



/ OMP # 00434
4-19-95
#12053
21PP

DATA EVALUATION REPORT
VINCLOZOLIN

Study Type: ONCOGENICITY FEEDING - MOUSE (83-2)

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Prepared for

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

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Biomedical and Environmental Information Analysis Section
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Task Order No. 94-18D

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[VINCLOZOLIN]

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Oncogenicity Study (83-2)

David G Anderson Date: 1/18/96
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DATA EVALUATION REPORT

STUDY TYPE: Oncogenicity Feeding - Mouse (83-2)

TOX. CHEM. NO.: 323C

P.C.CODE.: 113201

MRID NO.: 43254704

TEST MATERIAL: Vinclozolin, >99% a.i.

SYNONYMS: 3-(3,5-Dichlorophenyl)-5-ethenyl-5-methyl-2,4-oxazolidinedione; BAS 352F;
Ronilan

STUDY NUMBER: 80S0375/88112

SPONSOR: BASF Corporation

TESTING FACILITY: BASF Aktiengesellschaft Crop Protection, Product Safety, Dept. of
Toxicology, Ludwigshafen/Rhein, Germany

TITLE OF REPORT: Toxicology Study Report Carcinogenicity Study with Reg. No. 83
258-Vinclozolin in C57BL Mice Administration in the Diet for 18 Months

AUTHOR: W. Mellert

REPORT ISSUED: May 4, 1994 (Study Completion Date)

EXECUTIVE SUMMARY: In a 18-month oncogenicity feeding study (MRID# 43254704), vinclozolin was administered in the diet to 50 male and 50 female C57BL/6/JICO mice per group at 0, 15, 150, 3000, or 8000 ppm. Groups of 10 animals per sex were added and sacrificed after 12 months. The doses corresponded to average doses of about 0, 2.1, 20.6, 432, and 1225 mg/kg/day for males; and to 0, 2.8, 28.5, 557, and 1411 mg/kg/day for females. Two control groups each containing 50 mice per sex for the main study and 10 mice per sex for the satellite study were maintained to obtain more historical data for this strain of mouse. The treated groups were compared to each control group and, in some cases, to the combined control groups.

Mortality at 8000 ppm was 60% in males ($p < 0.001$) and 48% in females ($p < 0.001$) when compared with combined control groups. Mortality of males and females in the 18 month study

at 3000 ppm were increased significantly when compared to combined control groups but not when compared with the control group with the highest mortality and the increase in males was the same at 15, 150 and 3000 ppm and may not have been related to test material.

The most commonly reported effect in premature decedents was erosion and ulceration of the glandular stomach (highest in the 8000 ppm group). This occurred in all groups including the controls. Increased incidences of focal necrosis, pigment storage and bile duct proliferation in the liver were seen at 3000 ppm in both sexes. These findings at the 8000 ppm dose were increased in incidence and severity; additionally, increased incidences of basophilic foci, focal hyperplasia, and cellular alterations were also reported. Decreased weight gain was seen in both sexes at all dose levels. Body weight gain in control, 15, 150, 3000 and 8000 ppm dose groups were 8.5, 5.7, 6.7, 4.8 and 1.6 for males and 7.2, 6.1, 4.0, 4.4 and 2.4 g for females, respectively. The mean body weights were significantly less than control animals from the first week of the experiment at the 8000 ppm dose. The decreased weight gain was accompanied by decreased food intake especially at the beginning of the study; however, a decrease in overall food efficiency was seen, especially at the 2 highest doses. Male and female absolute and relative liver, kidney, brain and adrenal organ weights were increased at 3000 and 8000 ppm, but the increase was frequently equivocal at 15 and 150 ppm. The absolute and relative adrenal weights were significantly ($p < 0.01$) increased in both sexes at 3000 and 8000 ppm. The absolute and relative adrenal weights were also slightly increased in males at 15 and 150 ppm; however, there was not a good dose-relationship. An increased incidence of lipoidosis in the adrenal cortex and testicular Leydig cell hyperplasia, seminal vesicle, epididymis, prostate and uterine atrophy and/or reduced size were seen at 3000 and 8000 ppm and at 8000 ppm ovarian stromal hyperplasia and absence of follicles were seen. An increase in polymorphonuclear neutrophils and monocytes, and a decrease in the percent of lymphocytes were seen at 3000 and 8000 ppm. The increases in polymorphonuclear neutrophils and monocytes may have been secondary to the stress associated the increased stomach erosions and liver necrosis noted in these groups. These hematological changes, however, were seen primarily at 8000 ppm, are close to the normal range of values for mice. **The Lowest-Effect-Level (LEL) of 150 ppm (20.6 mg/kg/day for males; 28.5 mg/kg/day for females) was based on significantly decreased mean body weight gains that were greater than 10% less than the mean body weight gain of control animals. A No-Observable- Effect-Level (NOEL) of 15 ppm (2.1 mg/kg/day for males; 2.8 mg/kg/day for females) was identified.**

The incidence of hepatocellular carcinomas was significantly increased in females ($p < 0.001$) at 8000 ppm (22/50) when compared to either control group, 0/50 in control group 0 and 2/50 in control group 1). Most of the carcinomas (17/26) were observed at terminal sacrifice. Only one female developed hepatocellular carcinoma at 8000 ppm in the 12-month satellite study. In surviving males, the incidence of hepatocellular carcinomas was 3/20, $p \leq 0.05$, at 8000 ppm and 0/90 in combined controls. No additional treatment-related neoplastic findings were reported at any dose.

