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DATA EVALUATION REPORT

Reg.83 258 - VINCLOZOLIN

Study Type: ONCOGENICITY FEEDING - RAT (83-2A)

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by

Chemical Hazard Evaluation Group
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Oak Ridge, TN 37831
Task Order No. 94-18C

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Toxicology Branch I (7509C)

DATA EVALUATION REPORT

STUDY TYPE: Carcinogenicity Feeding - Rat; OPPTS 870.3200 (§83-2a)

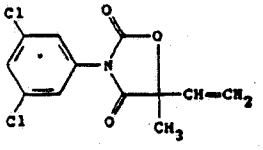
DP Barcode: D 222842
P.C. Code: 113201.
Rereg. Case: 2740

Submission Code: S468567
Tox. Chem. No.: 323C
Case: 819455

TEST MATERIAL (PURITY): Reg. No. 83 258 (Vinclozolin), 99.2% a.i..

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)

STRUCTURE:



STUDY NUMBER: 71S0375/88105; BASF No. 94/10279.

CITATION: Mellert, W. (May 2, 1994) Toxicology Study Report: Carcinogenicity Study With No. 83 258 - Vinclozolin in Wistar Rats Administration in the Diet for 24 Months. Laboratory name BASF Aktiengesellschaft, Department of Toxicology, Ludwigshafen/ Rhein, FRG. MRID# 43254703. Unpublished report to USEPA, OPP.

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

EXECUTIVE SUMMARY: In a carcinogenicity toxicity study (MRID# 43254703), groups of 50 male and 50 female Wistar rats were administered 0, 50, 500, or 3000 ppm (0, 2.3, 23, or 143 mg/kg/day, respectively, for males and 0, 3.0, 30, or 180 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 affected throughout the study in both sexes receiving 3000 ppm; the males weighed 25% less than control and females weighed 17% less than control at termination ($p < 0.01$ for both sexes compared with controls). Net body weight gain was reduced by 34% in males and 27% in females due in part to reduced food consumption (12 to 16% in males and 8 to 16% in females. However, since the relative efficiency of food

utilization was negative in males and was reduced 85% in females at 3000 ppm from study week 52 to 102 or termination, the body weight decrement in males and females was due in part to toxicity and only partly due to the reduced food consumption. These findings are consistent with the body weight decrement and food efficiency calculations seen in the chronic study in rats at 1500 and 4500 ppm (MRID# 43254701).

The absolute and relative weights of the testes were slightly decreased at 50 ppm and significantly increased ($p<0.01$) at 500 ppm (168 and 163%, respectively) and 3000 ppm (188 and 244%, respectively). Absolute and relative weights of the epididymides were depressed at all doses. The adrenal glands weights were elevated at all doses, but were significant ($p<0.01$) at 3000 ppm (191 and 262%, respectively). In females rats, organ weights did not show a clear dose-response or the effects were not biologically or statistically significant, except for the liver weights and the relative weight of the ovaries. The absolute (129%) and relative weights (156%) of the liver were increased ($p<0.01$) at 3000 ppm compared with the control group. The absolute (123 to 156%) and relative weights (121 to 190%) of the ovaries were increased at all three doses compared with the control group, but only relative weight at 3000 ppm was significant ($p<0.01$).

Vinclozolin/metabolite/degradation products competitively binds to the androgen receptor, and has antiandrogenic activity. In addition, vinclozolin interferes with lipid metabolism and/or storage. Lesions in the testes, epididymides, accessory organs, ovary, and adrenal glands are probably related to the antiandrogenicity and the effects of the test material on the lipid metabolism and/or storage. Testicular lesions showing increased incidences included interstitial edema (5/50, 6/50, 7/50, 12/50*), diffuse tubular atrophy (15/50, 14/50, 36/50*, 45/50*), tubular calcification (16/50, 20/50, 37/50*, 42/50*), cystic rete testis (3/50, 5/50, 3/50, 16/50*), and hyperplastic rete testis (1/15, 0/50, 0/50, 13/50*). (The values in parentheses represent the incidence/number of animals studied in control, 50, 500, and 3000 ppm, respectively; the asterisk (*) denotes a statistically significant difference ($p\leq0.05$ or $p\leq0.01$) compared with controls.) There were decreases in the incidence of focal tubular atrophy (37/50, 29/50, 22/50*, 9/50*) and focal Leydig cell hyperplasia (36/50, 39/50, 34/50, 11/50*). Accompanying the testicular lesions were azoospermia and oligospermia in the epididymides (13/50, 14/50, 41/50*, 49/50*), which occurred bilaterally in most 500- and 3000-ppm animals and degenerative lesions in accessory organs. Atrophy was noted in the seminal vesicle (3/50, 5/50, 16/50*, 31/50*) and coagulation gland (3/50, 5/50, 16/50*, 30/50*). Prostate effects included reduced secretion (4/50, 7/50, 12/50*, 21/50*), interstitial fibrosis (4/50, 9/50, 16/50*, 31/50*), and focal hyperplasia (11/50, 17/50, 25/50*, and 20/50*). The decreased prostatic secretion and interstitial cell fibrosis along with increased chronic inflammation, may be secondary to the antiandrogenicity of vinclozolin and the increased incidence of prostate adenoma at 500 and 3000 ppm. In female rats, statistically significant increased incidences of interstitial lipidosis in the ovaries occurred at all doses (2/50, 15/49*, 35/50*, 43/50*). There was also a dose-related increase in the severity of this lesion. Adrenal cortical lesions occurred in both sexes with incidences reaching statistical significance at 500 and 3000 ppm. Lipidosis occurred in males (1/50, 2/50, 33/50*, 50/50*) and females (0/50, 3/50, 12/50*, 50/50*) as did extra cortical nodules (males: 25/50, 32/50, 21/50, 40/50*; females: 19/50, 24/50, 40/50*, 45/50*). This increased

lipid accumulation in ovaries and adrenals may be related to the interference of vinclozolin with lipid metabolism and/or storage. The occurrence of lipogenic pigment did not show an increase in incidence, which was very high in all groups, but an increase in the severity rating at 3000 ppm was noted for both sexes. There was a **decrease** in the incidence of cystic degeneration in the adrenal cortex of female rats (50/50, 48/50, 49/50, 13/50*).

Liver cellular hypertrophy was not seen in either male or female controls, but the incidence was increased in treated male (0/50, 2/50, 12/50*, 44/50*) and female rats (0/50, 0/50, 11/50*, 44/50*). Eosinophilic foci, which were not seen in male control and in only one female control, showed a significantly increased incidence at all doses in males (0/50, 15/50*, 33/50*, 38/50*) and at the high dose in females (1/50, 0/50, 5/50, 38/50*). Basophilic and clear cell foci showed dose related **decreases.** The incidence of biliary cysts was increased in males (4/50, 5/50, 11/50*, 15/50*) and females (8/50, 10/50, 20/50*, 36/50*). Foam cell aggregates in the lungs also showed a significantly increased incidence in male rats at all doses (14/50, 27/50*, 24/50*, 40/50*). The incidence of lenticular degeneration was significantly increased in female rats at all doses (9/50, 23/50*, 49/50*, 50/50*) and at the two highest doses in male rats (11/50, 15/50, 39/50*, 50/50*). This lesion occurred bilaterally in almost all animals receiving 500 and 3000 ppm of the test material. In addition, lenticular calcification (males: 0/50, 1/50, 4/50, 46/50*; females: 0/50, 0/50, 12/50*, 48/50*) occurred in almost all high-dose animals.

Lesions occurred in the pancreas of both sexes (vacuolated acinar cells, 500 and 3000 ppm, skeletal muscle (focal fiber atrophy: 3000 ppm in males, dose-related increases in females), and iliac lymph nodes in males (pigment storage: 500 and 3000 ppm).

Several lesions showed statistically significant **decreased** incidences at 500 and/or 3000 ppm in males and females, some of which were possibly antiandrogen related or of undetermined cause.

The LOEL for systemic toxicity is 50 ppm (2.3 mg/kg/day for males and 3 mg/kg/day for females) based on eosinophilic foci in the liver and foam cell aggregates in the lung of male rats and lenticular degeneration of the eyes 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 (almost all benign) occurred in male rats at incidences of 23/50, 25/50, 47/50*, and 49/50* and prostate adenomas occurred with incidences of 0/50, 3/50, 7/50*, and 5/50* (controls, 50, 500, and 3000 ppm, respectively). There was no increase in the total number of treated male rats developing neoplasms compared with the controls. Adrenal cortical adenomas/carcinomas developed in 1/50, 2/50, 1/50 and 22/50* female rats; benign sex cord tumors (unilateral and bilateral) developed in 4/50, 7/49, 10/50, 29/50*; and adenocarcinomas of the uterus developed in 1/50, 0/39, 1/31, and 7/50*. The adrenal cortical tumor was malignant in one high-dose rat. The

development of the Leydig cell tumors are probably 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 prostate, adrenal cortical, ovarian, and uterine tumors may also be related to a hormonal imbalance and/or the effects of vinclozolin on lipid metabolism/storage. The maximum tolerated dose was exceeded in animals receiving 3000 ppm as evidenced by a significant reduction in growth, decreased relative food efficiency 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.

