thereafter.

Results - Body weights in both male and female rats showed a dose-related effect throughout the treatment period (Table 3). Body weights of males receiving 150 or 500 ppm were similar to or exceeded control values by as much as 12% (150 ppm) or 6% (500 ppm). The elevated body weights were statistically significant in 150-ppm males at almost all time points from weeks 22 to 82 and sporadically thereafter.

TABLE 3. SELECTED MEAN BODY WEIGHTS (g) OF MALE AND FEMALE RATS FED VINCLOZOLIN

Week	Males					Females				
	0	150	500	1500	4500	0	150	500	1500	4500
0	181.7	181.7 (100)[a]	181.8 (100)	180.7 (99)	180.4 (99)	147.7	148.0 (100)	147.1 (100)	148.0 (100)	146.7 (99)
1	235.3	236.3 (100)	238.2 (101)	232.8 (99)	206.1** (88)	169.2	171.8 (102)	172.2 (102)	171.0 (101)	151.9** (90)
4	340.5	347.5 (102)	345.2 (101)	339.1 (100)	304.8** (90)	219.6	223.0 (102)	219.2 (100)	219.8 (100)	206.8* (94)
8	423.0	435.5 (103)	431.8 (102)	422.2 (100	375.0** (89)	258.7	264.8 (102)	260.0 (101)	249.4 (96)	245.3 (95)
13	468.4	487.4 (104)	478.3 (102)	468.9 (100)	411.3** (88)	275.7	284.1 (103)	270.5 (98)	267.3 (97)	260.9 (95)
26	540.5	580.1* (107)	559.3 (103)	540.7 (100)	470.5** (87)	317.9	323.9 (102)	308.2 (97)	300.0 (94)	295.5* (93)
38	592.2	646.7* (109)	619.3 (105)	556.8 (94)	509.5** (86)	347.8	352.8 (101)	335.8 (97)	319.4* (92)	302.6** (87)
50	623.7	684.1* (110)	649.7 (104)	601.5 (96)	519.2** (83)	368.2	373.3 (101)	350.3 (95)	332.7* (90)	316.7** (86)
66	667.9	734.8* (110)	695.7 (104)	631.2 (95)	526.9** (79)	405.9	412.8 (102)	384.8 (95)	355.0** (87)	323.1** (80)
82	676.0	748.7* (111)	711.1 (105)	622.9 (92)	511.0** (76)	432.4	442.5 (102)	403.1 (93)	372.2** (86)	323.0** (75)
98	692.6	772.3 (112)	731.0 (106)	607.2* (88)	485.4** (70)	456.4	456.4 (100)	444.8 (97)	379.9** (83)	316.1** (69)
104	683.6	734.6 (107)	712.8 (104)	592.9 (87)	457.3** (67)	443.5	439.7 (99)	441.7 (100)	373.0** (84)	308.8** (70)
Wt. gain 0-104[b]	501.9	552.9 (110.2)	531.0 (105.8)	412.2 (81.2)	276.9 (55.2)	295.8	291.7 (98.6)	294.6 (99.6)	225.0 (76.1)	162.1 (54.8)
wt. gain 0-104[c]	504.2	550.9 (109.3)	529.7 (105.1)	414.6 (82.2)	277.9** (55.1)	295.4	290.9 (98.5)	294.3 (99.6)	224.9** (76.1)	161.0** (54.5)

Data taken from Tables 019-023, pp. 0094-0098 and Tables 154-158 pp 0229-0233 (males) and Tables 024-028, pp. 0099-0103 and Tables 159-163, pp 234-238 (females); MRID No. 432547-01.

[a]Numbers in parentheses are percent of control calculated by the reviewer. [b] Data calculated from the above table. [c] Data obtained from the body weight gain data in the submitted report for males, Table 158, page 0233 and for females, Table 163, page 0238. The reason for the discrepancy is unknown, but since the conclusions are unaltered, the discrepancy was ignored. 0.05; ** $p<0.01$; ANOVA plus Dunnett's test (two-sided)

Body weights in males receiving 1500 ppm were generally less than control (up to 13%) with statistical significance being achieved during weeks 94 and 98. In males receiving 4500 ppm, body weights were statistically significantly depressed throughout the study, especially during the second year (17 and 33% depression). Body weights in females receiving 150 or 500 ppm of test material were similar to control values. Females receiving 1500 ppm weighed significantly less than controls from week 34 to termination of the study and less than <90% of controls from week 62 to termination. Like males receiving 4500 ppm, female rats receiving 4500 ppm weighed less than controls throughout the study, with the depression ranging from 14 to 31% during the second year. Body weights at selected time points are presented in Table 3.

There was an apparent discrepancy between the body weight gain calculated from the body weight data and that calculated by the report authors (See Table 3, bottom). However, since the conclusion remained the same considering either data, the discrepancy was not investigated further.

Body weight gain also showed a dose-related effect. Cumulative body weight gain in males receiving 150 and 500 ppm exceeded that of controls throughout the study; statistical significance was achieved for 150-ppm males during weeks 22 to 66 and sporadically thereafter. Body weight gain in males receiving 1500 ppm was depressed from week 30 to termination with statistical significance being achieved only during weeks 94 and 98. In males receiving 4500 ppm, body weight gain was significantly depressed throughout the study. Weight gain in females receiving 150 or 500 ppm was similar to that of controls. However, weight gain was significantly less ($p<0.05$ or $p<0.01$) than controls from week 34 to termination in females receiving 1500 ppm and from weeks 1 to 6, 12, and 26 to termination in females receiving 4500 ppm. Net body weight gains (weeks 0-104) for each group are presented in Table 3.

3. Water and food consumption and compound intake and relative efficiency of food utilization

Daily water consumption (g/animal/day) was determined weekly for 13 weeks and monthly to termination starting at week 7 for males and 8 for females. In addition, one of the reviewers calculated the overall average water consumption (g/kg-rat) for week 0-13, 0-52 and 0-102. Food consumption for each cage was recorded once a week for 1 week during the first 14 weeks of the study and for 1 week at 4-week intervals thereafter. Food consumption was reported as g/animal/day. Food efficiency [(body weight gain in g/food consumption in g per unit time) × 100] was calculated. Food efficiency was also calculated by one of the reviewers in the same manner, but for study weeks 0-13, 0-52, 0-102 and 52-102. Compound intake (mg/kg/day) was calculated based on nominal concentration of the compound in the diet, food consumption, and body weight data. These values were presented for each day body weights and food consumption data were recorded.

Results -

a. Water consumption - Weekly water consumption (g/kg-rat) in control male and females rats slowly decreased to a minimum about 46 to 52 weeks and than increase such that by termination consumption was similar to the beginning consumption determined starting at weeks 7 and 8 (only selected data shown). Treated groups followed a similar pattern except that overall all water consumption was elevated, especially at the 2 highest dose levels (Table 4). Water consumption appeared elevated at 1500 and 4500 ppm in males (12% and 43%, respectively) and females (25% and 55%, respectively), starting at week 8 in males and females (the first week that water consumption was reported for males and females). The difference from control consumption remained elevated through week 13, 52 to termination at week 104, although weekly values were not always elevated and varied considerably. Water consumption at 1500 and 4500 ppm and week 13 was 13% and 47%, respectively, in males and 13% and 50%, respectively in females. At 1500 and 4500 ppm at week 52, it was elevated 21% and 42%, respectively in males and 15% and 9%, respectively in females. At week 102, at 1500 ppm and 4500 ppm, it was decreased 11% and elevated 52% in males and elevated 25% and depressed 16%, respectively in females.

   At 1500 and 4500 ppm, average water consumption by 13 weeks was 14% and 50%, respectively in males and 28% and 53%, respectively, in females and by 52 weeks the values were 6% and 38% in males and 23% and 30%, respectively in females and by week 102 the overall average for males is 4% and 50%,respectively, elevated over control consumption and the overall average for females in 14% and 16%, respectively.

b. Food consumption – Table 4 and 5 summarizes the average food consumption at selected times and relative efficiency of food utilization during treatment. Mean daily consumption rates fluctuated in animals receiving 150, 500, and 1500 ppm and did not show a consistent dose-related response. At 4500 ppm, food consumption was significantly reduced ($p < 0.05$ or 0.01) throughout the study. During the first week of the study, food consumption was reduced by 34 or 38% in male and female rats, respectively, receiving 4500 ppm of the test material. After the first week, the reduction in food consumption ranged from 5 to 17% of control values in males and 6 to 24% in females.

c. Compound consumption (time-weighted average) – The average compound intake calculated by the study authors was 7, 23, 71, and 221 mg/kg/day for male rats and 9, 29, 88, 257 mg/kg/day for female rats receiving 150, 500, 1500, or 4500 ppm, respectively, of test material in the diet (Table 1).

d. Food efficiency – The relative efficiency of food utilization in all groups showed a general decline during the study and appeared to be lower in treated groups than controls. In addition, the calculated efficiency over the study weeks 0-13, 0-52, 0-102 and 52-102 differed and an increase dose related pattern emerged (Bottom of Table 5). Weekly relative efficiency of food utilization did not differ significantly among controls and treated animals of either sex and showed only a general treatment-related pattern of decreases (Top of Table 5).

TABLE 4. AVERAGE FOOD CONSUMPTION (g/ANIMAL/DAY) BY MALE AND FEMALE RATS FED VINCLOZOLIN FOR 104 WEEKS

Week	Males					Females				
	0	150	500	1500	4500	0	150	500	1500	4500
1	24.8[a]	24.9	25.2	23.7*	16.3**	18.1[a]	18.8	18.6	17.3*	11.2**
4	25.9	26.6	26.4	26.6	24.6*	18.9	19.7	18.8	19.1	16.4**
8	26.0	26.8	27.3*	27.0	23.6**	19.5	20.4	19.3	19.1	17.5**
13	23.1	22.6	22.5	22.6	20.0**	16.0	16.9	16.3	15.9	15.0*
26	22.9	24.2	24.3*	23.7	20.8**	17.6	17.7	17.1	16.7*	15.2**
38	25.8	26.8	26.5	22.8*	23.5**	19.9	18.7	18.7	19.1	15.2*
50	26.6	27.4	26.8	25.3	23.3**	20.5	20.9	19.9	19.0*	17.1*
66	26.6	27.2	27.3	25.5	22.2**	21.2	21.0	20.4	19.3**	17.5**
82	27.5	28.4	28.5	25.7*	23.2**	22.7	23.4	21.1	20.2*	18.5*
98	26.9	30.0**	28.6	25.2	22.5**	22.7	21.7	22.3	20.2**	17.0**
104	26.3	28.0	27.4	25.1	22.3**	21.0	21.2	21.9	18.9*	17.3**
Male (20) water consumption at indicated study week, g/kg-rat. CV range was generally 13% to 30%, but 1 value was as high as 53%						Female (20) water consumption as indicated on study week, g/kg-rat. CV range was generally 12% to 27%, but 1 value was as high as 65%.				
Week 8[b] (CV)	62.2 (19%)	61.8 (16%)	66.2 (13%)	69.6 (18%)	88.8 (12)	82.7 (17%)	83.5 (28%)	93.8 (22%)	103.0 (28%)	128.4 (25%)
Week 13 (CV)	42.3 (23%)	42.3 (34%)	41.2 (23%)	48.0 (23%	63.5 (23%)	69.3 (21%)	63.4 (24%)	71.3 (19%)	84.5 (19%)	100.0 (30%)
Week 52 (CV)	41.8 (18%)	37.9 (19%)	41.2 (16%)	43.2 (41%)	59.3 (20%)	71.7 (30%)	74.2 (38%)	77.6 (28%)	82.4 (15%)	78.0 (25%)
Week 102 (CV)	67.4 (47%)	59.1 (31%)	55.2 (38%)	60.2 (43%)	102.7 (36%)	91.7 (27%)	79.8 (31%)	87.9 (29%)	114.6 (65%)	77.2 (33%)
Average male water consumption during the period indicated (g/kg-rat)						Average females water consumption during the period indicated. (g/kg-rat)				
0-13	54.6	54.9	57.7	61.9	80.1	80.8	79.2	86.0	104.3	124.9
0-52	47.0	44.0	46.5	50.4	65.1	73.2	71.7	78.7	89.7	94.6
0-102	49.6	45.7	47.2	51.7	74.5	78.3	75.1	80.7	90.1	91.5

[a] Data taken from Tables 001-010, page 76-85, MRID No. 432547-01. [b] = The first week water consumption was reported for both males and females, Table 011-018, pages 0086-0093. * = $p \leq 0.05$, ** = $p \leq 0.01$, Student's t-test calculated by the reviewer.

TABLE 5. AVERAGE FOOD EFFICIENCY BY MALE AND FEMALE RATS FED VINCLOZOLIN FOR 104 WEEKS

Week	Males					Females				
	0[a]	150	500	1500	4500	0	150	500	1500	4500
1	30.9[b]	31.4	32.0	31.4	22.4	16.9	18.0	19.3	18.9	6.2
4	15.0	16.1	14.7	14.5	16.6	11.1	10.6	9.8	9.6	12.2
8	8.9	10.4	10.9	9.6	9.0	6.8	6.7	7.0	2.9	6.2
13	1.4	0.2	0.1	0.5	-0.7	-5.0	-4.4	-3.0	-4.6	-2.3
26	2.2	3.2	2.8	2.1	1.1	1.0	0.8	0.2	0.1	0.5
38	2.1	2.6	2.7	-2.5	2.5	1.5	0.7	1.2	1.7	-0.2
50	0.3	0.9	0.3	1.3	0.3	1.5	1.7	1.2	1.2	0.0
66	1.3	0.9	1.1	1.4	-0.6	1.0	0.9	1.5	0.9	0.4
82	-0.3	-0.2	-1.3	-1.6	-0.8	0.0	0.3	-1.1	0.3	-0.2
98	-0.7	-0.5	-0.6	-0.3	-2.9	0.6	0.4	0.9	0.7	-0.4
104	-1.8	-7.2	-5.7	-2.4	-4.4	-3.8	-3.6	-1.0	-4.1	-3.0
Average male relative food efficiency[c] calculated for the study weeks indicated (% less than control)						Average female relative food efficiency[c] calculated for the study weeks indicated (% less than control)				
0-13	80.5	85.7	80.9	78.9	73.2 (9%)	49.4	50.8	49.2	46.9	52.3
0-52	15.8	17.2	16.3	15.5	14.0 (11%)	23.2	23.3	21.8	20.6	20.8 (10%)
0-102	17.9	18.9	18.4	15.2 (15%)	11.5 (36%)	13.6	13.6	14.2	11.2 (18%)	8.7 (36%)
52-102	4.1	5.1	4.7	-0.03	-3.3	6.9	6.6	8.7	4.5	-0.2

[a] Concentration of test material in the diet. [b] Data taken from Tables 029-038, page 104-113. MRID No. 432547-01. [c] = (g body weight gain/g food consumed per unit time) × 100 (weekly and for week 0-13, 0-52, 0-102 and 52-102.

The relative efficiency of food utilization for the first 13 weeks showed little change in dosed males or females, except males (9% less than control) at 4500 ppm may have been slightly lower than controls. In males, the trend remained after a 52 weeks

(11% lower at 4500 ppm than control) and became even more pronounced by study week 102, being 15% and 36%, respectively, lower than controls at the 1500 and 4500 ppm, respectively. In females at 1500 and 4500 ppm the food efficiency is 18% and 36% lower than control by week 102 (Table 5).

These effects on relative efficiency of food utilization at the 2 top dose levels appear to be marginal for the first year of the study. However, the efficiency calculations from week 52 to week 102 (termination) confirm the decrease in males and females at the 1500 and 4500 ppm levels (Table 5). These body weight and food efficiency data show that 90-day studies must be interpreted very carefully in determining an acceptable high dose level for testing in chronic studies and may be partly the reason that such a high dose level was tested.

4. Ophthalmoscopic examination

The eyes of all males and females in control and 4500-ppm dose groups were examined with an ophthalmoscope 1 day before treatment began. Due to the detection of abnormalities, the eyes of all males were examined starting on day 94 and all females starting on day 87 and continuing at 3-month intervals until the end of the study.

Results – Tables showing the results of the ophthalmoscopic examinations are presented in the Appendix of this DER (taken from MRID No. 432547-01; pages 53-56). Because abnormal findings in both eyes are more likely to be the result of treatment than abnormalities in only one eye, only those abnormalities occurring in both eyes will be described below. Abnormal findings were observed during the first ophthalmoscopic examination on day 87 in females and on day 94 in male rats . Cataracts were first seen on day 87 in 5/20 females receiving 4500 ppm of the test material, on day 293 in 6/19 females receiving 1500 ppm, on day 566 in 3/19 females receiving 500 ppm. The incidence increased to 19/20 in 4500-ppm females (day 201) with all 13 surviving having this abnormality; 18/18 in 1500-ppm females (day 661) with all 17 surviving having cataracts; and 9/15 in 500-ppm females (at termination). No controls or females receiving 150 ppm developed cataracts. Cataracts were first observed on day 201 in 11/20 male rats receiving 4500 ppm; all 20 males had this abnormality on day 566. In males receiving 1500 ppm, 5/19 had cataracts on day 398 and 14/19 on day 566. Cataracts were first observed on day 482 in 1/19 male receiving 500 ppm and in 3/19 on day 661. Cataracts did not develop in controls or 150-ppm males.

Bosselated (segmented and/or diffuse vacuolar changes) structures in lens were first observed in 14/20 females (highest incidence) receiving 4500 ppm, in 10/20 females receiving 1500 ppm on day 87, and in 1/20 500-ppm females on day 119. The highest incidence of bosselated structures occurred on day 119 in females receiving 1500 ppm (14/20), day 482 in 500-ppm females (8/20). Only one female receiving 150 ppm of the test material developed bosselated structures in both eyes (day 661), and none of the 20 control females developed this abnormality. The incidence of bosselated structures in the eyes of male rats followed the same pattern as in female

rats; the abnormality occurred in 13/20 males receiving 4500 ppm (day 94), 8/19 receiving 1500 ppm (day 398), and 4/19 receiving 500 ppm (day 398). Bosselated structures did not develop in males receiving 150 ppm or the corresponding controls. The decreased incidence of this finding was attributed to cataracts, which prevented its detection.

Bulbiform thickening of the lens occurred at peak incidences of 8/20 (day 119), 7/19 (day 293), and 7/20 (day 482) in females receiving 4500, 1500, or 500 ppm, respectively, and at peak incidences of 14/20 (day 201), 10/20 (day 293), and 5/19 (day 661) in males receiving 4500, 1500, or 500 ppm, respectively. Bulbiform thickening was not observed in the 150-ppm groups or in controls of either sex. The incidence of lens opacity (not otherwise specified, NOS) did not occur with a consistent dose- or duration-related pattern. However, cataracts may have prevented the detection of this abnormality.

Focal opacity was observed in all groups, but no dose-response relationship was observed. The highest incidences occurred in male rats fed 1500 ppm for 293 days and female rats fed 500 ppm for 482 days and 1500 ppm for 293 days. The presence of cataracts prevented detection of this abnormality.

5. Blood was collected from the retroorbital venous plexus of nonfasted nonanesthesized animals for hematology and clinical analysis on days 95, 186, 368, 557, and 724. All surviving animals were bled for hematologic and clinical analysis. The CHECKED (X) parameters were examined.

a. Hematology

X			X	
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
X	Blood clotting measurements			
X	(Thromboplastin time)			
	(Clotting time)			
	(Prothombin time)			

*Required for subchronic studies.

Results – Differences were noted in some hematologic parameters; except for thromboplastin time, the differences were not biologically significant or were not considered to be treatment-related. Slight decreases in red blood cell parameters that achieved statistical significance were observed in both male and female rats receiving the test material (Table 6). The decreases, however, were not consistently related to dose or duration of treatment. In addition, the decreases were $\leq 10\%$ relative to control values. There were no consistent or statistically significant effects on white cell parameters. The white blood cell count in male rats was 81, 70, 58, and 67%

(each dose group 150 to 4500 ppm, respectively) of the control value at the end of the study. The decreases were probably due to a slightly elevated white cell count in the controls and not to the test material. The platelet count was significantly ($p < 0.01$) decreased in male rats receiving 1500 ppm when measured on days 557 (12%) and 724 (16%) relative to control values; no statistically significant effect was observed at the other doses including the high-dose. Therefore, the decrease in platelet count is not considered to be due to the test material. Thromboplastin time (measured using the hepato quik test) was consistently decreased ($p < 0.01$) in female rats receiving 4500 ppm of the test material throughout the study; no effect was observed at other doses or in male rats.

TABLE 6. SELECTED HEMATOLOGIC VALUES IN MALE AND FEMALE RATS FED VINCLOZOLIN FOR 104 WEEKS

Group	Hb (mmol/L)	HCT (L/L)	RBC (10^{12}/L)	MCV (10^{-15} L)	MCH (fmol)	MCHC (mmol/L)	Hb (mmol/dL)	HCT (L/L)	RBC (10^{12}/L)	MCV (10^{-15}L)	MCH (fmol)	MCHC (mmol/L)
	Males						Females					
Day 95												
Control	9.55	0.384	7.95	48.24	1.20	24.88	9.41	0.380	7.65	49.55	1.23	24.82
150 ppm	9.40	0.381	7.98	47.59	1.18	24.75	9.35	0.378	7.56	49.97	1.24	24.75
500 ppm	9.51	0.383	7.97	47.99	1.19	24.84	9.36	0.375	7.54	49.57	1.24	25.00
1500 ppm	9.49	0.384	7.90	48.56	1.20	24.73	9.11	0.367	7.21*	51.42	1.28	24.80
4500 ppm	9.08**	0.371	7.53**	49.22	1.21	24.48*	8.85**	0.356**	7.28	48.82	1.21	24.84
Day 186												
Control	9.38	0.395	8.09	48.78	1.16	23.78	9.56	0.386	7.74	49.84	1.23	24.75
150 ppm	9.37	0.391	8.16	47.73	1.14	23.96	9.48	0.382	7.63	49.94	1.24	24.84
500 ppm	9.60	0.391	8.15	47.94	1.18	24.54	9.40	0.376	7.58	49.59	1.24	25.00
1500 ppm	9.49	0.383*	7.87	48.58	1.21	24.80	9.16**	0.369**	7.40**	49.76	1.24	24.85
4500 ppm	9.02	0.372**	7.56**	49.13	1.19	24.25	8.71**	0.354**	7.31**	48.37**	1.19**	24.62
Day 368												
Control	9.92	0.390	7.98	48.80	1.24	25.43	9.32	0.366	7.29	50.17	1.28	25.47
150 ppm	9.69	0.386	7.97	48.29	1.22	25.16	9.51	0.376	7.45	50.38	1.28	25.29
500 ppm	9.75	0.383	7.90	48.42	1.23	25.45	9.35	0.367	7.33	49.96	1.28	25.53
1500 ppm	9.52*	0.371	7.60	48.69	1.25	25.75	9.18	0.358	7.15	50.04	1.28	25.64
4500 ppm	8.94**	0.363**	7.40**	48.94	1.21	24.66*	8.60**	0.343**	7.16	47.79**	1.20**	25.10

TABLE 6.Continued

Group	Hb (mmol/L)	HCT (L/L)	RBC (10^{12}/L)	MCV (10^{-15}L)	MCH (fmol)	MCHC (mmol/L)	Hb (L/L)	HCT (L/L)	RBC (10^{12}/L)	MCV (10^{-15}L)	MCH (fmol)	MCHC (mmol/L)
	Males						Females					
Day 557												
Control	10.39	0.394	7.93	49.68	1.31	26.36	9.70	0.353	6.88	51.42	1.42	27.50
150 ppm	10.44	0.396	8.02	49.33	1.30	26.40	10.11	0.365	7.18	50.69	1.41	27.71
500 ppm	10.47	0.392	7.91	49.46	1.32	26.75	9.54	0.351	6.94	50.70	1.38	27.16
1500 ppm	10.16	0.382	7.66	50.10	1.35	26.82	9.61	0.352	6.97	50.45	1.38	27.31
4500 ppm	9.73	0.372	7.54	49.30	1.29	26.19	9.00*	0.332*	6.94	47.81**	1.30**	27.14
Day 724												
Control	9.49	0.397	7.21	56.34	1.35	23.92	8.92	0.359	6.64	54.42	1.36	24.96
150 ppm	9.98	0.418	7.89	52.88	1.27	23.93	9.59*	0.384	7.08	54.10	1.35	25.00
500 ppm	10.40	0.434	8.09	53.54	1.29	23.73	9.27	0.372	6.83	54.37	1.36	24.92
1500 ppm	10.07	0.420	7.99	52.62*	1.26	24.00	9.37	0.376	6.94	54.16	1.35	24.92
4500 ppm	9.78	0.407	7.92	51.27**	1.24	24.13	8.84	0.355	6.84	51.91**	1.30	24.93

Data taken from Tables 054-063, pages 129-138, MRID No. 432547-01.
* $p<0.05$; ** $p<0.01$; Dunnett's test (two-sided)

b. Clinical chemistry

Electrolytes
X Calcium*
X Chloride*
Magnesium*
X Phosphorus*
X Potassium*
X Sodium*

Enzymes
X Alkaline phosphatase (ALK)
Cholinesterase (ChE)
Creatinine phosphokinase*
Lactic acid dehydrogenase (LDH)
X Serum alanine aminotransferase (also SGPT)*
X Serum aspartate aminotransferase (also SGOT)*
X Gamma glutamyl transferase (GGT)
Glutamate dehydrogenase

Other
X Albumin*
X Blood creatinine*
X Blood urea nitrogen*
X Cholesterol*
X Globulins
X Glucose*
X Total bilirubin
X Total serum protein (TP)*
Albumin/globulin ratio
X Triglycerides
Serum protein electrophoresis

* Required for chronic studies.

Results - Serum enzyme levels showed effects in both male and female rats that appear to be treatment-related (Table 7). On day 95, serum γ-glutamyltransferase (SGGT) levels were elevated almost 14-fold in male rats receiving 4500 ppm of the test material and by almost 9-fold in female rats receiving the same dose. The level of this enzyme remained elevated throughout the study, with statistical significance ($p < 0.01$) being achieved at most time points. Serum aspartate aminotransferase (AST) levels were not significantly affected in male rats at any time point, but this enzyme showed slight decreases in female rats, with statistical significance being achieved in animals receiving 500 and 4500 ppm on day 368. Alanine aminotransferase (ALT) levels were also slightly decreased in both male and female rats. In males, the decreases in ALT levels were statistically significant at all doses on day 186 and 368, the two highest doses on day 557, but in none of the dose groups at termination. In females, the decreases in ALT levels were statistically significant in the animals receiving 500-4500 ppm on day 95 and 150-1500 ppm on day 368. Alkaline phosphatase (ALP) levels showed decreases in both male and female rats throughout the study. The decreases were statistically significant at all time points from day 186 to termination in high-dose male rats and all time points in high-dose female rats. The meaning of the depression in AST, ALT and ALP are unknown, but depressions in these enzymes are frequently seen in studies with vinclozolin.