This study receives a classification of **acceptable**, and it satisfies the guideline requirements for a oncogenicity feeding study in rodents (83-2).

COMPLIANCE: Special Review Criteria (40 CFR 154.7) None. Signed and dated quality assurance and GLP statements were present.

STUDY DEFICIENCIES: Food consumption and incidence data for gross and microscopic lesions were not analyzed statistically; average severity ratings were not calculated.

A. MATERIAL

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

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

2. Vehicle and/or positive control

   The test material was mixed directly with food; no other vehicle was used. A positive control was neither needed nor 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: 191 to 222 g (mean, 205 g); females:133 to 169 g (mean 151 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 light/12 h dark
Acclimation period: 11 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	50	50
1	50	2.3	3	50	50
2	500	23	30	50	50
3	3000	143	180	50	50

Data taken from page 29, MRID No. 432547-03. [a]Time-weighted average daily compound intake was reported by the authors (MRID No. 432547-03, p. 43).

Dose selection rationale: Dose selection was based on six 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 a 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. (5) Three-month study in rats (strain not specified) administered vinclozolin at concentrations of 300, 1000, or 3000 ppm: enlarged and whitish adrenal glands, vacuolization of pancreatic acinar cells at 300 ppm, some clinical pathologic effects, organ weight changes (adrenals, testes, liver, and kidney), microscopic effects in adrenal cortex, pituitary, testes, liver, and pancreas at 1000 and 300 ppm. (6) Clearly reduced body weight in the chronic study (MRID No. 432547-01) indicated that the maximum tolerated dose would be exceeded at 4500 ppm.

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. Storage conditions were not reported. Dietary concentrations of the test material were verified at the start of the study 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 150- and 4500-ppm preparations.

Results –

a. Homogeneity analysis – The 150-ppm samples varied by -5 to -9% of the target concentration and the 4500-ppm samples varied by +2 to +9%. 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 – 50 ppm: mean values for samples taken at study initiation (+1%), 3 months (-12%), 6 months (-16%), 21 months (-12%), and 24 months (-12%); all these were within 10% of the nominal concentration 500 ppm: analysis at initiation varied by -11% of the nominal concentrations; all others were within 10%; 3000 ppm: all samples were within 10% of the nominal concentration.

3. Diet

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

4. Statistics

Mean body weights were analyzed using the F-test (analysis of variance) and Dunnett's test for p-values greater than 0.05 (MRID No. 43254703, page 38). Mean organ weights were analyzed using Dunnett's test (MRID No. 43254703, page 482).

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. A comprehensive examination and palpations were conducted once a week.

Results – The most prominent signs of toxicity were the enlarged testes in 5, 4, 19, and 38 male rats, testes unpalpable in the scrotum in 2, 4, 18, and 16 male rats, and cataracts in 1, 2, 10, and 47 male rats fed 0, 50, 500 and 3000 ppm of the test material. Cataracts were observed in 1, 2, 2, and 49 females fed 0, 50, 500, and 3000 ppm. Piloerection and reduced general state were noted for all male rats receiving 3000 ppm; piloerection was noted in one control and reduced general state in no controls. Survival was not affected by treatment, and there was no significant difference in terminal survival rates in either male or female rats. Survival rates are summarized in Table 2.

2. Body weight

Individual animals were weighed once weekly up to week 14 and once every 4 weeks thereafter and at the end of the study.

Results – Body weights at selected time points and net body weight gain (weeks 0 to 104) are presented in Table 3. A dose-related effect on body weights was observed throughout the study. Both male and female rats receiving 50 or 500 ppm of the test material weighed 1% less to 7% more than the corresponding control group during the study. The body weights of both sexes receiving 3000 ppm were significantly depressed throughout the study. The males weighed 25% less than the controls and females weighed 17% less. Net body weight gain (week 0 to week 104) showed a dose-related decrease that was statistically significant throughout the study in animals receiving 3000 ppm. At termination, the high-dose males had gained 34% less weight than controls and females had gained 27% less. Male and female rats receiving the low dose gained 10 and 7%, respectively, more weight than the corresponding controls; statistical significance was achieved for the low-dose males at week 90 to termination (except week 98).

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

Week	0 ppm	50 ppm	500 ppm	3000 ppm
Males				
Week 26	100[a]	100	100	100
Week 52	96	98	100	100
Week 78	90	94	96	96
Week 91	88	88	88	92
Week 104	76	74	82	82
Females				
Week 26	100	100	100	100
Week 52	98	100	100	98
Week 78	88	94	98	96
Week 91	84	82	94	94
Week 104	76	60	74	84

Data were taken from Tables 75 and 76, pp. 131-132, MRID No. 432547-03. [a]Percent surviving; 50 animals per group at study initiation.

TABLE 3. SELECTED MEAN BODY WEIGHTS (g) OF MALE AND FEMALE RATS FED VINCLOZOLIN FOR 104 WEEKS

Week	Males				Females			
	0	50	500	3000	0	50	500	3000
0	205.7	205.4 (100)[a]	205.0 (100)	204.7 (100)	151.1	151.1 (100)	150.7 (100)	149.2 (99)
1	255.9	255.8 (100)	257.9 (101)	226.3** (88)	175.3	176.7 (101)	178.7 (102)	159.0** (91)
4	356.3	356.6 (100)	359.8 (101)	328.8** (92)	219.7	221.3 (101)	222.6 (101)	210.1** (96)
8	439.1	438.1 (100)	447.5 (102)	391.8** (89)	256.4	258.6 (101)	255.9 (100)	248.7 (97)
13	494.2	495.2 (100)	508.4 (103)	449.6** (91)	282.0	285.3 (101)	281.7 (100)	269.5** (96)
26	572.9	576.3 (101)	592.1 (103)	519.1** (91)	309.5	313.9 (101)	309.4 (100)	292.5** (95)
38	629.5	634.3 (101)	649.2 (103)	555.2** (88)	329.0	332.8 (101)	325.6 (99)	307.2** (93)
50	659.3	669.4 (102)	678.8 (103)	572.7** (87)	332.5	335.5 (101)	330.0 (99)	312.2** (94)
66	689.9	713.8 (103)	713.6 (103)	584.3** (85)	350.3	354.8 (101)	353.8 (101)	323.4** (92)
82	717.1	751.3 (105)	735.0 (102)	578.8** (81)	369.1	378.7 (103)	372.1 (101)	326.6** (88)
98	720.0	764.7 (106)	716.1 (99)	555.3** (77)	388.8	399.3 (103)	391.6 (101)	319.9** (82)
104	709.1	758.1 (107)	705.0 (99)	534.0** (75)	384.0	399.3 (104)	389.1 (101)	319.0** (83)
wt. gain 0-104	503.1[b]	552.9* (110)	499.7 (99)	330.0** (66)	232.5	249.2 (107)	238.8 (103)	170.7** (73)
Wt. gain 0-104	503.4[c]	552.7 (110)	500.0 (99)	329.3 (66)	232.9	248.2 (107)	238.4 (102)	169.8 (73)

Data taken from Tables 011-015, pp. 67-71 and Tables 021-025 pp. 77-81 (males) and Tables 016-020, pp. 72-76 and Tables 026-30, pp. 82-86 (females), MRID No. 432547-03. [a] Numbers in parentheses are percent of control calculated by the reviewer. [b] Body weight gain data taken from Table 025, page 081 for males and Table 030, page 086 for females. [c] Data calculated from the data in Table 3. Since the conclusions are the same using either data, the reason for the limited discrepancy was not investigated further. * $p<0.05$; ** $p<0.01$; ANOVA plus Dunnett's test (two-sided)

3. Food consumption and compound intake

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. The mean relative efficiency of food utilization was calculated on the bases of body weight gain over various periods per mean daily food consumption per kg body weight (as calculated from weekly or monthly averages) over a similar time period multiplied by 100 (Table 4b). 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. Food consumption - Table 4a summarizes the average food consumption at selected times during treatment. The authors did not calculate the total food consumed per animal during the study. There was no significant consistent effect on food consumption in rats fed 50 or 500 ppm of the test material. At the high-dose, however, food consumption showed a significant decrease ($p < 0.01$, student's t-test) throughout the study, with decreases ranging from 12 to 16% in males and 8 to 16% in females during the second year.

b. Compound consumption (time-weighted average) - The average compound intake calculated by the study authors was 2.3, 23, and 143 mg/kg/day for male rats and 3.0, 30, and 180 mg/kg/day for female rats receiving 50, 500, and 3000 ppm, respectively, of test material in the diet.

c. Food efficiency - Daily mean food efficiency, calculated weekly and/or monthly showed no consistent treatment-related patterns, and it did not differ significantly between controls and treated animals of either sex. Food efficiency in all groups showed a general decline during the study. However, one of the reviewers calculated the relative efficiency of food utilization over several time periods and found differences for the last half of the study (Table 4b). A distinct decrease in relative efficiency of food utilization occurred in males (negative) and females (84% less than control) at 3000 ppm from study weeks 52 to termination. Decreases may have been seen in males (39%), but not in females at 500 ppm at week 52-102. Slight decreases to no decreases in efficiency (especially in females) were seen at 3000 ppm in males and females during weeks 0 to 13, 13 to 52 and overall efficiency from weeks 0 to 102. These findings are similar to those seen in the 2-year chronic study in rats (MRID# 43254701), which were calculated on food efficiency per animal rather than per kg rat weight as in Table 4b. The decrease in relative efficiency of food utilization at 3000 ppm in males and females, especially between week 52 and 102, indicate that the body weight decrease was due to toxicity rather than decreased food consumption only and that dose level was adequate to test for carcinogenicity in male and female rats. Since the study was adequate at a dose level of 3000 ppm, the findings at 4500 ppm in the chronic study in rats (MRID# 43254701) were well above the dose levels required for an adequate study.