Other statistically significant serum chemistry changes were noted for both male and female rats (Table 8); all changes were not consistently related to dose or duration of treatment. Effects in males showing statistically and biologically significant changes (≥10% relative to controls) are as follows (occurred only in high-dose animals unless indicated): creatinine levels were elevated on days 95 (111% of the control value) and 368 (110%); cholesterol levels were elevated on days 95 (146%) and 186 (136%) and

TABLE 7. SELECTED SERUM ENZYME LEVELS IN MALE AND FEMALE RATS FED VINCLOZOLIN FOR 104 WEEKS

Enzyme	0 ppm	150 ppm	500 ppm	1500 ppm	4500 ppm	0 ppm	150 ppm	500 ppm	1500 ppm	4500 ppm
	Male					Female				
Day 95										
ALT (μkat/L)	0.79	0.75	0.80	0.84	0.77	0.76	0.70	0.62**	0.62**	0.64**
AST (μkat/L)	1.64	1.60	1.62	1.72	1.69	1.62	1.65	1.43	1.41	1.35
ALP (μkat/L)	4.63	4.79	4.89	4.68	4.32	4.20	3.92	3.50**	3.03**	2.93**
SGGT (nkat/L)	5	9	10	13	69**	8	7	8	18	71**
Day 186										
ALT (μkat/L)	1.07	0.88**	0.91**	0.83**	0.74**	0.80	0.74	0.70	0.73	0.70
AST (μkat/L)	1.75	1.64	1.79	1.57	1.49	1.61	1.48	1.46	1.38	1.41
ALP (μkat/L)	4.63	4.40	4.73	4.35	3.91**	3.36	3.28	3.02	2.54**	2.71**
SGGT (nkat/L)	5	4	1	6	54**	6	9	3	10	79**
Day 368										
ALT (μkat/L)	1.16	0.88**	0.89**	0.83**	0.76**	0.81	0.69*	0.69*	0.66*	0.70
AST (μkat/L)	1.57	1.53	1.70	1.48	1.52	1.88	1.70	1.36*	1.47	1.33*
ALP (μkat/L)	5.37	4.66	4.89	4.65	3.61**	3.09	3.21	2.75	2.42**	2.37**
SGGT (nkat/L)	2	3	2	0	10	0	0	3	1	10

TABLE 7. Continued

Enzyme	0.ppm	150 ppm	500 ppm	1500 ppm	4500 ppm	0 ppm	150 ppm	500 ppm	1500 ppm	4500 ppm
	Male					Female				
Day 557										
ALT (μkat/L)	0.96	0.85	0.88	0.70**	0.67**	0.78	0.77	0.65	0.88	0.63
AST (μkat/L)	1.34	1.36	1.44	1.42	1.24	1.35	1.36	1.39	1.31	1.39
ALP (μkat/L)	4.57	4.32	4.43	3.62**	2.77**	2.63	2.58	2.25	2.09**	1.66**
SGGT (nkat/L)	40	3	2	17	68	0	0	2	5	16**
Day 724										
ALT (μkat/L)	0.63	0.72	0.70	0.60	0.66	0.73	0.72	0.76	0.65	0.58
AST (μkat/L)	1.42	1.38	1.58	1.32	1.73	1.68	1.26	1.39	1.40	1.44
ALP (μkat/L)	4.12	3.99	3.91	3.31	2.80**	2.83	2.91	2.68	2.47	1.91**
SGGT (nkat/L)	6	9	4	8	113**	2	6	0	16	31**

Data taken from Tables 104-113, pages 179-188, MRID No. 432547-01.
*p<0.05; **p<0.01; ANOVA + Dunnett's test (two-sided)
ALT = alanine aminotransferase; AST = aspartate aminotransferase; ALP = alkaline phosphatase; SGGT = serum γ-glutamyltransferase

TABLE 8. SERUM CHEMISTRY VALUES IN MALE AND FEMALE RATS FED VINCLOZOLIN FOR 104 WEEKS

Group	Creat. (μmol/L)	Chol. (mmol/L)	T. Prot. (g/L)	Alb. (g/L)	Triglyc. (mmol/L)	Creat. (μmol/L)	Chol. (mmol/L)	T. Bil. (μmol/L)	K (mmol/L)	T. Prot. (gL)	Alb. g/L)	Glob. (g/L)
	Males					Females						
Day 95												
0 ppm	50.41	2.18	67.10	35.98	4.46	52.03	2.12	1.65	6.12	67.69	37.74	29.95
150 ppm	51.95	2.41	66.40	35.59	4.33	51.35	2.05	1.33	5.85	69.57	39.26	30.30
500 ppm	51.43	2.34	67.29	36.14	4.43	51.69	2.20	1.46	5.95	69.59	39.41	30.19
1500 ppm	52.97*	2.41	67.80	35.70	4.07	54.17	2.46	0.83	5.82	71.73**	40.38**	31.35
4500 ppm	55.81**	3.18**	68.74	36.56	2.83**	57.44**	4.35**	1.24	5.88	77.70**	41.29**	36.41**
Day 186												
0 ppm	53.53	2.51	70.25	36.08	5.02	53.12	2.33	0.00	6.11	69.74	40.53	29.21
150 ppm	52.28	2.71	70.87	36.15	5.66	50.94	2.18	0.00	6.20	68.47	40.33	28.14
500 ppm	53.96	2.58	72.17	36.86	5.64	52.21	2.28	0.00	6.18	70.71	41.69	29.02
1500 ppm	55.12	2.60	69.68	35.93	4.75	54.05	2.80*	0.02	6.26	72.83	42.37	30.46
4500 ppm	57.58**	3.42**	70.57	36.02	2.73**	55.48	4.52**	0.04	6.23	76.05**	41.95	34.10**
Day 368												
0 ppm	54.50	3.43	68.82	30.92	7.80	54.34	2.96	0.00	6.11	74.30	37.04	37.26
150 ppm	52.81	3.19	67.35	30.94	8.66	55.70	2.78	0.02	6.16	74.07	37.35	36.71
500 ppm	54.10	2.98	69.12	30.99	8.10	54.10	3.13	0.00	6.10	74.90	38.12	36.78
1500 ppm	55.07	2.87	68.18	31.63	6.83	57.71	3.52	0.02	6.38	77.72	39.24*	38.47
4500 ppm	59.78**	3.40	68.07	31.46	2.91**	60.15**	5.13**	0.04	6.18	82.21**	39.63*	42.59**

TABLE 8. Continue

Group	Creat. (µmol/L)	Chol. (mmol/L)	T. Prot. (g/L)	Alb. (g/L)	Trigly. (mmol/L)	Creat. (µmol/L)	Chol. (mmol/L)	T. Bil. (µmol/L)	K (mmol/L)	T. Prot. (g/L)	Alb. (g/L)	Glob. (g/L)
	Males					Females						
Day 557												
0 ppm	51.84	3.82	64.24	28.04	9.01	45.70	3.48	2.70	5.73	67.16	32.38	34.78
150 ppm	47.61	3.38	61.99	27.94	8.09	45.31	3.56	2.66	5.90	66.99	32.80	34.19
500 ppm	50.07	3.50	65.16	28.88	8.38	46.20	3.62	2.97	5.84	68.78	33.69	35.09
1500 ppm	50.44	3.01	62.85	29.03	6.36	49.82*	3.94	3.20	6.20*	71.18*	34.62*	36.56
4500 ppm	54.94	3.61	68.20*	31.02**	4.76*	51.05**	5.56**	3.17	6.05	75.30**	36.10**	39.21**
Day 724												
0 ppm	52.21	4.07	60.89	25.86	5.82	51.37	3.78	1.02	5.56	68.98	31.00	37.99
150 ppm	49.86	4.01	64.43	27.09	7.03	50.13	3.81	0.24**	5.77	68.28	31.71	36.57
500 ppm	52.78	4.06	65.58	27.91	7.19	52.11	3.89	0.55	5.85	70.83	32.92	37.91
1500 ppm	52.44	3.15*	65.00	29.53**	5.81	51.56	4.04	0.40*	6.23**	71.96	33.88**	38.08
4500 ppm	54.63	3.59	70.04**	30.55**	4.32	52.05	5.81**	0.24*	6.13**	73.99	34.27**	39.71

Data taken from Tables 114-133, pages 189-208, MRID No. 432547-01.
*p<0.05; **p<0.01; ANOVA + Dunnett's test (two-sided)
Alb. = albumin , Creat. = creatinine, Chol. = cholesterol, Glob. = globulin, K = potassium, T. Bil. = total bilirubin, T. Prot. = total protein, Trigly. = triglycerides

depressed on day 368 (77%) at 1500 ppm; albumin levels were elevated on day 557 (111%) at 4500 ppm and on day 724 at 1500 (114%) and 4500 ppm (118%); total protein levels were increased on day 724 (115%); triglyceride levels were depressed on days 95 (63%), 186 (54%), 368 (37%), and 557 (53%). Effects in female rats showing statistically ($p \leq 0.05$) and biologically significant ($\geq 10\%$ relative to controls) changes are as follows (only in high-dose animals unless indicated): creatinine levels were elevated on days 95 (110%), 368 (111%), and 557 (112%); total protein levels were elevated on days 95 (115%), 358 (111%), and 557 (112%); globulin levels were elevated on days 95 (122%), 186 (117%), 368 (114%), and 557 (113%); albumin was increased on days 557 (111%) and 724 (111%), total bilirubin decreased on day 724 (24 to 54% at all doses); cholesterol levels were increased on days 95 (205%), 186 (194%), 368 (173%), 557 (160%), and 724 (154%) at 4500 ppm and on day 186 (120%) at 1500 ppm; and potassium increased on day 724 at 1500 (112%) and 4500 ppm (110%).

SGGT, while not a liver specific enzyme was probably elevated due to the combined effects of the test material on the liver (necrosis) and lipid metabolism/storage.

6. Urinalysis*

Urine was collected from all male and female rats on days 89, 180, 362, 551, and 719. The CHECKED (X) parameters were examined.

X			X		
X	Appearance*		X	Glucose*	
	Volume*		X	Ketones*	
	Specific gravity*		X	Bilrubin*	
X	pH		X	Blood pigments 0	
	Sediment (microscopic)*		X	Nitrate	
X	Protein*		X	Urobilinogen	

* Not required for subchronic studies.

Results – The incidence of elevated urobilinogen levels in the urine of male rats receiving 4500 ppm of vinclozolin was significantly increased on day 89 (6/20 vs 1/20 in controls, $p < 0.05$), 180 days (6/20 vs 0/20, $p < 0.01$), and 551 days (7/20 vs 1/17, $p < 0.05$). Only 1/15 males receiving 4500 ppm male had elevated urinary urobilinogen levels at termination. Urinary urobilinogen levels were significantly elevated in females (12/19, $p < 0.01$) receiving 1500 ppm of vinclozolin for 362 days. No other parameters showed statistically significant changes that could be related to dose or duration of treatment.

7. Sacrifice and pathology

All animals that died before termination, sacrificed due to moribundity, or killed on schedule by carbon dioxide asphyxiation were subject to gross pathological examination, and the CHECKED (X) tissues were collected for histological examination. The animals were fasted 16 to 24 h before sacrifice. All gross lesions and all tissues from control and 4500-ppm groups were examined histopathologically. In addition the all gross lesions, lungs, liver, kidneys, adrenal glands, testes (and accessory organs), ovaries, musculature, and eyes from 150-, 500-, and 1500-ppm groups were examined histopathologically. The (XX) organs were weighed.

X		X		X	
Digestive system		Cardiovasc./Hemat.		Neurologic	
	Tongue	X	Aorta*	XX	Brain*+
X	Salivary glands*	X	Heart*	X	Periph. 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 n.)*
X	Jejunum*	X	Thymus*	Glandular	
X	Ileum*	Urogenital		X	Adrenal gland*
X	Cecum	XX	Kidneys*+	X	Lacrimal gland
X	Colon*	X	Urinary bladder*	X	Mammary gland*
X	Rectum*	XX	Testes*+	X	Parathyroids*
XX	Liver*+	XX	Epididymides	X	Thyroids*
	Gall Bladder*	X	Prostate	Other	
X	Pancreas*	X	Seminal vesicle	X	Bone*
Respiratory		X	Ovaries*+	X	Skeletal muscle*
X	Trachea*	X	Uterus*	X	Skin*
X	Lung*	X	Vagina	X	All gross lesions and
	Nose				
	Pharynx				
	Larynx				

* Required for chronic studies.
+ Organ weight required for chronic studies.

Results -

a. Organ weight - Organ weight data are summarized in Table 9. In male rats receiving 4500 ppm of the test material, the mean absolute weight of the liver (133% of the control weight) and adrenal gland (252%) were significantly ($p<0.01$) increased and the brain weight ($p<0.01$) was decreased. The

corresponding relative weights were significantly ($p<0.01$) increased for liver (197%), kidney (137%), brain (134%), and adrenal gland (393%) in high-dose males. The relative kidney weight (117%) was also increased ($p<0.01$) in males receiving 1500 ppm. The absolute (143-253%) and relative weights (130-355%) of the testes was increased at all dose levels, but statistical significance was achieved only for the relative weight in the high-dose animals. There were no statistically significant effects on organ weights in male rats receiving 150 or 500 ppm of the test material. The effect on kidney and brain weight may have been due to the decreased terminal body weights. The increased absolute weight of the liver, testes, and adrenal glands appear to be treatment-related and not due to changes in body weight; the increased relative weights may have been due in part to decreased body weight.

In female rats receiving 4500 ppm, absolute weights of the liver (140% of the control weight) and adrenal gland (135%) were also elevated ($p<0.01$), and the absolute brain weight (90%) was decreased ($p<0.01$). Relative weights of the same organs (liver, 203%; adrenal gland, 139%; brain, 130%) and the kidney (145%) were elevated at 4500 ppm. In females receiving 1500 ppm, the relative weights of the liver (120%), kidney (123%), and brain (112%) were significantly increased ($p<0.01$). At 500 ppm, the absolute weight of the kidney (114%) was increased ($P<0.05$). There were no other statistically significant effects on organ weights at 500 ppm and none at 150 ppm. As noted in male rats, the relative weights may have been due in part to decreased terminal body weights, but the increased absolute weights of the liver and adrenal gland appear to be treatment-related.

TABLE 9. ABSOLUTE (g) AND RELATIVE (%) ORGAN WEIGHTS IN MALE AND FEMALE RATS FED VINCLOZOLIN FOR 104 WEEKS

Organ	Dietary concentration (ppm)				
	0 ppm	150 ppm	500 ppm	1500 ppm	4500 ppm
Males					
Liver (g)	18.7 ± 2.54[a] 2.9 ± 0.34[b]	19.2 ± 2.77 2.7 ± 0.35	19.3 ± 2.38 2.9 ± 0.37	17.8 ± 2.52 3.2 ± 0.28	24.8 ± 4.35** 5.7 ± 0.92**
Kidneys (g)	3.8 ± 0.47 0.60 ± 0.10	4.1 ± 0.42 0.58 ± 0.08	4.2 ± 0.46 0.62 ± 0.08	3.9 ± 0.46 0.70 ± 0.07**	3.5 ± 0.63 0.82 ± 0.15**
Testes (g)	4.9 ± 2.25 0.77 ± 0.42	7.0 ± 4.9 1.0 ± 0.70	12.4 ± 14.81 1.80 ± 2.30	11.5 ± 6.13 2.00 ± 1.0	11.6 ± 4.87 2.73 ± 1.28**
Brain (g)	2.2 ± 0.08 0.35 ± 0.05	2.3 ± 0.07 0.33 ± 0.04	2.3 ± 0.11 0.35 ± 0.06	2.2 ± 0.08 0.39 ± 0.05	2.0 ± 0.08** 0.47 ± 0.05**
Adrenal glands (mg)	99.8 ± 17.1 0.015 ± 0.003	99.8 ± 18.3 0.014 ± 0.003	135.9 ± 114.4 0.021 ± 0.021	127.1 ± 33.5 0.023 ± 0.005	251.1 ± 65.1** 0.059 ± 0.018**
Females					
Liver (g)	12.6 ± 1.92 3.0 ± 0.22	12.9 ± 1.64 3.1 ± 0.44	14.2 ± 4.19 3.4 ± 0.67	12.7 ± 2.44 3.6 ± 0.37**	17.7 ± 2.07** 6.1 ± 0.88**
Kidney (g)	2.9 ± 0.32 0.69 ± 0.09	2.9 ± 0.35 0.72 ± 0.12	3.3 ± 0.41* 0.80 ± 0.15	3.0 ± 0.42 0.85 ± 0.12**	2.9 ± 0.21 1.00 ± 0.11**
Brain (g)	2.1 ± 0.11 0.50 ± 0.08	2.0 ± 0.1 0.50 ± 0.07	2.1 ± 0.1 0.51 ± 0.07	2.0 ± 0.1** 0.56 ± 0.08**	1.9 ± 0.1** 0.65 ± 0.04**
Adrenal gland (mg)	167.8 ± 57.0 0.04 ± 0.014	134.1 ± 34.2 0.032 ± 0.007	152.2 ± 43.7 0.037 ± 0.011	142.1 ± 43.0 0.04 ± 0.011	226.3 ± 40.6** 0.078 ± 0.015**

Data taken from pp. 1120-1123, MRID No. 432547-01.
[a]Absolute weights ± standard deviation.
[b]Relative organ weights (% of terminal body weight) ± standard deviation.
*P<0.05; **p<0.01; Dunnett's test (two-sided)

b. Gross pathology - Notable gross lesions are summarized in Table 10. Several gross lesions showed treatment-related increases in incidences in both male and female rats. Neither sex fed vinclozolin at 150 ppm showed statistically significant increases in the incidence of gross lesions. Statistically significant ($p \leq 0.05$ or $p \geq 0.01$) increases in the incidences were seen at one or more doses for the following lesions in male and/or female rats: liver masses, lung focus, hyperemia of the iliac lymph nodes, enlarged or discolored adrenal glands, cataracts in the eyes, testicular enlargement or masses, reduced size of the epididymides, seminal vesicles, and prostate, and ovarian masses. The liver and lung lesions occurred at significantly increased incidences only at the highest doses, but the lesions were seen in both sexes. Enlarged adrenal glands was a prominent lesion in males fed 4500 ppm and in females fed 1500 ppm and 4500 ppm; it occurred bilaterally in all animals at the two highest doses except for one female rat. The incidence of cataracts showed a dose-related increase in both sexes, with statistical significance being achieved at the two highest doses. Additionally, this lesion occurred bilaterally in all females and most of the males receiving the two highest doses. The gross observation of cataracts upon necropsy coincides with detection of this lesion by ophthalmic examination; however, all lesions detected by ophthalmoscopic examination were not confirmed in all individual animals during gross examination. The incidences of testicular enlargement or masses and reduced sizes of accessory organs in male rats showed dose-related increases, which achieved statistical significance at the two highest doses. In addition, the incidences of testicular masses and reduced size of the epididymis were increased in animals receiving 500 ppm of vinclozolin. In female rats, ovarian masses showed a dose-related increase in incidence (statistically significant at 4500 ppm), but the significantly increased incidences of ovarian cysts only at 500 ppm and enlarged uterus only at 1500 ppm indicate that these lesions were not treatment related. Female rats also showed a significant decrease in the incidence of pituitary masses at the two highest doses.

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TABLE 10. SELECTED GROSS PATHOLOGIC LESIONS IN RATS FED VINCLOZOLIN FOR 104 WEEKS

Organ/Lesions	Dietary concentration (ppm)				
	0 ppm	150 ppm	500 ppm	1500 ppm	4500 ppm
Number of animals in each group	20	20	20	20	20
Males					
Liver Mass	1[a]	0	2	2	7*
Lungs Focus	3	0	5	8	12**
Iliac lymph nodes Hyperemia	3	7	8	10*	10*
Adrenal glands Enlarged (unilateral/bilateral) Discoloration	1 0	0 1	1 4*	4 10**	16** 19**
Eyes cataracts (bilateral)	0	0	3	7**	14**
Testes Enlarged, unilateral Enlarged, bilateral Mass, unilateral/bilateral	5 0 8	4 3 13	10 4 17**	10 8** 19**	5 11** 20**
Epididymides Reduced size	0	2	5*	9**	13**
Seminal vesicles Reduced size	4	5	5	13**	16**
Prostate Reduced size	1	3	4	12**	16**
Females					
Liver Focus	8	8	6	6	15**
Lungs Focus	5	3	4	6	12*
Adrenal glands Enlarged (unilateral/bilateral) Discoloration	5 0	1 0	6 6*	15** 16**	17** 18**
Eyes Cataracts (bilateral)	0	0	0	7*	20**
Pituitary Mass	18	14	17	9	9
Ovary Cyst Mass, unilateral/bilateral	3 1	2 1	11** 2	6 4	0 6*
Uterus Enlarged	2	7	7	8*	5

Data taken from text Tables 1-6, pages 1085-1088 and from pages 1124-1131; MRID No. 432547-01.
[a]Number of animals having a lesion.
* $p \leq 0.05$, ** $p \leq 0.01$, [Fisher exact tests conducted by reviewer (Number Cruncher Statistical System, Version 5.03)].

c. Microscopic pathology -

1) Non-neoplastic - The incidences of nonneoplastic lesions in the liver, lungs, pancreas, skeletal muscle, eyes, and adrenal gland were elevated in male and female rats fed vinclozolin. In addition, lesions in kidney, testes, and accessory sex organs showed increased incidences in male rats as did ovarian lesions in female rats. The incidences of nonneoplastic lesions are summarized in Tables 11 and 12.

The liver lesions in male and female rats consisted of cellular hypertrophy, single cell necrosis, altered foci, and bile duct proliferations. The incidences of cellular hypertrophy, which occurred in the centrilobular region of the liver, and single cell necrosis were significantly elevated in the 1500- and 4500-ppm dose groups of both sexes compared with the corresponding control groups. The severity of the lesions also increased with dose; the only cases of the "severe" form of cellular hypertrophy and "moderate" necrosis occurred in the animals receiving 4500 ppm of the test material and one female control. Among the different types of altered foci, the eosinophilic foci showed a significantly elevated incidence at the two highest doses in both male and female rats. Eosinophilic foci did not occur in control rats of either sex; one female each fed 150 or 500 ppm of the test material developed the lesion. The incidence of basophilic foci was decreased at 4500 ppm in females, and the incidence of clear cell foci was decreased in both sexes at 4500 ppm. The incidence of bile duct proliferation showed only a marginally significant increase ($p=0.06$) in incidence in male rats receiving 4500 ppm. The average severity rating for this lesion decreased with dose. Vinclozolin did not result in a significant effect on the bile duct in female rats. The lack of a dose-response relationship for either incidence or severity in male rats and the lack of an effect in female rats suggest that bile duct proliferation is not a treatment-related lesion.
The incidence of foam cell aggregates in the lungs of male rats did not show a clear dose-response relationship, but the incidence was significantly elevated in high-dose male rats. A dose-related effect was observed in female rats. The average severity of the lung lesion increased with dose in male rats but not in female rats. Nevertheless, the lesion appear to be treatment-related in both sexes. According to the study author, foam cell aggregates roughly corresponded to some of the gross lesions described as a "focus."

The incidence of urothelial hyperplasia (renal pelvis) increased in a dose-related manner in male rats; the severity of this lesion showed only a modest increase in animals receiving 4500 ppm compared with controls. The number of male rats with calcification of the renal pelvis increased significantly at 4500 ppm, but there was no increase in severity. The study author did not consider the renal lesions to be treatment related, but the incidence shows a strong dose-related trend ($p<0.001$, Cochran-Armitage trend test conducted by the reviewer). This lesion is commonly associated with severe spontaneous

nephropathy (Boorman, Eustis, Elwell, Montgomery, and MacKenzie, 1990.

TABLE 11. INCIDENCE OF NONNEOPLASTIC MICROSCOPIC LESIONS IN MALE RATS FED VINCLOZOLIN FOR 104 WEEKS

Organ/Lesions	Dietary concentration (ppm)				
	0	150	500	1500	4500
Liver					
Cellular hypertrophy	1/20 (1.0)[a]	1/20 (3.0)	2/20 (2.0)	17/20** (1.82)	20/20** (2.95)
Single cell necrosis	1/20 (2.0)	2/20 (1.5)	3/20 (2.0)	7/20* (1.71)	20/20** (2.25)
Altered foci	15/20	15/20	17/20	15/20	16/20
Eosinophilic foci	0/20	0/20	1/20	9/20**	15/20**
Clear cell foci	11/20	12/20	13/20	12/20	2/20**
Bile duct proliferation	13/20 (2.38)	11/20 (1.91)	14/20** (1.90)	8/20 (1.63)	18/20 (1.83)
Lungs					
Foam cell aggregates	10/20 (1.40)	7/20 (1.57)	15/20 (1.60)	14/20 (1.71)	16/20* (2.19)
Kidneys					
Urothelial hyperplasia (U/B)	5/20 (2.0)	8/20 (1.75)	11/20* (1.82)	13/20** (1.83)	16/20** (2.50)
Bilateral	2/20	3/20	3/20	9/20*	15/20**
Calcification, renal pelvis (U/B)	9/20 (1.78)	7/20 (1.86)	12/20 (1.33)	12/20 (1.92)	17/20** (1.47)
Bilateral	2/20	3/20	3/20	5/20	9/20
Pancreas					
Vacuolated acinar cells	8/20 (1.38)	13/20 (1.08)	16/20** (2.06)	19/20 **(3.58)	20/20** (3.40)
Exocrine atrophy	1/20	9/20 **	8/20**	3/20	2/20
Skeletal muscle					
Focal fiber atrophy	3/20 (2.0)	5/20 (1.80)	5/20 (2.0)	6/20 (1.33)	16/20** (2.38)
Heart					
Myocardial fibrosis	18/20	4/9	4/6	3/6	2/20**
Eyes					
Degeneration of lens (U/B)	1/20 (1)	4/20 (2.5)	19/20 **(2.05)	19/20** (3.21)	20/20** (3.85)
Bilateral	0/20	4/20*	14/20**	19/20**	19/20**
Lenticular calcification (U/B)	0/20 (0)	0/20 (0)	1/20 (2)	7/20** (2.29)	18/20** (2.61)
Bilateral	0/20	0/20	0/20	4/20*	14/20**
Serous fluid accumulation	0/20	0/20	3/20	3/20	5/20**
Adrenal Gland (cortex)					
Lipidosis	0/20 (0)	0/20 (0)	5/20* (2.20)	14/20** (2.57)	20/20** (4.05)
Lipogenic pigment	18/20 (1.78)	18/20 (1.94)	20/20 (2.15)	18/20 (1.78)	20/20 (2.70)
Extracortical nodules (U/B)	11/20	9/20	15/20	14/20	20/20**
Bilateral	3/20	2/20	5/20	5/20	14/20**
Pituitary					
Focal hyperplasia	11/20	4/11	4/17*	0/13**	5/20*
Diffuse hyperplasia	0/20	1/11	7/17**	7/13**	7/20**