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

Week	Males				Females			
	0	50	500	3000	0	50	500	3000
1	26.9	26.4	27.6	18.5**	19.6	19.9	20.2*	12.9**
4	27.3	27.7	27.9	26.3**	19.9	20.2	20.3	18.3
8	27.8	28.1	28.3	24.8**	19.7	20.0	19.7	19.2**
13	26.5	26.9	27.3*	25.3**	19.0	19.1	19.0	18.0**
26	25.9	26.4	26.8*	24.6**	18.3	18.6	18.6	17.4**
38	27.1	26.9	27.1	24.1**	18.3	18.7	18.4	17.1**
50	26.9	27.6	27.0	24.0**	19.0	19.0	19.2	17.5**
66	28.5	28.7	27.5*	24.2**	19.9	20.5	20.3	18.7**
82	27.8	28.7	28.1	23.9**	20.6	21.6	20.8	18.1**
98	28.1	28.3	27.7	24.0**	20.8	20.7	20.6	17.4**
104	28.1	28.3	28.0	24.1**	20.2	21.2	20.6	18.1**

Data taken from Tables 001-005, pp. 57-61 (males) and Tables 006-010, pp. 62-66 (females); MRID No. 432547-03.
* $p<0.05$; ** $p<0.01$; Students's t-test calculated by the reviewer.

Table 4b. RELATIVE EFFICIENCY OF FOOD UTILIZATION [(g Change BWt)/(g/kg-rat/day)] BY MALE AND FEMALE RATS FED VINCLOZOLIN FOR 104 WEEKS.

Study weeks	Male				Females			
	0	50	500	3000	0	50	500	3000
0-102	10.6	11.7	10.5	7.00	10.6	10.6	10.6	10.6
0-13	31.5	31.5	32.8	26.5	12.1	11.9	6.92	11.7
13-52	8.11	8.53	8.49	5.90	1.89	1.89	1.80	1.62
52-102	2.55	4.50	1.56	-1.29	1.75	1.95	1.75	0.28

Statistical analysis was not conducted on the data for week 0-102, 0-13, 13-52 and 52-102 in Table 4b. Data was calculated by the reviewer, based on body weights; Tables 011-015, pages 067-071 for males and Tables 016-020, pages 072-076 and food consumption Tables 001-005, pages 057-061 for males and Tables 005-010, pages 060-066, pages 062-066 for females.

4. Ophthalmoscopic examination
The eyes of all males and females in control group and in the lowest dose group (50-ppm) were examined with an ophthalmoscope 1 day before treatment began and at 3-month intervals throughout the study.

Results – No treatment-related abnormalities of the eyes were detected with the ophthalmoscope. Cataracts were detected in one male control (bilateral) and in two 50-ppm males (one bilateral and one unilateral). In females, cataracts were detected in one control (unilateral) and in three 50-ppm females (bilateral in one and unilateral in two).

5. Blood smears were prepared for differential white cell counts following decapitation. Smears were prepared from animals killed because of moribundity and from all control and high-dose animals surviving to termination. The CHECKED (X) parameters were examined.

a. Differential white cell count*

X	
X	Leukocyte count (WBC)
	Eosinophils
X	Basophils
X	Band Cells
X	Polymorphonuclear neutrophils
X	Lymphocytes
X	Monocytes

*Required for oncogenicity studies.

Results – No treatment-related effect on differential blood counts were seen in either male or female rats receiving 3000 ppm of the test material compared with the corresponding controls. The morphology of the white and red blood cells was not affected by treatment with vinclozolin.

b. Clinical chemistry – Evaluation of clinical chemistry parameters is not required for oncogenicity studies (83-2).

6. Urinalysis – Oncogenicity test guidelines (83-2) do not require analysis of urine samples.

7. Sacrifice and pathology

All animals that died before termination, sacrificed due to moribundity, or killed on schedule by decapitation under carbon dioxide anesthesia were subject to gross pathological examination, and the CHECKED (X) tissues were collected for histological examination. All gross lesions and all tissues from control and the 3000-ppm groups

were examined microscopically. In addition all gross lesions, lungs, liver, pancreas, kidneys, adrenal glands, testes (and accessory organs), ovaries, musculature, bone marrow (femur), and eyes from the 50- and 500-ppm groups were also examined microscopically. The (XX) organs were weighed. Organs with obvious masses (liver in males, adrenal gland in both sexes and ovaries in females) were excluded from calculations of mean organ weights.

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	eye (optic n.)*
X	Jejunum*	X	Thymus*	Glandular	
X	Ileum*	Urogenital		X	Adrenal gland*
X	Cecum	X	Kidneys*+		Lacrimal gland
X	Colon*	X	Urinary bladder*	X	Mammary gland*
X	Rectum*	X	Testes*+	X	Parathyroids*
XX	Liver*+	X	Epididymides	X	Thyroids*
X	Pancreas*	X	Prostate	Other	
Respiratory		X	Seminal vesicle	X	Bone*
X	Trachea*	X	Ovaries*+	X	Skeletal muscle*
X	Lung*	X	Uterus*	X	Skin*
	Nose	X	Vagina	X	All gross lesions and
	Pharynx				
	Larynx				

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

Results -

a. Organ weight - Mean organ weight data are summarized in Table 5. In male rats, feeding of vinclozolin did not affect the mean absolute liver weight; the mean relative weight was significantly ($p<0.01$) elevated (141%) at 3000 ppm, but unaffected at the other doses. The absolute and relative weights of the testes were slightly decreased at 50 ppm and significantly increased ($p<0.01$) at 500 ppm (168 and 163%, respectively) and 3000 ppm (188 and 244%, respectively). Absolute and relative weights of the epididymides were depressed at all doses; statistical significance ($p<0.01$) was achieved at 500 ppm (66 and 68%, respectively) and 3000 ppm (45 and 63%, respectively). The adrenal glands weights were elevated at all doses, with statistical significance being achieved at 3000 ppm (191 and 262%, respectively). Absolute weights of the kidney (90%) and brain (93%) were significantly depressed at the high dose, whereas the relative weights were significantly elevated (123 and 156%, for kidney and brain weights, respectively). The effects on the testes, epididymides, and adrenal glands weights are considered to be treatment-related. The magnitude of the effect on the absolute kidney and brain weights is too small to be considered biologically significant. The increased

relative liver, kidneys, and brain weights are probably due to depressed body weights and not to treatment with vinclozolin.

In female rats, organ weights did not show a clear dose-response or the effects were not biologically or statistically significant, except for the liver weights and the relative weights of the ovary. The absolute (129%) and relative weights (156%) of the liver were significantly ($p < 0.01$) increased at 3000 ppm compared with the control group; the effects appear to be dose-related. The absolute (123 to 154%) and relative weights

TABLE 5. ABSOLUTE AND RELATIVE (%) ORGAN WEIGHTS IN MALE AND FEMALE RATS FED VINCLOZOLIN FOR 104 WEEKS

Organ	Dietary concentration (ppm)			
	0 ppm	50 ppm	500 ppm	3000 ppm
Males				
Liver (g)	19.9 ± 2.87[a] 2.9 ± 0.41[b]	20.4 ± 2.86 2.9 ± 0.46	19.4 ± 2.67 2.87 ± 0.34	20.6 ± 2.19 4.1 ± 0.46**
Kidneys (g)	4.2 ± 0.41 0.62±0.09	4.1±0.36 0.57±0.06*	4.09±0.42 0.61±0.1	3.8±0.42** 0.76±0.11**
Testes (g)	5.9±4.29 0.91±0.77	5.5±3.54 0.77±0.55	9.9±7.25** 1.48±1.06**	11.1±4.34** 2.22±0.86**
Epididymides (g)	1.29±0.20 0.19±0.04	1.24±0.19 0.17±0.03	0.85±0.24** 0.13±0.04**	0.58±0.16** 0.12±0.03**
Brain (g)	2.29±0.11 0.34±0.05	2.29±0.08 0.32±0.03	2.26±0.09 0.34±0.05	2.14±0.096** 0.43±0.05**
Adrenal glands (mg)	88.8±19.3 0.013±0.003	93.3±16.8 0.013±0.003	105.6±39.6 0.016±0.009	169.4±51.7** 0.034±0.013**
Females				
Liver (g)	11.6±1.79 3.2±0.34	11.56±2.03 3.1±0.37	12.1±1.49 3.3±0.40	15.0±2.19** 5.0±0.65**
Kidneys (g)	2.7±0.27 0.75±0.11	2.7±0.28 0.74±0.10	2.9±0.25* 0.80±0.12	2.7±0.24 0.92±0.11**
Ovaries (g)	0.139±0.161 0.039±0.047	0.192±0.196 0.053±0.057	0.171±0.136 0.047±0.037	0.217±0.160 0.074±0.058**
Brain (g)	2.04±0.07 0.57±0.08	2.02±0.07 0.55±0.08	2.02±0.08 0.56±0.08	1.87±0.09** 0.63±0.07*
Adrenal glands (mg)	192.1±76.0 0.054±0.02	180.5±139.0 0.05±0.042	133.0±62.2* 0.036±0.014	225.8±129.3 0.077±0.046**

Data taken from pp. 528-531, MRID No. 432547-03. [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)

(121 to 190%) of the ovaries were increased at all three doses compared with the control group, but statistical significance was achieved only for relative weight at 3000 ppm. The absolute adrenal glands weight was decreased at 50 (94% of control, N.S.) and 500 ppm (69% of control, $p<0.05$) and increased at 3000 ppm (117% of control, N.S.); the relative weights showed a corresponding decrease at 500 ppm (67% of control, N.S.) and an increase at 3000 ppm (143%, $p<0.01$). The absolute kidney weight showed an increase at 500 ppm (107% of control, $p<0.05$) that was not biologically significant and no effect at 3000 ppm; the relative weight showed a statistically significant ($p<0.01$) increase at 3000 ppm (123% of control). Mean brain weights were affected only in rats receiving 3000 ppm, wherein the absolute weight was decreased (92%, $p<0.01$) and the relative weight was increased (111%, $p<0.05$) compared with the control group.

b. Gross pathology – The incidences of notable gross lesions are summarized in Tables 6 (male rats) and 7 (female rats). Treatment-related gross lesions were observed in the liver, lungs, adrenal glands, and eyes of both sexes; the pituitary, testes, epididymides, seminal vesicles, and prostate of males; and the ovaries of females. In addition, the lymph nodes (iliac) in male rats also appear to be a target for the test material. The incidences of gross lesions in these organs were dose-related with statistical significance achieved at one or more doses. The incidences of gross lesions in the pituitary and mammary gland **decreased** in female rats, and the incidences of some lesions in the testes of males and the adrenal glands of female rats also **decreased** with dose. The **decreased** incidences of pituitary masses in females rats did not show a clear dose-response; statistical significance was achieved at low- ($p<0.05$) and high-dose ($p<0.01$), but not at the intermediate dose. Compression of the brain in the region of the pituitary was a prominent lesion in female controls, probably due to the pituitary masses. The lesion showed a negative trend, with statistical significance achieved at the high dose ($p<0.01$). There was also an unexplained statistically significant decrease in the incidence of enlarged adrenal glands in females receiving 500 ppm of the test material. The decrease is probably a chance occurrence, because the first chronic toxicity study (MRID No. 432547-01) showed that the adrenal glands (unilateral/bilateral combined) of 85% of the females receiving 500 ppm were enlarged ($p<0.01$). Generally, gross lesions and effective doses observed in the current study and the first chronic toxicity study are in agreement.

TABLE 6. SELECTED GROSS PATHOLOGIC LESIONS IN MALE RATS FED VINCLOZOLIN FOR 104 WEEKS

Organ/Lesions	Dietary concentration (ppm)			
	0 ppm	50 ppm	500 ppm	3000 ppm
Number of animals in each group	50	50	50	50
	Males			
Liver				
Focus	12[a]	20*	22*	38**
Lungs				
Focus	10	7	6	24**
Iliac lymph nodes				
Hyperemia	9	6	22**	26**
Adrenal glands				
Enlarged (unilateral/bilateral)	1	0	4	33**
Discoloration	0	0	8**	42**
Pituitary				
Enlarged	3	4	9	12**
Eyes				
Cataracts (bilateral)	1	1	3	18**
Testes				
Mass, unilateral	14	9	15	6*
Mass, bilateral	6	11	30**	43**
Mass, unilateral/bilateral	20	20	45**	49**
Cystic degeneration	4	5	16**	11*
Focus	7	7	4	0**
Epididymides				
Reduced size	3	4	29**	45**
Seminal vesicles				
Reduced size	3	4	16**	38**
Prostate				
Reduced size	3	1	13**	39**

Data taken from text Tables 1-7, pages 485-490 and from pages 532-537, MRID No. 432547-03.
[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)].

TABLE 7. SELECTED GROSS PATHOLOGIC LESIONS IN FEMALE RATS FED VINCLOZOLIN FOR 104 WEEKS

Organ/Lesions	Dietary concentration (ppm)			
	0 ppm	50 ppm	500 ppm	3000 ppm
Number of animals in each group	50	50	50	50
	Females			
Liver				
Cyst	7[a]	8	18**	24**
Focus	9	12	10	39**
Lungs				
Focus	1	1	0	10**
Adrenal glands				
Enlarged (unilateral/bilateral)	8	4	1*	34**
Cyst	12	11	8	0**
Discoloration	0	1	5	43**
Focus	14	10	5*	1**
Eyes				
Cataracts (bilateral)	0	1	1	49**
Pituitary				
Mass	25	16*	18	5**
Brain				
Compression	23	15	17	5**
Ovary				
Mass, unilateral/bilateral	2	7	7	17**
Mammary gland				
Mass	13	11	5*	4*

Data taken from text Tables 1-7, pages 485-490 and from pages 532-537, MRID No. 432547-03.

[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, epididymides, seminal vesicles, coagulation gland, and prostate showed increased incidences in male rats and lesions in the ovary in female rats. The incidences of nonneoplastic lesions are summarized in Tables 8 (males) and 9 (females).

The liver lesions consisted of cellular hypertrophy, eosinophilic foci, and biliary cysts.
The incidence of cellular hypertrophy showed a clear dose-response relationship in both sexes, with statistical significance ($p<0.01$) achieved at 500 and 3000 ppm. The incidence of all altered foci combined in males receiving 500 ppm was significantly elevated over that of controls; at other doses and in female rats, the incidence was similar to controls. Evaluation of each type of foci revealed that eosinophilic foci exhibited a clear positive dose-related trend, with the incidence achieving statistical significance at all doses in male rats and at the high dose in female rats. Eosinophilic foci were not seen in male controls and in only one female control. Basophilic and clear cell foci, which were common lesions in controls of both sexes, exhibited treatment-related negative trends. Significantly fewer ($p<0.01$) high-dose animals of both sexes developed clear cell foci and fewer ($p<0.01$) intermediate- and high-dose animals of both sexes developed the basophilic foci than the corresponding controls. Biliary cysts appear to be a treatment-related lesion showing a clear positive dose-response relationship. Significantly ($p<0.01$) greater numbers of animals receiving 500 and 3000 ppm of vinclozolin developed this lesion than the corresponding control groups. Peripheral fatty lesions developed in significantly fewer male and female rats receiving 3000 ppm than in the control groups.

Foam cell aggregates in the lungs, which correspond to the gross lesion described as foci, occurred in a significantly greater number in male rats at all three doses ($p<0.01$), and the increased incidence was dose-related ($p<0.001$, Cochran-Armitage trend test conducted by the reviewer). In female rats, the incidence of foam cell aggregates was significantly increased ($p<0.01$) only at 3000 ppm. Incidences in animals receiving 50 or 500 ppm of the test material were comparable with those of the control groups.

The incidences of urothelial hyperplasia and calcification of the renal pelvis showed clear dose-response relationships, but the average severity did not show a clear increase with dose. The incidences of both lesions were significantly increased at 500 ($p<0.05$) and 3000 ($p<0.01$) compared with the incidences of the controls. In addition, both lesions occurred bilaterally in significantly more treated animals than in controls, particularly urothelial hyperplasia in the intermediate- and high-dose groups and calcification of the renal pelvis in the high-dose group. Although the average severity ratings were comparable between treated groups and controls, the bilateral occurrence of the lesions along with the positive dose-response relationships suggest that both lesions are treatment related.