TABLE 11. Continued

Organ/Lesions	Dietary concentration (ppm)				
	0	150	500	1500	4500
Testes					
Interstitial edema	13/20	12/20	7/20	2/20**	1/20**
Focal tubular atrophy	14/20	9/20	8/20	2/20**	2/20**
Diffuse tubular atrophy	5/20	7/20	14/20*	18/20**	20/20**
Tubular calcification	5/20	12/20*	17/20**	15/20**	18/20**
Cystic rete testis	0/20	0/20	0/20	2/20	13/20**
Hyperplastic rete testis	0/20	0/20	0/20	0/20	4/20*
Focal Leydig cell hyperplasia	16/20	13/20	17/20	10/20*	9/20*
Epididymis					
Atrophy	3/20	6/20	8/20	17/20**	20/20**
Azoospermia/oligospermia	6/20	8/20	15/20**	18/20**	20/20**
Granuloma	0/20	0/20	0/20	1/20	4/20*
Epithelial vacuolization	1/20	2/20	4/20	7/20*	1/20
Seminal Vesicle					
Atrophy	2/20 (3.0)	2/20 (2.67)	1/18 (4.0)	11/20** (3.27)	16/18** (3.75)
Coagulation Gland					
Atrophy	1/20 (2.0)	2/20 (3.5)	1/18 (5.0)	11/20** (3.36)	16/18** (3.88)
Prostate					
Reduced secretion	1/20 (2.0)	1/20 (3.0)	6/18* (2.58)	9/20** (2.78)	17/18** (3.41)
Interstitial fibrosis	0/20 (0.0)	5/20* (2.0)	7/18** (2.0)	11/20** (2.36)	13/18** (3.0)
Hyperplasia, NOS	2/20	2/20	3/18	8/20*	4/18
Acinar concretion	17/20 (2.35)	15/20 (2.60)	17/18 (2.47)	18/20 (2.39)	4/18** (2.00)

Data taken from Text Tables 7, 10, 11, 12, 13, 14, 15, 17, 18, 22, 23, 25, 27, and 28 (pages 1089-1109) and the pathology report on pages 1143-1151; MRID No. 432547-01. [a]Number of animals having a lesion/number of animals examined; numbers in parentheses are the average severity grade: 1 = minimum, 2 = slight, 3 = moderate, 4 = severe, and 5 = extreme (or grades 1, 2, 3, 4, and 5).
* $p \leq 0.05$, ** $p < 0.01$[Fisher exact tests, calculated by the reviewer (Number Cruncher Statistical System, Version 5.03)]
NOS = not otherwise specified. U/B = unilateral/bilateral

TABLE 12. INCIDENCE OF NONNEOPLASTIC MICROSCOPIC LESIONS IN FEMALE RATS FED VINCLOZOLIN FOR 104 WEEKS

Organ/Lesions	Dietary concentration (ppm)				
	0	150	500	1500	4500
Liver					
Cellular hypertrophy	1/20 (4.0)[a]	0/20 (0)	0/20 (0)	17/20 **(1.94)	19/20** (2.79)
Single cell necrosis	1/20 (2.0)	0/20 (0)	2/20 (2.0)	6/20* (1.50)	16/20** (1.69)
Altered foci	12/20	8/20	8/20	12/20	14/20
Eosinophilic foci	0/20	1/20	1/20	4/20*	14/20**
Basophilic foci	9/20	6/20	6/20	4/20	3/20*
Clear cell Foci	6/20	3/20	4/20	7/20	0/20**
Bile duct proliferation	15/20 (1.53)	11/20 (1.82)	12/20 (1.75)	13/20 (1.46)	15/20 (1.80)
Lungs					
Foam cell aggregates	6/20 (1.50)	5/20 (1.20)	8/20 (1.25)	12/20 (1.50)	16/20** (1.69)
Pneumonitis	1/20	2/20	4/20	4/20	6/20*
Kidney					
Interstitial nephritis	10/20	12/20	11/20	8/20	4/20*
Pancreas					
Vacuolated acinar cells	4/20 (1.25)	6/20 (1.17)	16/20** (1.94)	20/20** (3.30)	20/20** (2.30)
Skeletal muscle					
Focal fiber atrophy	0/20 (0)	0/20 (0)	0/20 (0)	0/20 (0)	7/20** (2.0)
Heart					
Myocardial fibrosis	16/20	5/5	4/5	0/3*	6/20**
Eyes					
Degeneration of lens (U/B)	3/20 (2.33)	4/20 (2.25)	20/20** (2.60)	19/20** (3.58)	20/20** (4.10)
Bilateral	1/20	2/20	18/20**	19/20**	20/20**
Lenticular calcification (U/B)	0/20	1/20 (1.0)	7/20** (1.71)	17/20** (2.35)	17/20** (2.88)
Bilateral	0/20	1/20	2/20	11/20**	11/20**
Serous fluid accumulation	2/20	1/20	3/20	2/20	9/20*
Adrenal Gland					
Cystic degeneration	18/20	15/20	12/19*	4/20**	1/20**
Lipidosis	0/20 (0)	1/20 (2.0)	2/19 (2.0)	20/20** (3.25)	20/20** (4.20)
Lipogenic pigment	20/20 (2.40)	20/20 (2.70)	19/19 (2.26)	20/20 (2.70)	20/20 (3.55)
Extracortical nodules (U/B)	8/20	6/20	10/19	11/20	18/20
Bilateral	1/20	0/20	2/19	3/20	7/20*
Mammary Gland					
Glandular cysts	17/20	5/9	9/10	2/9**	6/18**
Ovaries					
Interstitial cell lipidosis	1/20 (2.0)	15/20** (2.33)	19/20** (2.42)	20/20** (3.00)	20/20** (3.95)
Stromal hyperplasia	17/20 (2.47)	19/20 (2.53)	20/20 (2.75)	20/20 (2.75)	20/20 (3.35)
Uterus					
Cyst(s)	5/20	4/13	10/17**	2/13	5/20
Cervix					
Fibrosis	2/20	5/12*	9/13**	8/11**	1/20

Data taken from Text Tables 7, 8, 14, 15, 17, 18, 19, 22, 27, and 28 (pages 1089-1109) and the pathology report on pages 1152-1160; MRID No. 432547-01.

[a]Number of animals having a lesion/number of animals examined; numbers in parentheses are the average severity grade: 1 = minimum, 2 = slight, 3 = moderate, 4 = severe, and 5 = extreme (or grades 1, 2, 3, 4, and 5).

* $p \leq 0.05$, ** $p < 0.01$ [Fisher exact tests, calculated by the reviewer (Number Cruncher Statistical System, Version 5.03)]

U/B = unilateral/bilateral

Pathology of the Fisher Rat, pp. 140). There appeared to be no association between urothelial hyperplasia (5/20 vs 16/20, control vs 4500 ppm) and chronic nephropathy (11/20 vs 7/20, control vs 4500 ppm), which showed a decreased incidence at 4500 ppm. Urothelial hyperplasia occurred in all female control rats and in almost all treated rats, and calcification of the renal pelvis occurred in 16 or more female rats in each group including controls (results not presented in Table 12). The results suggest that urothelial hyperplasia is not a treatment-related lesion in females. The incidence of interstitial nephritis, however, showed a dose-related decrease, which was significant at 4500 ppm in female rats.

The number of male and female rats with vacuolated acinar cells in the pancreas and the severity of the lesion increased in a dose-related manner. In the control groups, 8 males and 4 females developed the lesion compared with all male and females rats receiving 4500 ppm and 19 males and all females receiving 1500 ppm. The lesions were minimal to slight in the control and 150-ppm groups, whereas in males and females fed $\geq$500 ppm, the lesions were moderate and/or severe. Exocrine atrophy was seen at a high incidence in male rats receiving 150 and 500 ppm; the lack of a dose-response relationship makes it difficult to attribute this lesion to treatment. The incidence of exocrine atrophy was not elevated in female rats.

Skeletal muscle atrophy (focal) was another lesion showing a significantly increased incidence in male rats receiving the highest dose (16/20) compared with controls (3/20). There was no notable increase in the incidence at the other doses. The severity of the lesion in animals receiving 4500 ppm was also increased, but only slightly. This lesion was seen in the thigh muscle, but not in the muscles taken with the spinal cord segments. In female rats, focal atrophy of the skeletal muscle was seen in seven animals receiving 4500 ppm of the test material and in none of the controls or other dose groups. Myocardial fibrosis occurred in significantly fewer male and female rats receiving 4500 ppm and in females receiving 1500 ppm.

Treatment-related adrenal cortical lesions were induced in male rats receiving $\geq$500 ppm and in female rats receiving 1500 and 4500 ppm of vinclozolin. The incidence and severity of lipidosis (accumulation of lipids in the cytoplasm of enlarged cortical cells) increased with dose in both male and female rats. Lipidosis accounted for discoloration and enlargement of the adrenal glands observed during necropsy (Table 8). The occurrence of lipogenic pigment in the adrenal cortex was found in almost all animals in each dose group including controls; this lesion was more severe in both sexes receiving 4500 ppm than in the corresponding control group. Although no male controls and only one female control developed the severest form of this lesions, 4 males and 11 females fed 4500 ppm of vinclozolin developed the severest form. The incidence (unilateral/bilateral combined) of extracortical nodules (clusters of cortical cells extending through the fibrous capsule into the periadrenal fat) also showed a dose-related increase in both sexes. The increased incidence of bilateral nodules in both sexes compared with their respective controls (3 vs 14, control vs 4500-ppm males; 1 vs 7, control vs 4500-ppm females) suggests that this lesions is treatment related. These nodules may be precursors to adrenal cortical neoplasms. Cystic

degeneration of the adrenal cortex, which occurred in 90% of the female controls, occurred in significantly fewer females fed doses ≥500 ppm. In male rats, the decreased incidence of cystic degeneration did not show a clear dose-response or achieve statistical significance (data not presented in the table). Focal hyperplasia in the adrenal cortex was observed in both sexes (control and treated), but the effect was not treatment-related (data not presented in the tables).

The eyes exhibited a pronounced effect due to exposure to vinclozolin. Cataracts were detected by ophthalmoscopic examination and during necropsy. Microscopic lesions were described as lens degeneration, lenticular calcification (mineralization) within the degenerated lens fibers, and accumulation of serous fluid in the anterior chamber of the eyes. The incidences and severity of lens degeneration and calcification were increased significantly in females at doses ≥500 ppm compared with controls. In male rats, the incidences were increased significantly at doses ≥500 ppm for lens degeneration and at ≥1500 ppm for lens calcification. In male rats receiving 150 ppm of vinclozolin, four showed evidence of lens degeneration in both eyes, whereas none of the controls showed evidence of lens degeneration in both eyes. If only bilateral lens degeneration is compared, the incidence in 150-ppm males is significantly elevated compared with controls (0/20 vs 4/20, $p=0.05$). Therefore, the effect at 150 ppm is considered to be treatment related. It should be noted that only 3 animals of a total of 120 (males and females combined) receiving doses ≥500 ppm showed no evidence of lens degeneration. The effect on serous fluid accumulation was not as pronounced as other effects on the eyes; the only significant increase occurred in male and female rats fed 4500 ppm of the test material.

Vinclozolin had pronounced toxic effects on the testes, epididymis, and accessory sex organs in male rats compared with controls. The incidence of diffuse (or subtotal) tubular atrophy was dose-related, and statistical significance was achieved at doses ≥500 ppm. Calcification accompanied the tubular atrophy. The incidence of this lesion did not show a clear dose-response relationship, but was statistically significant in all treated groups compared with controls. The study author noted that the rete testis was not found microscopically in all animals. Nevertheless, cystic ducts were observed in 2 rats receiving 1500 ppm and 13 receiving 4500 ppm; hyperplasia in the rete testis was observed in 4 rats receiving 4500 ppm. The incidences of interstitial edema and focal Leydig cell hyperplasia occurred with dose-related decreases. Focal Leydig hyperplasia was probably related to the development of Leydig cell neoplasms. All the testicular lesions are considered to be treatment related.

Lesions in the epididymis and accessory organs accompanied the effects in the testes. Generally, these lesions showed dose-response relationships and are considered to be treatment related. Epididymal effects showing significantly increased incidences compared with controls and dose-response relationships consisted of atrophy (1500 and 4500 ppm), azoospermia/oligospermia (≥500 ppm), and granuloma (4500 ppm). The incidence of epithelial vacuolization was significantly increased at 1500 ppm but not at other doses. Atrophy of the seminal vesicle and coagulation gland occurred with significantly increased incidences at 1500 and 4500 ppm. Prostate lesions

occurred with significantly increased incidences at all doses (interstitial fibrosis), at doses ≥500 ppm (reduced secretion), or at 1500 ppm only (hyperplasia). The incidence of acinar concretions in the prostate was significantly decreased at 4500 ppm, but not at other doses.

In female rats, the incidence of ovarian interstitial cell lipidosis was dose related and statistically significant at all doses tested compared with control animals. The severity of the lesion also increased with dose. Stromal hyperplasia occurred at a high incidence at all doses including controls. This lesion did show a dose-related increase in severity, which achieved statistical significance ($p < 0.01$) at the highest dose. Uterine cysts occurred in 10/17 females receiving 1500 ppm compared with only 5/20 controls; no significant increase in the incidence of this lesion occurred at other doses. The incidence of cervical fibrosis was significantly increased at 150, 500, and 1500 ppm but not at 4500 ppm (1/20 vs 2/20 for controls). The lack of clear dose-response relationships suggest that the lesions in the uterus and cervix are not treatment related.

Diffuse hyperplasia of the pituitary lesions occurred with a significantly increased incidence in male rats receiving doses of 500 ppm or more, whereas focal hyperplasia occurred with significantly decreased incidences at the same doses. The latter lesion showed no clear dose-response relationship. Vinclozolin had no notable effect on nonneoplastic lesions in the pituitary of female rats. Females did show a decrease in the incidence of glandular cysts in the mammary gland, which achieved statistical significance at the two highest doses.