Vacuolation of acinar cells was seen in the pancreas (treatment-related lesion) of a large number of male (35 and 48, $p<0.01$) and female rats (45 and 48, $p<0.01$)

receiving 500 or 3000 ppm, respectively, of the test material. The corresponding incidence in the controls was very low (six males and four females). The average severity ratings for the lesion showed a strong dose-response relationship in both sexes. The lesions were minimal to slight in controls (both sexes), minimal to moderate in 50-ppm males, minimal to moderate in 500-ppm males, and minimal to extreme in 3000-ppm males (extreme in 1 and severe in 20). In female rats, vacuolation of pancreatic acinar cells was minimal to severe (moderate in 15 and severe in 1) in females receiving 500 ppm of the test material and minimal to severe in those receiving 3000 ppm (moderate in 24 and severe in 8).

Focal fiber atrophy of the skeletal muscle occurred in only four females (N.S.) receiving 3000ppm, two receiving 500 ppm of the test material, and in none receiving 50 ppm or the controls. This finding appeared to be dose related, but statistical significance was not achieved at the high dose. In male rats, focal fiber atrophy of the skeletal muscle occurred in 11 controls and a comparable number of animals receiving 50 ppm (12) or 500 ppm (15). The lesion occurred in 23 male rats ($p < 0.01$) receiving 3000 ppm. The average severity ratings did not show a clear dose response; however, the lesions were severe in two 500-ppm and one 3000-ppm males and in none of the controls or 50-ppm males. Focal fiber atrophy is considered to be a treatment related lesion in male rats and possibly in female rats.

A prominent lesion that occurred in male and female rats fed vinclozolin was degeneration of the lens. The increasing incidences and average severity ratings showed positive dose-response relationships. The incidence was significantly ($p < 0.01$) increased in females at all dose levels and in males at 500 and 3000 ppm. Lenticular degeneration occurred bilaterally in all 50 male and 50 female rats fed 3000 ppm of the test material. At 500 ppm, lenticular degeneration occurred bilaterally in more than 90% of the animals (39 males and 49 females) that developed the lesion and in 60% of the 50-ppm females that developed the lesion . In addition, 36 females and 17 males receiving 3000 ppm developed the severe form of the lesion, whereas it did not progress beyond the moderate form in the controls. Lenticular calcification was also a prominent finding in the eyes; the incidences and the average severity ratings were dose-related. This lesion did not occur in controls of either sex or in females receiving 50 ppm of the test material; it occurred in one male receiving 50 ppm, in 4 males (N.S.) and in 12 females ($p < 0.01$) receiving 500 ppm, and in almost all males (46) and females(48) receiving 3000 ppm. Like lenticular degeneration, calcification also occurred bilaterally in most instances. Serous fluid accumulation in the anterior chamber was another finding accompanying the degeneration of the lens. The incidence of this lesion showed a dose-response relationship. It occurred at all doses in both sexes, but statistical significance was achieved only at the high-dose in females and at the intermediate and high doses in males.

Pigment storage in the iliac lymph nodes was a common lesion in male rats; the incidence was significantly increased in intermediate- (35/44 treated vs. 19/32 controls, $p < 0.01$) and high-dose male rats (40/49 treated compared with 19/32

controls, $p<0.01$). Pigment also accumulated in the renal lymph nodes (8, 2, 13, 18 male rats, for 1, 50, 500, and 3000 ppm, respectively). This accumulation of pigment may not be related to treatment, because it did not occur at increased incidences in lymph nodes at other sites.

Other treatment-related lesions occurred in reproductive or endocrine organs of male and female rats receiving vinclozolin. Adrenal cortical lesions occurring with significantly elevated incidences at one or more doses and showing dose-related trends included lipidosis (accumulation of lipids in the cytoplasm of enlarged cortical cells), focal hypertrophy, focal hyperplasia, and extra cortical nodules. The incidence of lipidosis was significantly increased ($p<0.01$) in male and female rats receiving 500 and 3000 ppm of the test material; the average severity was also increased at the high dose. Focal hypertrophy showed significantly ($p<0.05$ or $p<0.01$) increased incidences at 3000 ppm in males and 500 and 3000 ppm in females. The incidence of focal hyperplasia was significantly increased at all doses in male rats, but only at the 3000-ppm dose in female rats. The incidence of unilateral/bilateral extra cortical nodules (clusters of cortical cells extending through the fibrous capsule into the periadrenal fat) was significantly ($p<0.01$) increased in male rats receiving 3000 ppm and in females at the 500 and 3000 ppm. This lesion occurred bilaterally in 31 of 45 (69%) high-dose female rats that developed the lesion and in 19 of 40 (48%) high-dose male rats that developed the lesion. At 500 ppm; extra cortical nodules occurred bilaterally in only 25% of the females and 33% of the males that developed the lesion. Lipogenic pigment was a prominent lesion occurring with comparable incidences in all groups (including controls) of both sexes. The average severity rating was markedly increased in high-dose animals; 21 males and 17 females developed the moderate grade of the lesion and 14 males and 24 females developed the severe grade. The lesion did not progress beyond the moderate grade in the controls.

Vinclozolin also had a notable effects on the testes and accessory organs of male rats and the ovaries of female rats. In male rats, the effects occurred primarily at the intermediate and high dose. Interstitial edema in the testes developed in 12 high-dose male rats compared with 5 controls ($p<0.05$). The incidences of diffuse tubular atrophy and tubular calcification in the testes were significantly increased ($p<0.01$) in the animals fed 500 and 3000 ppm of the test material. The background rates for these lesions were high with 15 or 16 controls developing the lesion, and there was no increase in the average severity ratings. The incidences of focal tubular atrophy and focal Leydig cell hyperplasia were very high in control animals (37 and 36 animals, respectively). The incidences showed a marked decrease, especially at 3000 ppm, with only 9 animals developing focal tubular atrophy and 11 developing focal Leydig cell hyperplasia. Cystic ducts in the rete testes was seen in 16 high-dose males compared with 3 controls ($p<0.01$), and hyperplasia of the rete testes was seen in 13 high-dose male rats compared with 1 control ($p<0.01$). The incidences of all the testicular lesions were positively or negatively dose-related and are considered to be related to treatment.

Lesions in the epididymides and accessory organs accompanied the effects seen in the testes. The gross lessions (masses) generally correlated with the microscopic lesions, which showed a dose-response relationship; the incidences were statistically significant at one or more doses; and the effects are considered to be related to treatment. Azoospermia and oligospermia within the epididymides occurred in 49 male rats ($p < 0.01$) receiving 3000 ppm and 41 ($p < 0.01$) receiving 500 ppm of the test material compared with only 13 controls. This finding occurred bilaterally in almost all high-dose animals and in the majority of intermediate dose animals. Atrophy of the seminal vesicle and coagulation gland occurred with significantly increased incidences in rats receiving 500 and 3000 ppm of the test material compared with the control incidences. Prostate lesions (reduced secretion, interstitial fibrosis, and focal/multifocal hyperplasia) occurred with significantly increased incidences ($p \leq 0.05$ or $p < 0.01$) at 500 and 3000 ppm. The incidence of acute inflammation showed a negative trend with statistical significance attained at the high dose, and the incidence of chronic inflammation showed a significant increase ($p < 0.05$) at the intermediate dose, but not the high dose.

The most prominent lesion occurring in the reproductive organs of female rats was lipidosis in the interstitial cells of the ovary, the incidence of which showed a clear dose-response relationship and statistical significance ($p < 0.01$) at all doses of vinclozolin. The average severity rating did not show a clear dose-response relationship, but there was a progressive increase in the number of animals presenting the moderate form of the lesion (1, 9, 17, and 26 females in the 0-, 50-, 500- and 3000-ppm groups, respectively). The severe grade of the lesion occurred in 13 females receiving 3000 ppm of the test material, and in none of the other dose groups. The incidence of ovarian cysts (not otherwise specified, NOS) was also significantly increased ($p \leq 0.05$ or $p < 0.01$) at all doses. However, the incidence of ovarian cysts was very high in controls (30/50), and it did not show an increase between 500 and 3000 ppm suggesting that the increased incidence is not related to treatment. The incidence of fibrosis of the uterine cervix showed a negative dose-related trend, with statistical significance noted at the high dose (1/50 vs 12/50 in controls, $p < 0.01$).

Table 8. INCIDENCE OF NONNEOPLASTIC MICROSCOPIC LESIONS IN MALE RATS FED VINCLOZOLIN FOR 104 WEEKS

Organ/Lesions	Dietary concentration (ppm)			
	0	50	500	3000
Liver				
Cellular hypertrophy	0/50[a] (0.00)	2/50 (2.00)	12/50** (2.08)	44/50** (2.95)
Peripheral fatty changes	20/50 (2.50)	27/50 (2.37)	27/50 (1.96)	6/50** (2.50)
Altered foci	34/50	38/50	44/50**	39/50
Eosinophilic foci	0/50	15/50**	33/50**	38/50**
Basophilic foci	29/50	25/50	11/50**	8/50**
Clear cell foci	25/50	24/50	33/50	3/50**
Biliary cysts	4/50	5/50	11/50*	15/50**
Lungs				
Foam cell aggregates	14/50 (2.00)	27/50** (1.89)	24/50** (1.96)	40/50**1.98)
Kidneys				
Urothelial hyperplasia (U/B)	7/50 (2.43)	14/50 (2.16)	15/50* (2.47)	23/50** (2.52)
Bilateral	2/50	5/50	8/50*	9/50*
Calcification, renal pelvis (U/B)	12/50 (2.17)	14/50 (2.43)	21/50* (2.10)	31/50** (2.42)
Bilateral	2/50	5/50	4/50	14/50**
Pancreas				
Vacuolated acinar cells	6/50 (1.50)	9/50 (1.33)	35/50** (2.00)	48/50** (3.35)
Skeletal muscle				
Focal fiber atrophy	11/50 (1.64)	12/50 (2.00)	15/50 (2.40)	23/50** (2.09)
Heart				
Myocardial fibrosis	43/50 (2.23)	10/14 (2.30)	11/12 (2.45)	26/50** (1.65)
Eyes				
Degeneration of lens (U/B)	11/50 (2.18)	15/50 (2.13)	39/50** (2.72)	50/50** (3.32)
Bilateral	6/50	8/50	36/50**	50/50**
Lenticular calcification (U/B)	0/50 (0.00)	1/50 (3.00)	4/50 (2.00)	46/50** (2.59)
Bilateral	0/50	0/50	1/50	38/50**
Serous fluid accumulation	6/50	10/50	14/50**	19/50**
Iliac lymph nodes				
Pigment storage	19/32	22/30	35/44*	40/49*
Adrenal Gland (cortex)				
Lipidosis	1/50 (3.00)	2/50 (3.50)	33/50** (2.09)	50/50** (4.12)
Lipogenic pigment	48/50 2.21)	49/50 (2.06)	48/50 (1.98)	47/50 (2.96)
Focal hypertrophy	15/50	23/50	16/50	34/50**
Focal hyperplasia	10/50	19/50*	21/50**	23/50**
Extracortical nodules (U/B)	25/50	32/50	21/50	40/50**
Bilateral	7/50	8/50	7/50	19/50**

Data taken from Text Tables 9-33, pages 492-517) and the pathology summary tables on pages 552-560 and 577-584, MRID No. 432547-03. [a]Number of animals having a lesion/number of animals examined; numbers in parentheses are the average severity grades: 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

TABLE 8. Continued

Organ/Lesions	Dietary concentration (ppm)			
	0	50	500	3000
Testes				
Interstitial edema	5/50	6/50	7/50	12/50*
Focal tubular atrophy	37/50 (1.68)	29/50 (1.76)	22/50** (2.45)	9/50** (3.00)
Diffuse tubular atrophy	15/50 (4.00)	14/50 (3.79)	36/50** (3.97)	45/50** (3.98)
Tubular calcification	16/50 (2.06)	20/50 (1.80)	37/50** (1.84)	42/50** (2.00)
Cystic rete testis	3/50 (2.00)	5/50 (2.00)	3/50 (2.00)	16/50** (3.00)
Hyperplastic rete testis	1/50 (2.00)	0/50 (0.00)	0/50 (0.00)	13/50** (2.54)
Focal Leydig cell hyperplasia	36/50 (2.19)	39/50 (2.00)	34/50 (2.24)	11/50** (1.82)
Epididymis				
Azoospermia/oligospermia (U/B)	13/50	14/50	41/50**	49/50**
Bilateral only	3/50	5/50	27/50**	46/50**
Seminal Vesicle				
Atrophy	3/50 (3.00)	5/50 (2.40)	16/50** (3.19)	31/50** (3.13)
Coagulation Gland				
Atrophy	3/50 (2.67)	5/50 (2.20)	16/50** (2.69)	30/49** (3.10)
Prostate				
Reduced secretion	4/50 (2.75)	7/50 (2.00)	12/50* (2.00)	21/50** (2.43)
Interstitial fibrosis	4/50 (1.75)	9/50 (2.00)	16/50** (2.19)	31/50** (2.84)
Focal/multifocal hyperplasia	11/50 (2.09)	17/50 (2.24)	25/50** (2.08)	20/50* (2.25)
Acute inflammation	7/50	7/50	2/50	1/50**
Chronic inflammation	5/50	8/50	12/50*	11/50

Data taken from Text Tables 9-33, pages 492-517) and the pathology summary tables on pages 552-560 and 577-584, MRID No. 432547-03. [a]Number of animals having a lesion/number of animals examined; numbers in parentheses are the average severity grades: 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

TABLE 9. INCIDENCE OF NONNEOPLASTIC MICROSCOPIC LESIONS IN FEMALE RATS FED VINCLOZOLIN FOR 104 WEEKS

Organ/Lesions	Dietary concentration (ppm)			
	0	50	500	3000
Liver				
Cellular hypertrophy	0/50[a] (0.00)	0/50 (0.00)	11/50** (2.00)	44/50** (2.61)
Peripheral fatty changes	14/50 (2.14)	23/50* (2.04)	34/50** (2.06)	6/50* (1.83)
Altered foci	32/50	31/50	32/50	39/50
Eosinophilic foci	1/50	0/50	5/50	38/50**
Basophilic foci	28/50	26/50	16/50**	8/50**
Clear cell Foci	21/50	19/50	23/50	4/50**
Biliary cysts	8/50	10/50	20/50**	36/50**
Lungs				
Foam cell aggregates	7/50 (1.57)	8/50 (1.13)	9/50 (1.56)	28/50** (1.86)
Pancreas				
Vacuolated acinar cells	4/50 (1.25)	2/50 (2.00)	45/50** (2.16)	48/50** (2.79)
Skeletal muscle				
Focal fiber atrophy	0/50 (0.00)	0/50 (0.00)	2/50 (1.50)	4/50 (1.50)
Heart				
Myocardial fibrosis	33/50 (2.12)	9/20* (2.11)	4/14** (1.75)	15/50** (1.67)
Eyes				
Degeneration of lens (U/B)	9/50 (2.11)	23/50** (2.26)	49/50** (2.67)	50/50** (3.72)
Bilateral	4/50	14/50**	46/50**	50/50**
Lenticular calcification (U/B)	0/50 (0.00)	0/50 (0.00)	12/50** (1.75)	48/50** (2.69)
Bilateral	0/50	0/50	6/50**	36/50**
Serous fluid accumulation	4/50	5/50	2/50	34/50**
Adrenal Gland				
Cystic degeneration	50/50	48/50	49/50	13/50**
Lipidosis	0/50 (0.00)	3/50 (3.00)	12/50** (2.50)	50/50** (4.08)
Lipogenic pigment	49/50 (2.41)	45/50 (2.31)	49/50 (2.39)	48/50 (3.35)
Focal hypertrophy	22/50	13/50	13/50*	45/50**
Focal hyperplasia	22/50	22/50	30/50	46/50**
Extracortical nodules (U/B)	19/50	24/50	40/50**	45/50**
Bilateral	4/50	5/50	10/50	31/50**

Data taken from Text Tables 9-33, pages 492-517) and the pathology summary tables on pages 552-560 and 577-584, MRID No. 432547-03. [a]Number of animals having a lesion/number of animals examined; numbers in parentheses are the average severity grades: 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

TABLE 9. Continued

Organ/Lesions	Dietary concentration (ppm)			
	0	50	500	3000
Brain				
Focal compression	20/50	14/27	17/25	5/50**
Dilated ventricle	13/50	9/27	7/25	5/50*
Mammary Gland				
Glandular cysts	42/50	17/27*	12/23**	27/50**
Ovaries				
Follicle absent	34/50	17/50	11/50	12/50
Interstitial cell lipidosis	2/50 (2.50)	15/49**	35/50**	43/50**
Cysts (NOS)	30/50	(2.60)	(2.49)	(3.21)
		39/49*	43/50**	43/50**
Cervix				
Fibrosis	12/50	7/35	4/30	1/50**

Data taken from Text Tables 9-33, pages 492-517) and the pathology summary tables on pages 552-560 and 577-584, MRID No. 432547-03. [a]Number of animals having a lesion/number of animals examined; numbers in parentheses are the average severity grades: 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

In addition, glandular cysts developed in the mammary gland of female rats. The incidence of the lesion showed a negative dose-related trend in which statistical significance was achieved at all doses. Pituitary lesions did not occur at significantly elevated or lower incidences in females than in controls. However, the incidence of focal compression of the brain was observed in significantly fewer high-dose females than in controls. This lesion corresponds to the gross lesion associated with the presence of pituitary masses.

2) Neoplastic – The incidences of notable neoplastic lesions for males and females are summarized in Table 10. Benign Leydig cell tumors developed in all groups of male rats. The incidence at 500 and 3000 ppm was significantly ($p < 0.01$) greater than in controls. Two male rats receiving 3000 ppm developed malignant Leydig cell tumors, whereas no malignant lesions developed in controls or the other dose groups. Prostate adenomas occurred in each group receiving vinclozolin, but in none of the controls. A clear dose-response relationship was not observed, but the incidence achieved statistical significance at the intermediate and high dose. The total number

of male rats developing neoplasms, whether benign or malignant, was similar in treated groups and controls.