2) Neoplastic – The incidence of notable neoplastic lesions are summarized in Table 13. Although the background incidence of Leydig cell tumors in the testes of male rats was very high (55%), treatment with vinclozolin caused a dose-related increase in the number of animals developing Leydig cell tumors. Statistical significance was achieved for doses ≥500 ppm. These tumors occurred bilaterally in almost all treated animals compared with an approximate equal distribution between unilateral and bilateral in control animals. They were benign except for one malignant tumor occurring unilaterally in the 4500-ppm group. Hepatocellular carcinomas also developed in nine male rats fed 4500 ppm and in one male fed 1500 ppm; no hepatocellular carcinomas developed in controls or the other treated groups. In female rats, hepatocellular carcinomas developed in one animal fed 150 ppm and in one fed 4500 ppm of vinclozolin. The total number of male rats with tumors or benign tumors was similar for all groups; the total with malignant tumors was significantly elevated in the 4500-ppm group compared with the control.

In female rats, adrenal cortical tumors developed in six animals fed 4500 ppm and in one fed 1500 ppm of the test material. These tumors occurred unilaterally and

TABLE 13. NEOPLASTIC LESIONS IN MALE AND FEMALE RATS FED VINCLOZOLIN FOR 104 WEEKS

Organ/Lesions	Dietary concentration (ppm)				
	0	150	500	1500	4500
Males					
Testes					
Leydig cell tumors/benign	11/20[a]	12/20	17/20*	19/20**	20/20**
/malignant	0/20	0/20	0/20	1/20	1/20
Liver					
Hepatocellular carcinoma	0/20	0/20	1/20	1/20	9/20**
Total animals with tumors	20/20	17/20	19/20	20/20	20/20
Total animals with benign tumors	19/21	16/20	19/20	19/20	20/20
Total animals with malignant tumors	3/20	5/20	3/20	6/20	13/20**
Females					
Adrenal cortex					
cortical adenoma/carcinoma	0/20	0/20	0/19	1/20	6/20**
cortical carcinoma	0/20	0/20	0/19	1/20	1/20
Ovaries					
Benign sex cord tumor (U/B)	0/20	0/20	2/20	4/20*	10/20**
Benign luteoma	0/20	0/20	0/20	1/20	0/20
Granulosa cell tumor	4/20	3/20	2/20	2/20	1/20
Total ovarian tumors	4/20	3/20	4/20	7/20	11/20*
Pituitary					
Adenoma	14/20	12/20	12/20	6/18*	5/20**
Carcinoma	0/20	0/20	0/20	1/20	1/20
Mammary gland					
Fibroadenoma	5/20	5/9	3/10	6/9	1/18
Total animals with tumors	19/20	19/20	16/20	16/20	20/20
Total animals with benign tumors	19/20	17/20	14/20*	14/20*	17/20
Total animals with malignant tumors	2/20	9/20*	3/20	3/20	9/20*

Data taken from Text Tables 9, 16, 18, 20, 30, and 31 (pages 1091, 1098, 1100, 1102, 1111, and 1112) and the pathology report on pages 1143-1160; MRID No. 432547-01.
[a]Number of animals showing a lesion/number of animals examined.
*$p \leq 0.05$, ** $p \leq 0.01$ [Fisher exact tests, calculated by the reviewer (Number Cruncher Statistical System, Version 5.03)]

were benign except for one tumor in each affected group. There was no increase in the incidence of adrenal cortical tumors in male rats; one control developed an adenoma. Benign sex cord tumors developed in the ovaries of two females fed 500 ppm, four fed 1500 ppm and ten fed 4500 ppm; none occurred in controls or the 150-ppm group. The

total incidence of ovarian tumors was significantly increased at 4500 ppm. The incidences of pituitary adenomas (statistically significant at 1500 and 4500 ppm) and mammary fibroadenomas (nonsignificant at 4500 ppm) were decreased in female rats. The decrease for fibroadenomas did not achieve statistical significance at any dose and there was no clear dose-response relationship. In females, the overall tumor incidence was similar in treated groups and controls; significant increases in the incidences of malignant tumors were noted for the 150- and 4500-ppm groups; the benign tumor incidence was significantly decreased only in the 500- and 1500-ppm groups compared with controls.

D. DISCUSSION

Groups of 20 male and 20 female Wistar rats were given vinclozolin in their diet at concentrations of 0, 150, 500, 1500, or 4500 ppm continuously for 2 years. Calculated doses reported by the study author were 0, 7, 23, 71, and 221 mg/kg/day, respectively, for male rats and 0, 9, 29, 88, and 257 mg/kg/day, respectively, for female rats. Vinclozolin had no statistically significant effect on survival in either male or female rats. Body weights were markedly reduced in males and females fed 4500 (33 and 30%, respectively) and moderately reduced at 1500 ppm (13 and 16%, respectively). Body weight gain was reduced by about 45% in both sexes receiving 4500 ppm and 18 and 24%, respectively, in males and females receiving 1500 ppm. Food consumption was significantly reduced throughout the study in both males and females fed 4500 ppm and at some time points in animals fed 1500 ppm; the reduced food consumption probably contributed in part to the reduced body weight gain during the study. The overall relative efficiency of food utilization from week 52 to 102 in males and females showed a biologically significant dose related decrease at 1500 and 4500 ppm. In control, 1500 and 4500 ppm, respectively, the values for males are 4.1, -0.03 and -3.3 and for females the values are 6.9, 4.5 and -0.2. It should be noted that the decrement in food efficiency becomes clear only when the overall efficiencies were average over weeks 0-102 and weeks 52-102. The increase in water consumption (g/kg-rat) was most pronounced the last year of the last year of the study. This water consumption decreased or increased over control consumption in males and females depending on the time period included, but the overall all effect was an increase in water consumption. It appears that with prolonged exposure to the anti-androgen, vinclozolin, some homeostatic mechanisms fail and results in organ systems showing tumors and other histopathology at several dose levels and especially at the highest dose level. In several instances, there were dose related decreases in histological findings. A similar phenomena was noted in the 18-month study in mice (MRID# 43254704). The mechanism for these decreases is unknown, but a perturbing normal hormonal androgen levels may be involved.

Several hematologic and clinical chemistry changes were noted. The hematologic changes appeared to be either unrelated to treatment or biologically insignificant. The study authors attributed many of the clinical chemistry changes to induction of microsomal enzymes by the study authors, but some changes appeared to be indicative of liver toxicity (increased glutamyl transferase, cholesterol, albumin, total protein, and globulin). The clinical chemistry changes indicative of liver toxicity were confirmed by statistically significant increased absolute and relative weight of the liver in both sexes, gross lesions,

and microscopic findings (hepatocellular hypertrophy and necrosis and eosinophilic foci). The serum levels of ALT, AST, and ALP were depressed; there is no known cause for the decreases in these enzymes. The increase in serum creatinine levels may have been due to muscle atrophy, which showed an increased incidence and severity in male and female rats fed 4500 ppm. Urine urobilinogen levels in the urine of male rats were elevated throughout treatment also suggesting a toxic effect on the liver, possibly the increased incidence of bile duct proliferation. No other treatment-related changes in urine parameters were noted.

Vinclozolin administered orally to male and female rats had a distinctive and pronounced effects on the eyes manifested by different types of lenticular lesions or abnormalities. Abnormalities detected by ophthalmoscopic examination consisted of cataracts, bosselated structures, bulbiform thickening, and focal opacities. Cataracts were detected in all male and female rats receiving 1500 and 4500 ppm that survived to termination. Bosselated structures and bulbiform thickening were seen at high incidences during the early part of the study, but could not be detected during the later stages because of the formation of cataracts. The study author did not know the pathognomonic relevance of bosselated structures; he stated, however, that they were not related to formation of cataracts. The author further noted that bulbiform thickening and focal opacities were somehow related to and "clearly" indicative of the formation of cataracts. The eye lesions observed during necropsy were described only as cataracts; however, not all cataracts detected during ophthalmoscopic examination were detected during necropsy. Cataracts were described microscopically as lenticular degeneration (degeneration of the lenticular fibers). This lesion was observed in all groups including controls and in more animals than indicated by ophthalmoscopic or gross examination. It occurred bilaterally in one control female and in none of the control males. Even with considering only the bilateral lesions, a treatment-related effect is observed at all doses in male rats; therefore, a no-observed-effect level (NOEL) cannot be established for this lesion in males. The other microscopic lesion of the eye was lenticular calcification (mineral deposition within the degenerated lenticular fibers), which showed a significantly increased incidence at the two highest doses in both sexes and a dose-related increase in severity. It appears that lenticular toxicity was due to the systemic route, because there was no evidence of a direct effect due to dust or particles from the feed getting into the eyes. Further, the effects may be species specific, because the eyes of male and female C57Bl/6 mice fed vinclozolin at concentrations 15, 150, 3000, or 8000 ppm were not affected (MRID No. 432547-04).

According to the study author, vinclozolin binds competitively with the androgen receptor and thus has antiandrogenic activity. The effects on the testes, epididymis, and sex accessory organs can be attributed to its antiandrogenic properties. At necropsy, the testes appeared to be enlarged, whereas the epididymis and accessory organs appeared to be smaller than normal. Microscopically, the testes showed evidence of seminiferous tubular atrophy and calcification; concomitantly, the epididymis shows evidence of atrophy and either pronounced reduction or complete absence of spermatozoa. In addition, the seminal vesicles and coagulation gland atrophied and the prostate degenerated (reduced secretion and fibrosis). The enlargement of the testes was probably due to over stimulation by luteinizing hormone in response to the blocked androgen negative feedback, and atrophy of

the epididymis and accessory organs was probably due to the lack of androgenic stimulation. The incidences of seminiferous tubular calcification and fibrosis in the prostate are significantly increased in male rats at doses of vinclozolin including the lowest (150 ppm). These results show effects at the lowest dose level tested in male rats. Because vinclozolin competitively binds to the androgen receptor, it probably has antianabolic activity. The increased incidence of muscle atrophy observed at 4500 ppm in male and female rats could be due to antianabolic activity of the test material. Additionally, part of the reduced body weight gain may be due to this activity.

Other organs showing treatment-related effects include the lungs (males and females), kidneys (males), pancreas (males and females), adrenal cortex (males and females), and ovaries (females). Lipidosis in the adrenal cortex (Boorman, Eustis, Elwell, Montgomery, and MacKenzie, 1990. Pathology of the Fisher Rat, pp. 516) and ovary may be associated with inhibition of steroidogenesis, which in turn may be due to the hormone antagonistic effect of vinclozolin. The extracortical nodules in the adrenal gland are preneoplastic lesions, which the study author classified as a neoplasm depending on its size. The incidence and severity of lipidosis in the ovarian interstitial cells was dose-related; statistical significance was achieved at the lowest dose; therefore, a NOEL cannot be established for this lesion in female rats. Stromal hyperplasia of the ovary was a common lesion in female rats including controls, but the degree of hyperplasia increased with dose of vinclozolin. Foam cell aggregates (lungs) are also common lesions, particularly in male rats, associated with the gross lesions described as "focus." This lesion may be identical to alveolar histiocytosis or alveolar lipidosis (clusters of lipid-containing alveolar macrophages) that accumulate lipid due to decreased removal or catabolism of phospholipids (Boorman, Eustis, Elwell, Montgomery, and MacKenzie, 1990. Pathology of the Fisher Rat, pp. 346). Vacuolation of acinar cells in the pancreas is probably a degenerative lesion; it occurred in male and female rats receiving $\geq$500 ppm of the test material. There was no gross lesion associated with the microscopic lesion in the pancreas.

Male rats developed hyperplasia of the renal urothelium (epithelium of the renal pelvis); the lesion was predominately bilateral, the incidence showed a clear dose-response relationship, and the severity was slightly elevated at the highest dose. In some strains of rats, urothelial hyperplasia appears to be associated with severe spontaneous nephropathy (Boorman, Eustis, Elwell, Montgomery, and MacKenzie, 1990. Pathology of the Fisher Rat, pp. 140). In this study, the incidence of nephropathy decreased with dose (11/20 vs 7/20, control vs 4500 ppm), and there was no dose-related effect on severity, suggesting that nephropathy was not associated with urothelial hyperplasia. Therefore, the strong dose-response relationship and no obvious association with nephropathy suggest that urothelial hyperplasia is a treatment-related effect.

Some lesions occurring with significantly decreased incidences included hepatic clear cell (males and females) and basophilic foci (females), interstitial nephritis (females), cystic degeneration of the adrenal cortex (females), myocardial fibrosis (males and females), glandular cysts in mammary tissue (females), focal hyperplasia of the pituitary (males), interstitial edema in the testes (males), and acinar concretions in the prostate gland

(males). The spontaneous development of these lesions in a large number of controls is probably due to age-related effects; their decreased occurrence in treated groups is probably due to alteration in hormonal status (antiandrogenic activity of vinclozolin) for endocrine and reproductive organs or to other unknown causes for the remaining organs. There was no significant life shortening resulting from treatment with vinclozolin, so the decreased incidences cannot be attributed to reduced numbers of animals at risk during the late stage of the study. The marked reductions in weight gain or possible antianabolic effects of vinclozolin may have contributed to the lowered incidences. It should also be noted that the reduced incidences of these lesions are not of the magnitude or biological significance to attribute a beneficial effect to vinclozolin.

In conclusion, nonneoplastic treatment-related effects were observed at all doses in both male and female rats fed vinclozolin at concentration of 150, 500, 1500, and 4500 ppm. Therefore, a NOEL cannot be established for either sex. A second study using lower doses was conducted to establish a NOEL (MRID 432547-02). The microscopic examination findings in the epididymides, seminal vesicles, coagulating glands, Leydig cell tumors/hyperplasia and reduced prostate secretion and possibly interstitial fibrosis of the prostate are related to decreased androgen stimulation in these organs/tissues. These findings indicate a functional deficit which probably relates to a lack of androgen stimulation caused by the inhibition of the androgen receptors by vinclozolin/metabolite/degradation products. The currently routinely used analytical methods for LH levels may not be as sensitive to perturbation as these functional deficits. The malignancies in Leydig cells at high dose levels may be due to excess proliferation and stimulation of these cells in the 2-year study. The adrenal gland lipidosis in males and females and the interstitial cell lipidosis of ovaries are probably due to the effect of vinclozolin on aspects of lipid/cholesterol metabolism/storage. Perhaps even the eye cataracts/opacities and other lens degeneration may be indirectly with effects of vinclozolin on lipid/cholesterol metabolism/storage.

Evaluation of the neoplastic lesions revealed that male rats developed a significantly ($p<0.01$) elevated incidence of Leydig (interstitial) cell tumors in the testes at 500, 1500, and 4500 ppm. The antiandrogenic activity of vinclozolin probably contributed to the development of the Leydig cell tumors, i.e. resulting in stimulation of testicular interstitial cell proliferation by luteinizing hormone. Statistically significant interstitial fibrosis of the prostate especially at $\geq$ 1500 ppm may be involved in the slight elevation of prostate hyperplasia at these dose levels. The tumors occurred at a high incidence in control rats; they were all benign except for one; and they usually occurred bilaterally. Hepatocellular carcinomas occurred at a significantly ($p<0.01$) elevated incidence in male rats fed 4500 ppm of vinclozolin; no adenomas were identified. There was a corresponding increase in the incidence of altered foci (preneoplastic lesion). In female rats, adrenal cortical tumors (mostly adenomas) occurred at the highest dose (4500 ppm), and sex-cord benign tumors occurred at a significantly increased incidence 1500 and 4500 ppm. The adrenal tumors were almost all unilateral and one-half the ovarian tumors at 4500 ppm were unilateral. In addition, there was a significant increase in preneoplastic adrenal cortical lesions in female rats. Although the data showed a clear carcinogenic response at 4500 ppm, the maximum tolerated dose (MTD) was also clearly exceeded in male and female rats at this dose as

evidenced by a pronounced depression in body weight gain and toxicity affecting multiple organs. Hepatocellular carcinogenesis at the highest dose may be due in part to a toxic response in the liver; however, the development of malignant tumors as opposed to benign tumors (adenomas) and the absence of treatment-related hyperplasia suggest the carcinogenic activity was not due to liver toxicity. Effects related to the antiandrogenicity of vinclozolin cannot be ruled out. Regarding the testicular tumors, the incidence was significantly increased at doses below the MTD; however, an indirect effect due to stimulation of cell proliferation by luteinizing hormone cannot be ruled out. In female rats, the increased incidence of adrenal tumors occurred only at the dose exceeding MTD; the increased incidence of sex cord tumors occurred at the MTD and at the dose exceeding the MTD. These tumors were mostly benign, and they occurred in organs that are targets for pituitary hormones suggesting that the carcinogenic response is related to a hormone imbalance rather than to a direct effect of the test material.

E. STUDY DEFICIENCIES

This study showed toxicity at the lowest dose in both male and female rats; this deficiency was corrected by conducting a supplementary study (MRID No. 43254702) using lower doses. Incidence data for gross and microscopic lesions were not analyzed statistically.

APPENDIX

Contained in this appendix are:

1. A copy of the Executive Summary for MRID# 43254701 from the contractor, 3 pages.
2. A copy of the methods used in statistical analyses by the registrant (MRID# 43254701, pages 44,45 and 1083.
3. Copies of Tables of the findings at ophthalmoscopic examination from the submitted report (MRID# 43254701, pages 53-56).

Below is the original executive summary written by the contractor. The current executive summary at the beginning of the DER was shortened and modified by the David G Anderson

EXECUTIVE SUMMARY: In a chronic toxicity study, groups of 20 male and 20 female Wistar rats were administered 0, 150, 500, 1500, or 4500 ppm (0, 7, 23, 71, or 221 mg/kg/day, respectively, for males and 0, 9, 29, 88, or 257 mg/kg/day, respectively, for females) of vinclozolin in their diets for 104 weeks.

Survival was not significantly affected in either male or female rats fed vinclozolin. Growth was markedly reduced in both sexes fed the test material. During the second year of treatment with 4500 ppm, male rats weighed 17-33% less than corresponding controls, and females weighed 14-30% less than controls. Total body weight gain was reduced by about 45% in both sexes receiving 4500 ppm, due in part to reduced food consumption (5-17% in males and 6-24% in females). In females receiving 1500 ppm, the total body weight gain was 76.1% of the control value ($p < 0.01$), whereas the males gained 82.2% as much weight as corresponding controls (N.S.). Food efficiency fluctuated considerably, but was generally reduced in both sexes receiving 4500 ppm compared with controls.

Increases in absolute (143-253%) and relative testes weights (130-355%) occurred at all doses, but statistical significance was achieved only for the relative weight in the male rats fed 4500 ppm. Statistically significant increases occurring in the relative kidney weights at 1500 and 4500 ppm and in relative brain weights also at 1500 (females only) and 4500 ppm were probably due to decreased body weight. In male and female rats, adrenal toxicity accompanied the weight change of the organ.

Vinclozolin affected multiple targets including the eyes, testes and male accessory organs, ovaries, adrenal gland, liver, lungs, pancreas, kidneys (males only), and skeletal muscle in both sexes. The lesions discussed below are considered to be treatment-related. Incidences or numbers of animals developing lesions are listed in the following order: control, 150, 500, 1500, and 4500 ppm; the asterisk (*) denotes a statistically significant difference ($p \leq 0.05$ or $p \leq 0.01$) compared with controls.

Ophthalmoscopic examination showed the eyes to be affected by treatment with vinclozolin as early as day 87 of treatment. At various times during treatment bilateral lesions such as cataracts, bosselated structures in the lens, bulbiform thickening of the lens, and focal opacity were seen in both sexes receiving doses of 500 ppm or more. The lesions detected by ophthalmoscopic examination were described as cataracts (bilateral) during gross examination of the same treated group (0, 0, 3, 7*, 14* for males and 0, 0, 0, 7*, 20* for females). The incidence of the corresponding microscopic lesion (lenticular degeneration, bilateral occurrence) was significantly elevated in males at all doses (0/20, 4/20*, 14/20*, 19/20*, and 19/20*) and in females at doses $\geq$ 500 ppm (1/20, 2/10, 18/20*, 19/20*, 20/20*). In addition, increased incidences were observed for lenticular calcification in males (0/20, 0/20, 0/20, 4/20*, 14/20*), females (0/20, 1/20, 2/20, 11/20*, 11/20*) and for serous fluid accumulation in the anterior chamber of the eyes in males (0/20, 0/20, 3/20, 3/20, 8/20*) and females (2/20, 1/20, 3/20, 2/20, and 9/20*). There was no evidence that the eye lesions were due to dust or particles

from the feed.

The testes showed a dose-related increase in relative weight ($p < 0.01$ at 4500 ppm) and a corresponding increase in the incidence of enlargement (bilateral, $p < 0.01$, 1500 and 4500 ppm) was noted during necropsy. Microscopically, several lesions were observed to have significantly increased incidences, notably tubular calcification (5/20, 12/20*, 17/20*, 15/20*, 18/20*) and diffuse tubular atrophy (5/20, 7/20, 14/20*, 18/20*, 20/20*). There was a significant decrease in the incidence of focal Leydig cell hyperplasia at 1500 (10/20) and 4500 ppm (9/20) compared with controls (16/20). Cysts (0/20, 0/20, 0/20, 2/20, 13/20*) and hyperplasia (0/20, 0/20, 0/20, 0/20, 4/20*) was observed in the rete testes. Accompanying the testicular lesions were statistically significant increases in the incidences of gross and microscopic lesions of the epididymis (notably reduced size or atrophy and azoospermia/oligospermia at doses ≥500 ppm), seminal vesicle (reduced size or atrophy at doses ≥1500 ppm), coagulation gland (atrophy at doses ≥1500 ppm), and prostate (reduced size or reduced secretion at doses ≥500 ppm). In addition, interstitial fibrosis was a notable lesion in the prostate, showing a dose-related increase in incidence (0/20, 5/20*, 7/20*, 11/20*, 13/20*) and severity. In female rats, a statistically significant increase in the incidence of interstitial lipidosis in the ovaries occurred at all doses (1/20, 15/20*, 19/20*, 20/20*, 20/20*). There was also a dose-related increase in the severity of this lesion. Vinclozolin has antiandrogenic activities; therefore, the lesions in the testes, male accessory organs, and ovaries may be related to a hormonal imbalance due to excessive stimulation by luteinizing hormone (LH) or by inhibition of steroidogenesis.

Other organs showing dose-related and statistically significant increased incidences of lesions at one or more doses are the liver of both sexes (cellular hypertrophy, single cell necrosis, and eosinophilic foci (1500 and 4500 ppm), kidney of male rats (urothelial hyperplasia, ≥500 ppm; renal pelvis calcification, 4500 ppm), lungs of both sexes (foam cell aggregates, 4500 ppm), pancreas of both sexes (vacuolated acinar cells, ≥500 ppm), skeletal muscle of both sexes (focal fiber atrophy, 4500 ppm), and adrenal gland of both sexes (lipidosis, ≥500 ppm for males and ≥1500 ppm for females, extracortical nodules, 4500 ppm).

In addition to the microscopic lesions, statistically ($p \leq 0.05$) and biologically significant (≥10% change compared with controls) changes occurred in serum levels of γ-glutamyltransferase activity (increased up to 14-fold in males and 9-fold in females at 4500 ppm), creatinine (up to 112% of control levels, both sexes), cholesterol (up to 146% in males and up to 205% in females), total bilirubin (up to 54%, females), albumin (up to 118%), total protein (up to 115%), and globulin (up to 112%, females). Serum alkaline phosphatase, aspartate aminotransferase, and alanine aminotransferase activities were depressed by 15 to 34% in male and female rats receiving 4500 ppm of vinclozolin; the cause was unknown.

Incidences of lesions showing significant decreases were clear cell foci in the liver (male and female, 4500 ppm), basophilic foci in the liver (females, 4500 ppm), interstitial nephritis (females, 4500 ppm), cystic degeneration of the adrenal cortex (females, ≥500

ppm), myocardial fibrosis (male, 4500 ppm; females, ≥1500 ppm), glandular cysts in mammary tissue (females, ≥1500 ppm), focal hyperplasia of the pituitary (males, ≥500 ppm), interstitial edema in the testes (males, ≥1500 ppm), and acinar concretions in the prostate (males, 4500 ppm).

The LOEL for systemic toxicity is 150 ppm (7 mg/kg/day for males and 9 mg/kg/day for females) based on bilateral lenticular degeneration of the eyes, seminiferous tubular calcification in the testes, and interstitial fibrosis in the prostate of male rats and interstitial cell lipidosis in the ovaries of female rats. There is no corresponding NOEL, because the lowest dose tested is the LOEL.

This study showed some evidence of carcinogenicity probably involving a hormonal imbalance. Leydig cell tumors (mostly benign) occurred in male rats at incidences of 11/10, 12/20, 17/20*, 19/20*, and 20/20* and hepatocellular carcinomas at incidences of 0/20, 0/20, 1/20, 1/20, 9/20* (controls, 150, 500, 1500, and 4500 ppm, respectively). In addition, the total number of 4500-ppm male rats with malignant tumors at any site (13/20 vs 3/20) was significantly increased. Females had significantly increased incidences of adrenal cortical tumors (0/20, 0/20, 0/20, 1/20, 6/20*), benign sex cord tumors in the ovaries (0/20, 0/20, 2/20, 4/20*, 10/20*), and all ovarian tumors combined (4/20, 3/20, 4/20, 7/20, 11/20*). The incidences of pituitary adenomas in females rats (14/20, 12/20, 12/20, 6/18*, 5/20**) and mammary fibroadenomas (5/20, 5/9, 3/10, 6/9, 1/18) were decreased. The development of the Leydig cell tumors may be due indirectly to the antiandrogenic activity of vinclozolin, which disrupts the feedback mechanism for luteinizing hormone (LH), resulting in overstimulation of the Leydig cells by LH and not to a direct effect of the test material on the testes. The development of adrenal cortical and ovarian tumors may also be related to a hormonal imbalance. The maximum tolerated dose was clearly exceeded in animals receiving 4500 ppm as evidenced by marked reduction in growth and induction of numerous nonneoplastic lesions. However, the development of Leydig cell, adrenal cortical, and ovarian tumors is probably not due to the excessive toxicity, but to a hormonal imbalance. Except for one male rat in the 1500-ppm group, hepatocellular carcinomas developed only in male rats fed 4500 ppm. The lack of adenomas either accompanying or preceding the hepatocellular carcinomas suggests that the development of hepatocellular carcinomas is a direct effect of vinclozolin and not an indirect effect of hepatocellular toxicity. However, the induction of hepatocellular carcinomas was not confirmed in the carcinogenicity study (MRID No. 432547-03) where male rats fed 3000 ppm for 104 weeks did not develop hepatocellular carcinomas at a significantly increased incidence.

This study and the supplementary study using lower doses (MRID No.432547-02) combined receives a classification of **core - minimum**, and they satisfy the guideline requirements for a chronic rodent feeding study (83-1). The classification for this study alone is supplementary upgradable. Toxicity was observed at all doses; therefore, a NOEL could not be established from this study alone. This deficiency was corrected when the supplementary study was conducted using lower doses (MRID No. 432547-02). All pertinent endpoints were evaluated; however, the study author did not summarize all data satisfactorily as incidence data and severity ratings were not analyzed statistically.