In female rats, increased tumor incidences were noted for the adrenal cortex, ovaries, and uterus. Decreased incidences were noted for the pituitary and mammary gland. Adrenal cortical adenomas occurred in 21 female rats ($p<0.01$) receiving 3000 ppm of the test material compared with only 1 control; 2 females receiving 50 ppm and 1 receiving 500 ppm developed adenomas. Carcinomas were observed in one female receiving 3000 ppm and none developed in the other treated groups or controls. The incidence of benign sex cord tumors (unilateral/bilateral)showed a positive dose-related trend with statistical significance observed for females receiving 3000 ppm ($p<0.01$) only. Other ovarian tumors were seen, but the incidences did not achieve statistical significance. Uterine adenocarcinomas occurred in seven females receiving 3000 ppm, in one control, and in one receiving 500 ppm. Adenomas and fibroadenomas (combined) of the mammary glands occurred in 11/27 females ($p<0.01$) receiving 50 ppm compared with 6/50 controls and only 3/23 and 4/49 females receiving 500 and 3000 ppm, respectively. In addition, a negative dose-related trend was observed for pituitary adenomas, with only 16 high-dose females ($p<0.01$) developing this neoplasm compared with 31 controls. The incidence in the other treated groups was similar to that of controls. Finally, there was no significant difference between the treated groups and controls in the total number of animals having a neoplasm at any site.

3) Historical Control Data - Data from 2-year studies in the Wistar rat conducted from 1984 to 1994 and submitted in support of the carcinogenicity study in rats.

TABLE 10. HISTORICAL CONTROL DATA FROM BASF; APPROX. 1250 WISTAR RATS/SEX IN APPROX. 29 2-YEAR STUDIES.

Tumor type	Males		Females	
	Mean	Range	Mean	Range
Hepatocellular adenomas	8.3%	0 to 30%	2.8%	0 to 20%
Hepatocellular carcinomas	3.5%	0 to 10%	0.7%	0 to 5%
Adenal cortical adenomas	1.8%	0 to 5.1%	2.7%	0 to 15%
Adenal cortical adenocarcinomas	0.4%	0 to 2.0%	0.4%	0 to 4.0%
Prostate adenomas	1.0%	0 to 12%	-	-
Prostate carcinomas	0.24%	0 to 5%	-	-
DATA FROM HANOVER DATA BASE (EUROPEAN); APPROX. 1300 WISTAR RATS/SEX IN 24 2-YEAR STUDIES.				
Testicular Leydig cell adenomas	17.7%	2.0 to 52.5%	-	-
Uterine adenocarcinomas	-	-	1.9%	0 to 10%
Ovarian sex cord, stromal, mixed, benign	-	-	0.4%	0 to 2.0%
Ovarian granulosa cell, benign	-	-	1.9%	0 to 5.2%
Ovarian granulosa cell, malignant	-	-	0.4%	0 to 2.0%

TABLE 10. NEOPLASTIC LESIONS IN MALE AND FEMALE RATS FED VINCLOZOLIN FOR 104 WEEKS

Organ/Lesions	Dietary concentration (ppm)			
	0	50	500	3000
Males				
Liver				
Adenoma, hepatocellular	0	1	1	3
Carcinoma, hepatocellular	1	1	5	2
Testes				
Leydig cell tumors, benign	23/50[a]	25/50	47/50**	48/50**
Leydig cell tumors, malignant	0/50	0/50	0/50	2/50
Leydig cell tumors, total	23/50	25/50	47/50**	49/50**
Prostate				
Adenoma	0/50	3/50	7/50**	5/50*
Total No. of animals with tumors	44/50	43/50	50/50	50/50
No. with benign tumors	42/50	36/50	49/50	48/50
No. with malignant tumors	15/50	20/50	16/50	13/50
Females				
Adrenal cortex				
Cortical adenoma	1/50	2/50	1/50	21/50**
Cortical carcinoma	0/50	0/50	0/50	1/20
Adenoma/carcinoma	1/50	2/50	1/50	22/50**
Pituitary				
Adenoma	31/50	23/50	29/50	16/50*
Ovaries				
Benign sex cord tumor (U/B)	4/50	7/49	10/50	29/50**
Sertoli cell tumor, benign	0/50	1/49	0/50	2/50
Granulosa cell tumor, benign	2/50	0/49	4/50	2/50
Granulosa cell tumor, malignant	0/50	1/49	1/50	0/50
Thecoma, malignant	0/50	0/49	0/50	2/50
Uterus				
Adenocarcinoma	1/50	0/39	1/31	7/50*
Mammary gland				
Fibroadenoma/adenoma	6/50	11/27**	3/23	4/49
Adenocarcinoma	7/50	0/27	3/23	0/49**
Total no. of animals with tumors	44/50	43/50	43/50	48/50
No. with benign tumors	41/50	38/50	41/50	46/50
No. with malignant tumors	19/50	11/50	17/50	15/50

Data taken from Text Tables 11, 15, 21,23, 24, 25, and 33 (pages 494, 499, 505, 508, 509, 510, and 517) and the pathology summary tables on pages 561-572, MRID No. 432547-03. [a]Number of animals with 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)]

DISCUSSION: Groups of 50 male and 50 female Wistar rats were given vinclozolin in their diet at concentrations of 0, 50, 500, or 3000 ppm continuously for 2 years. Calculated doses reported by the study author were 0, 2.3, 23, and 143 mg/kg/day, respectively, for male rats and 0, 3, 30, or 180 mg/kg/day,respectively, for female rats. Survival of the groups of animals given vinclozolin was similar to that of controls. Clinical signs of toxicity included enlarged or unpalpable testicles and cataracts in the eyes of animals fed 500 and 3000 ppm of the test material; these effects were consistent with the gross and microscopic lesions observed. Terminal body weights were significantly ($p<0.01$) reduced in male and female rats (25 and 17%, respectively) fed 3000 ppm, and body weights at 50 and 500 ppm slightly exceeded or were similar to the control animal weights. Body weight gain was significantly reduced during the entire study in animals receiving 3000 ppm of the test material, with net weight gain being reduced by 34 and 27% in males and females, respectively. Food consumption was reduced by 12 to 16 % in 3000-ppm males and 8-16% in 3000-ppm females, possibly accounting for some, but not all, of the decreased weight gain. However, relative efficiency of food utilization was decreased at 3000 ppm relative to control values. This indicates that the part of the severe body weight was due to toxicity. The findings on food efficiency are consistent with those found in the chronic rat study (MRID# 432547-01), which showed reduced food efficiency from week 52 to 104 at 1500 and 4500 ppm. These findings appear to indicate that the hormonal imbalance induced by prolonged exposure to the anti-androgen, vinclozolin, are principally expressed during the last year of exposure. The only clinical pathology tests performed were a differential white blood cell count and red and white blood cell morphology. These tests showed that vinclozolin was not toxic to blood cell elements.

This study confirmed most of the results obtained in the first chronic toxicity study in which male and female rats were administered dietary vinclozolin at concentrations ranging from 150 to 4500 ppm for 2 years (MRID No. 432547-01). Treatment-related toxic effects occurred primarily in the eyes, reproductive organs, and endocrine organs. In addition, toxicity was also observed in the liver, lungs, kidneys, pancreas, and skeletal muscle.

The study author noted that vinclozolin competitively binds to the androgen receptor, effectively inactivating the receptor and producing an antiandrogenic effect. The effects on the testes, epididymides, accessory organs, ovaries, and adrenal glands are probably due to the antiandrogenic effect of the test material. Effects on the testes were noted during clinical examination, and during necropsy and microscopic examination primarily at doses ≥ 500 ppm. During necropsy, the testes contained masses (probably tumors) and showed evidence of cystic degeneration; the microscopic lesions consisting of diffuse tubular atrophy, tubular calcification (both showed a dose-related increase in incidence but not average severity), and cystic and hyperplastic rete testes. Testicular effects are probably due to the over production of pituitary hormones due to the lack of feedback from testosterone. Effects in the epididymides were manifested by a pronounced reduction or complete absence of spermatozoa. Corresponding effects in the accessory organs (probably due to lack of androgenic stimulation) consisted of degeneration of the prostate (reduced secretion and fibrosis) and atrophy of the seminal vesicles and coagulation gland. These possible antiandrogen induced response may be related to the statistically significantly increase

prostate adenomas at 500 and 3000 ppm. The lack of effects at 50 ppm confirms the NOEL established in the second chronic study for lesions in the testes, epididymides, and accessory organs.