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3.9. PATHOLOGY

For description of the methods used see separate PATHOLOGY REPORT. (Vol. III)

3.10. STATISTICAL EVALUATION

The statistical evaluation of the data was carried out on the computing systems of the Department of Toxicology (Dr. H.D. Hoffmann responsible).

3.10.1. Clinical examinations

Means and standard deviation for the variables food consumption, water consumption, body weight, food efficiency and test substance intake were calculated for the animals of each test group. They were printed out in the summary and individual value tables, with the exception that for food efficiency and test substance intake only summary tables were prepared.

For the parameter body weight a parametric one-way analysis of variance was done via the F-test (ANOVA) (2). If the resulting p-value was equal or less than 0.05, a comparison of each dose group with the control group was carried out. These comparisons were performed simultaneously via Dunnett's test (3, 4) for the hypothesis of equal means. If the results of this test were significant, labels (* for $p \leq 0.05$, ** for $p \leq 0.01$) were printed together with the group mean in the tables. Both tests were performed two-sided.

3.10.2. Clinical chemistry and hematology

Means and standard deviations have been calculated for each test group and tabulated together with the individual values. In order to test if the results of the individual dose groups differed statistically significantly from the results of the control group, the means for the dose groups, excepting the differential blood count, were compared with those for the control group using the analysis of variance (ANOVA (2) and DUNNETT's test (3, 4)).

Significances resulting from the statistical comparison have been indicated in the tables on means.

3.10.3. Urinalyses

With the exception of volume, color and turbidity the scale for the urine parameters is divided into 4 sections (0, 1, 2, 3).
For the parameter "Nitrite" only a division in two sections (0, 1) is made.

The parameters, which were recorded in 4 sections, were sorted into 2 classes. This was done for the statistical analysis.

A pairwise comparison of each dose group with the control was carried out using Fisher's exact test [6] for the hypothesis of equal proportions. If the results of this test are significant, labels (* for $p \leq 0.05$, ** for $p \leq 0.01$) were printed in the tables.

3.11. RETENTION OF RECORDS

The study protocol, the raw data, the reserve sample and the specimens, as well as the original of this report, are stored at BASF Aktiengesellschaft for at least the period of time specified in the GLP regulations.

The specimens will be retained only as long as the quality of the material allows evaluation.

(Details concerning responsibilities or locations of archiving can be seen from the respective SOP and from the raw data.)

MATERIALS AND METHODS

Statistical Evaluation

The calculations were carried out on the computer systems of the Department of Toxicology (Dr. H.D. Hoffmann responsible).

Mean and standard deviation were calculated for the statistical evaluation of the study for the variables of terminal body weight and of absolute and relative organ weights (related to terminal body weight) of the animals in each test group and tabulated together with the individual values (absolute and relative organ weights).

The statistical evaluation was carried out using the DUNNETT test (1, 2) for a simultaneous comparison of several dose groups with a control group.

If the results of this test are significant, p-markers (* for $p \leq 0.05$, ** for $p \leq 0.01$) were printed together with the group mean in the tables.

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(1) DUNNETT, C.W. (1955):
A multiple comparison procedure for comparing several treatments with a control
J. Amer. Statist. Assoc. 50, 1096-1121

(2) DUNNETT, C.W. (1964):
New tables for multiple comparisons with a control
Biometrics 20, 482-491

Table 4.2.7.1.

BOSSELATED STRUCTURE OF LENS

Males

Day of examinations		94	125	201	293	398	482	566	661	720
		No. of animals with finding/ No. of alive animals examined								
Group 0	one-sided	0/20	0/20	0/20	0/20	1/20	2/20	2/17	2/15	1/13
0 ppm	both sided	0/20	0/20	0/20	0/20	0/20	1/20	1/17	1/15	1/13
Group 1	one-sided	0/20	0/19	0/18	0/18	0/18	0/18	0/17	0/16	0/13
150 ppm	both sided	0/20	0/19	0/18	0/18	0/18	0/18	0/17	0/16	0/13
Group 2	one-sided	0/20	0/20	1/19	0/19	1/19	0/19	1/19	4/19	0/15
500 ppm	both sided	0/20	0/20	0/19	0/19	4/19	4/19	4/19	4/19	0/15
Group 3	one-sided	4/20	6/20	0/20	2/20	0/19	0/19	2/19	0/15	0/14
1,500 ppm	both sided	4/20	2/20	4/20	7/20	8/19	8/19	1/19	1/15	0/14
Group 4	one-sided	1/20	1/20	1/20	2/20	1/20	1/20	0/20	0/19	0/15
4,500 ppm	both sided	13/20	11/20	2/20	2/20	2/20	2/20	0/20	0/19	0/15

Females

Day of examinations		87	119	201	293	398	482	566	661	720
		No. of animals with finding/ No. of alive animals examined								
Group 0	one-sided	0/20	0/20	0/20	1/20	3/20	4/20	4/20	4/17	5/17
0 ppm	both sided	0/20	0/20	0/20	0/20	0/20	0/20	0/20	0/17	0/17
Group 1	one-sided	0/20	0/20	0/20	0/20	0/20	2/20	2/19	1/18	3/16
150 ppm	both sided	0/20	0/20	0/20	0/20	0/20	0/20	0/19	1/18	0/16
Group 2	one-sided	1/20	2/20	3/20	1/20	2/20	3/20	3/19	5/16	2/15
500 ppm	both sided	0/20	1/20	1/20	5/20	7/20	8/20	6/19	2/16	0/15
Group 3	one-sided	2/20	1/20	5/19	3/19	3/19	5/19	0/19	0/18	0/17
1,500 ppm	both sided	10/20	14/20	13/19	9/19	6/19	3/19	1/19	0/18	0/17
Group 4	one-sided	0/20	1/20	0/20	0/19	0/19	0/19	0/18	0/15	0/13
4,500 ppm	both sided	14/20	3/20	1/20	1/19	1/19	1/19	0/18	0/15	0/13

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Table 4.2.7.2.

BULBIFORM THICKENING

Males

Day of examinations		94	125	201	293	398	482	566	661	720
		No. of animals with finding/ No. of alive animals examined								
Group 0	one-sided	0/20	0/20	0/20	0/20	0/20	0/20	0/17	0/15	0/13
0 ppm	both sided	0/20	0/20	0/20	0/20	0/20	0/20	0/17	0/15	0/13
Group 1	one-sided	0/20	0/19	0/18	0/18	0/18	0/18	0/17	0/16	0/13
150 ppm	both sided	0/20	0/19	0/18	0/18	0/18	0/18	0/17	0/16	0/13
Group 2	one-sided	0/20	0/20	0/19	0/19	2/19	2/19	0/19	0/19	0/15
500 ppm	both sided	0/20	0/20	0/19	0/19	1/19	2/19	4/19	5/19	0/15
Group 3	one-sided	0/20	0/20	0/20	1/20	0/19	0/19	2/19	0/15	0/14
1,500 ppm	both sided	0/20	0/20	0/20	10/20	9/19	8/19	1/19	1/15	0/14
Group 4	one-sided	1/20	0/20	0/20	2/20	1/20	1/20	0/20	0/19	0/15
4,500 ppm	both sided	0/20	4/20	14/20	3/20	3/20	3/20	0/20	0/19	0/15

Females

Day of examinations		87	119	201	293	398	482	566	661	720
		No. of animals with finding/ No. of alive animals examined								
Group 0	one-sided	0/20	0/20	0/20	0/20	0/20	0/20	0/20	0/17	0/17
0 ppm	both sided	0/20	0/20	0/20	0/20	0/20	0/20	0/20	0/17	0/17
Group 1	one-sided	0/20	0/20	0/20	0/20	0/20	0/20	0/19	1/18	1/16
150 ppm	both sided	0/20	0/20	0/20	0/20	0/20	0/20	0/19	0/18	0/16
Group 2	one-sided	0/20	0/20	0/20	1/20	0/20	4/20	5/19	5/16	2/15
500 ppm	both sided	0/20	0/20	0/20	5/20	0/20	7/20	5/19	2/16	0/15
Group 3	one-sided	0/20	0/20	0/19	2/19	3/19	5/19	0/19	0/18	0/17
1,500 ppm	both sided	0/20	0/20	0/19	7/19	6/19	3/19	1/19	0/18	0/17
Group 4	one-sided	0/20	1/20	0/20	0/19	0/19	0/19	0/18	0/15	0/13
4,500 ppm	both sided	0/20	8/20	0/20	1/19	1/19	1/19	0/18	0/15	0/13

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Table 4.2.7.3.

OPACITIES

Males

Day of examinations		94	125	201	293	398	482	566	661	720
		No. of animals with finding/ No. of alive animals examined								
Group 0 - 0 ppm	one-sided	1/20	0/20	0/20	0/20	0/20	2/20	6/17	4/15	2/13
	both sided	0/20	0/20	0/20	0/20	0/20	0/20	0/17	1/15	3/13
Group 1 150 ppm	one-sided	0/20	0/19	0/18	0/18	0/18	1/18	2/17	5/16	2/13
	both sided	0/20	0/19	0/18	0/18	0/18	1/18	2/17	1/16	3/13
Group 2 500 ppm	one-sided	0/20	0/20	2/19	0/19	0/19	2/19	6/19	5/19	4/15
	both sided	0/20	0/20	0/19	0/19	1/19	1/19	1/19	0/19	2/15
Group 3 1,500 ppm	one-sided	0/20	0/20	7/20	6/20	2/19	1/19	1/19	1/15	0/14
	both sided	0/20	0/20	1/20	6/20	6/19	7/19	2/19	1/15	0/14
Group 4 4,500 ppm	one-sided	0/20	0/20	0/20	2/20	1/20	1/20	0/20	0/19	0/15
	both sided	0/20	0/20	1/20	4/20	3/20	3/20	0/20	0/19	0/15

Females

Day of examinations		87	119	201	293	398	482	566	661	720
		No. of animals with finding/ No. of alive animals examined								
Group 0 0 ppm	one-sided	0/20	0/20	0/20	1/20	1/20	1/20	1/20	1/17	3/17
	both sided	0/20	0/20	0/20	0/20	1/20	1/20	1/20	0/17	0/17
Group 1 150 ppm	one-sided	0/20	0/20	1/20	1/20	3/20	2/20	1/19	2/18	4/16
	both sided	0/20	0/20	0/20	0/20	0/20	0/20	1/19	1/18	0/16
Group 2 500 ppm	one-sided	0/20	0/20	0/20	3/20	4/20	3/20	4/19	5/16	2/15
	both sided	0/20	0/20	0/20	0/20	6/20	10/20	7/19	1/16	0/15
Group 3 1,500 ppm	one-sided	0/20	0/20	1/19	4/19	3/19	5/19	0/19	0/18	0/17
	both sided	0/20	0/20	0/19	9/19	7/19	4/19	2/19	0/18	0/17
Group 4 4,500 ppm	one-sided	1/20	0/20	0/20	0/19	0/19	0/19	0/18	0/15	0/13
	both sided	0/20	0/20	0/20	1/19	1/19	1/19	0/18	0/15	0/13

Table 4.2.7.4.

CATARACTS

Males

Day of examinations		94	125	201	293	398	482	566	661	720
		No. of animals with finding/ No. of alive animals examined								
Group 0	one-sided	0/20	0/20	0/20	0/20	0/20	0/20	0/17	0/15	0/13
0 ppm	both sided	0/20	0/20	0/20	0/20	0/20	0/20	0/17	0/15	0/13
Group 1	one-sided	0/20	0/19	0/18	0/18	0/18	0/18	0/17	0/16	0/13
150 ppm	both sided	0/20	0/19	0/18	0/18	0/18	0/18	0/17	0/16	0/13
Group 2	one-sided	0/20	0/20	0/19	0/19	1/19	0/19	1/19	3/19	2/15
500 ppm	both sided	0/20	0/20	0/19	0/19	0/19	1/19	1/19	3/19	3/15
Group 3	one-sided	0/20	0/20	0/20	1/20	0/19	1/19	1/19	1/15	0/14
1,500 ppm	both sided	0/20	0/20	0/20	0/20	5/19	6/19	14/19	13/15	14/14
Group 4	one-sided	1/20	2/20	1/20	1/20	2/20	1/20	0/20	0/19	0/15
4,500 ppm	both sided	0/20	0/20	11/20	13/20	15/20	16/20	20/20	19/19	15/15

Females

Day of examinations		87	119	201	293	398	482	566	661	720
		No. of animals with finding/ No. of alive animals examined								
Group 0	one-sided	0/20	0/20	0/20	0/20	0/20	0/20	0/20	0/17	0/17
0 ppm	both sided	0/20	0/20	0/20	0/20	0/20	0/20	0/20	0/17	0/17
Group 1	one-sided	0/20	0/20	0/20	0/20	0/20	0/20	1/19	1/18	2/16
150 ppm	both sided	0/20	0/20	0/20	0/20	0/20	0/20	0/19	0/18	0/16
Group 2	one-sided	0/20	0/20	0/20	0/20	1/20	1/20	2/19	3/16	3/15
500 ppm	both sided	0/20	0/20	0/20	0/20	0/20	0/20	3/19	6/16	9/15
Group 3	one-sided	0/20	0/20	2/19	3/19	3/19	5/19	0/19	0/18	0/17
1,500 ppm	both sided	0/20	0/20	0/19	6/19	9/19	10/19	17/19	18/18	17/17
Group 4	one-sided	0/20	3/20	0/20	0/19	0/19	0/19	0/18	0/15	0/13
4,500 ppm	both sided	5/20	10/20	19/20	18/19	18/19	18/19	18/18	15/15	13/13

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