The prominent lesion in the adrenal gland of both sexes and ovary of females was lipidosis, the incidences of which were clearly dose-related. 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 hormonal antagonistic effect of vinclozolin or effects resulting from the effects on lipid metabolism/storage. The incidence of adrenal cortical lipidosis was significantly increased in both sexes at doses $\geq$500 ppm, whereas the incidence of ovarian lipidosis was significantly increased at all doses. In the second chronic study (MRID No. 432547-02), lipidosis did not occur in the ovaries of control or treated female rats fed 25 or 50 ppm of the test material. Therefore, in contrast to the second chronic study, the results of the current study does not established a NOEL for ovarian interstitial cell lipidosis. The extracortical nodules in the adrenal cortex are probably preneoplastic lesions, which the study author classified as neoplasms depending in their size. This lesion is commonly seen in controls (50% of males and 40% of females), showed significantly increased incidences in males at the high dose and in females at the intermediate and high doses. There was also significant ($p \geq 0.05$) increases in the incidence of focal hyperplasia in the adrenal gland of males at all doses and in females at 3000 ppm. Because the incidence in male rats is statistically significant at all doses and dose-related, the lesion appear to be treatment-related. However, the incidence of focal hyperplasia in the adrenal cortex of male controls in the first chronic study was 15% (MRID No. 432547-01, pp. 1149) and 45% in the second chronic study (MRID No. 432547-02, pp. 779). The incidences in treated animals in the current study range from 38 to 46%, which is comparable to the range of the control incidences in the chronic studies. Consequently, focal hyperplasia should not be considered a treatment-related lesion in male rats. In females, however, the incidence at the high dose (92%) exceeds the range (20 to 45%) of controls in all three studies (20%, MRID No. 432547-01, pp. 1158; 45%, MRID No. 432547-02, pp. 779), suggesting that focal hyperplasia in the adrenal cortex of female rats is treatment-related. Focal hypertrophy was not seen in controls or treated rats in the first chronic study, but 30% of male controls and 35% of female controls in the second study exhibited this lesion. In the present study, the control incidences were 30 and 44% in male and female rats, respectively, compared with 68% and 90%, respectively, at 3000 ppm. The lack of a clear dose-response in either sex, especially females, suggests that focal hypertrophy is not a treatment-related lesion. Lipogenic pigment in the adrenal cortex is a common age-related lesion in both sexes; however, its incidences did not increase; the dose-related increase in the average severity suggested that the lesion is treatment-related.

Focal fiber atrophy is a degenerative lesion that occurred in skeletal muscle. The incidence of focal fiber atrophy in female rats did not show a statistically significant increase at any dose, but statistical significance was achieved in males receiving the high dose. The incidences were dose-related in both sexes. The lesions in both sexes should also be considered treatment-related, because it is probably due to antianabolic effects associated with test material. Vacuolation of acinar cells is a degenerative pancreatic lesion that showed a dose-related increase in incidence and average severity in both sexes. The liver was also

particularly affected by treatment with vinclozolin, with cellular hypertrophy and eosinophilic foci developing in males and females. The incidences of both lesions were dose-related and statistically significant at one or more doses. The incidence of eosinophilic foci was statistically significant at all doses in male rats. In the first and second chronic studies, eosinophilic foci were not seen in male rats receiving ≤500 ppm of the test material, except for one rat in the 500-ppm group (MRID No. 432547-01, pp. 1143; MRID No. 432547-02, pp. 774). Unlike the chronic studies, this study does not establish a NOEL for eosinophilic foci in male rats. Biliary cysts occurred with statistically significant increased incidences in both sexes receiving the intermediate and high doses. The author associated this lesion with cellular hypertrophy that may have compressed the smaller bile ducts leading to progressive dilation of the ducts. The kidneys of male rats showed the same effects as those seen in the first chronic study, urothelial hyperplasia and calcification of the renal pelvis. Again there appears to be no association with chronic nephropathy suggesting that these lesions are treatment-related. Foam cell aggregates in the lungs is a common lesion corresponding to the gross lesion described as a "focus". The incidence of foam cell aggregates showed a dose-related increase in male (statistically significant at all doses) and female rats, but not in the average severity ratings. A NOEL was not established for this lesion in male rats. Foam cell aggregates 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). Further analysis may establish a relationship with the effects on lipid metabolism/storage by vinclozolin.

Cataracts noted during clinical observations were confirmed during necropsy and microscopic examination (lenticular degeneration) of the eyes. The incidence and average severity rating of lenticular degeneration were dose-related and statistically significant at the intermediate and high dose in male rats and at all doses in female rats. Therefore, a no-observed-effect level (NOEL) cannot be established for lenticular degeneration in females. In contrast, the second chronic study showed no increase in the incidence of lenticular degeneration in female rats receiving 50 ppm of the test material for 2 years (MRID No. 432547-02). Lenticular calcification was observed at statistically significant increased incidences in males receiving the high-dose and in females receiving the intermediate and high doses. However, serous fluid accumulation was observed at the two highest doses in males and only at the high dose in females. These results also suggest that the eyes of female rats may be more sensitive to the toxic effects of vinclozolin than the eyes of male rats.

Nonneoplastic lesions occurring with decreased incidences in both male and female rats included hepatic clear cell and basophilic foci, peripheral fatty changes (not described in the first chronic study), and myocardial fibrosis of the heart. Lesions occurring with decreased incidences only in males included focal tubular atrophy and focal Leydig cell hyperplasia in the testes and acute inflammation in the prostate. Lesions occurring with decreased incidences in only female rats were cystic degeneration of the adrenal cortex, focal compression and dilated ventricles in the brain, glandular cysts in the mammary gland, and fibrosis of the cervix. 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 may be due to alterations in hormonal status (antiandrogenic activity of vinclozolin) for

endocrine and reproductive organs and to 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 a reduction in the number of animals at risk during the late stage of the study. The reductions in weight gain or possible antianabolic effects of vinclozolin may have contributed to the lowered incidences. The decreased incidences are not of a magnitude or biological significance to attribute a beneficial effect to vinclozolin.

The reasons for these test material related statistically significant decreases in the usual age related pathology are unknown, but some of them may be related the decreased androgen stimulation caused by the vinclozolin/metabolites/degradation product inhibition of androgen receptors.

In conclusion, treatment-related nonneoplastic effects were observed at all doses in male (eosinophilic foci in the liver and foam cell aggregates in the lung) and female rats (lenticular degeneration, and ovarian interstitial cell lipidosis). Therefore, a NOEL cannot be established for either sex. The second chronic study established a NOEL at 50 ppm the lowest dose used in the current study. The reason for the discrepancy cannot be determined: the calculated time-weighted-average doses were similar; the ages at study initiation were the same; the average body weight of the males at study initiation was about 11% greater in the current study than in the second chronic study (MRID No. 432547-02, pp. 23); the average body weights were similar in females; and compound consumption at 50 ppm was similar throughout the studies. There is no obvious explanation for the difference in the NOEL for the two studies.

Male rats fed 50, 500, or 3000 ppm of vinclozolin had a significantly increased incidence of Leydig (interstitial) cell tumors in the testes at the intermediate and high doses. These results confirm those obtained in the first chronic study, which showed a significantly increased incidence at 500, 1500, and 4500 ppm. The antiandrogenic activity of vinclozolin probably contributed to the development of the Leydig cell tumors, i.e. causing a hormonal imbalance by stimulating the production of luteinizing hormone which in turn caused proliferation of interstitial cells in the testes. The tumors occurred at a high incidence in control rats; they were malignant only in two high-dose animals and occurred bilaterally in most animals. Hyperplasia of the Leydig cells (probably preneoplastic) was also a common age-related lesion in male rats. This study also showed a low incidence of prostate adenomas at the intermediate and high doses. A clear dose-response was not observed, but considering the hormonal responsiveness of the organ, the adenomas are considered to be treatment-related. The increased severity in the chronic inflammation and fibrosis may also have been exacerbated by the decreased prostate secretions indirectly resulting from the antiandrogenicity. The increase in prostate interstitial fibrosis is stated to be an integral part of the chronic inflammation, past and present, and maybe be related to the increased incidence of prostate adenoma. In female rats, significantly increased incidences of adrenal cortical tumors, ovarian sex cord tumors (benign), and uterine adenocarcinomas occurred at the high-dose. That these ovarian benign tumors may be related to abnormal lipidosis seen in the ovaries, leading to dysplastic cellular growth and/or tumor formation is speculative, but possible. The adrenal cortical tumors were all benign except in one animal. The first chronic study did not show a statistically significant increase in uterine adenocarcinomas at

4500 ppm, but in the current study the neoplasm was increasaed at 3000 ppm. Statistically significant decreased incidences were observed for pituitary adenomas and mammary gland fibroadenoma/adenoma and adenocarcinomas. Although the data showed a clear carcinogenic response at 3000 ppm, the maximum tolerated dose (MTD) was also clearly exceeded in male and female rats at this dose as evidenced by a statistically and biologically significant depression in body weight gain and toxicity affecting multiple organs. Exceeding the MTD may not have affected the carcinogenic response of the target organs, because the mechanism may involve a hormonal imbalance.