This study is core-guideline and satisfies the guideline requirements for a 83-2 oncogenicity feeding study in mice.

Comments by the RfD/QA Peer Review Committee:

Some members of the RfD/QA Committee objected to the NOEL of 15 ppm for males (2.1 mg/kg/day) and females (2.8 mg/kg/day) and considered that the NOEL should be 150 ppm (20.6 and 28.5 mg/kg/day for males and females, respectively) with the LEL being 3000 ppm

(432 and 557 mg/kg/day for males and females, respectively) based on the failure of a good dose response at 0, 15 and 150 ppm in the body weight and body weight gain data and efficiency of food utilization. However, after acknowledging that mouse body weight gain and food efficiency data in a mouse feeding study are sometimes difficult to interpret, the committee considered that since the NOEL of 1.2 mg/kg/day from the rat studies was lower and acceptable, the NOEL in the mouse study was mute. The committee considered the matter no further.

Special Review Criteria (40 CFR 154.7) None

A. MATERIALS

1. Test material: Vinclozolin

Description: crystalline solid

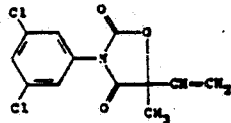
Lot/Batch No.: N 183

Purity: >99 % a.i.

Stability of compound: The stock compound was analyzed at the end of the study and shown to still be >99%.

CAS No.: 50471-44-8

Structure:



2. Vehicle and/or positive control

No vehicle was used. Test material was mixed directly with feed; no positive control was included.

3. Test animals

Species: mouse

Strain: C57BL/6/JICO

Age and weight at study initiation: males were 53 - 55 days old; females were 55 - 57 days old; mean weights: males 23 g (18.5 - 25.2 g) main experimental groups, and 23 g (20.4 - 24.2 g) satellite groups; females 19 g (15.5 - 20.6 g) main experimental groups, and 19 g (14.8 - 21.7 g) satellite groups.

Source: IFFA CREDO Botte Postale 0109, F-69592 L'Arbresle Cedex, France

Housing: The mice were housed singly in type MI Makrolon cages with mesh wire tops supplied by BECKER & CO. The floor area per cage was about 200 cm². Bedding material, type 3/4, was supplied by SSNIFF, and was regularly assayed for contaminants including chlorinated hydrocarbons and heavy metals.

Environmental conditions:

Temperature: 20-24°C

Humidity: 30-70%

Air changes: not provided
 Photoperiod: 12 hours light/12 hours dark
 Acclimation period: 13-15 days

B. STUDY DESIGN

1. Animal assignment

Animals were assigned with the aid of a computerized randomization list according to body weight to the test groups in Table 1. Satellite animals were utilized for hematology and interim examinations after 12 months of treatment.

TABLE 1. STUDY DESIGN					
Dose Group	Doses (mg/kg/day)			No. Animals	
	Dietary Conc. Both Sexes (ppm)	Approximate Dosage Achieved mg/kg/day (Mean)		Male	Female
		Males	Females		
0 Control 18 mo.	0	0	0	50	50
0S Control 12 mo.	0	0	0	10	10
1 Control 18 mo.	0	0	0	50	50
1S Control 12 mo.	0	0	0	10	10
2 Main Study	15	2.1	2.8	50	50
2 Satellite	15	2.0	3.0	10	10
3 Main Study	150	20.6	28.5	50	50
3 Satellite	150	21.2	26.9	10	10
4 Main Study	3000	432	557	50	50
4 Satellite	3000	436	557	10	10
5 Main Study	8000	1225	1411	50	50
5 Satellite	8000	1243	1479	10	10

Data were extracted from MRID No. 432547-04, pp. 0030, 0031, and 0047.

Dose selection rationale: Data from two previous studies were available. The test substance was given to 50 NMRI mice per sex in doses of 162, 486, 1458 and 4374 ppm

in the diet for 112 weeks. Significantly decreased body weight gain and decreased food consumption in males were seen at 1458 and 4374 ppm. No other dose dependent findings were reported and no carcinogenic potential of the test substance was found. In a 3-month feeding study, 10 C57BL mice/sex/group were given food containing 100, 1000, or 5000 ppm vinclozolin. Slightly (8%) reduced body weight compared to controls was reported in males at 5000 ppm. The high dose also resulted in hematological changes including increased hemoglobin, hematocrit, mean cell volume, mean corpuscular hemoglobin, leukocytes, and lymphocytes in both sexes, and increased erythrocytes in males. Changes in clinical chemistry values were seen at 5000 ppm including increased alanine aminotransferase activity in both sexes; increased protein, globulins, and alkaline phosphatase activity in males; decreased glucose in males; and decreased triglycerides and cholesterol in both sexes. Increased absolute and relative liver weights with histological changes were reported in both sexes, and histological changes in the adrenal glands of both sexes, and in the testes and ovaries occurred at 5000 ppm. At 1000 ppm, relative liver weights were increased, histochemical changes were seen in the adrenal glands of both sexes, and increases in hemoglobin, mean cell volume and mean corpuscular hemoglobin were seen in females. No substance-induced findings were seen at 100 ppm.

The selection of the high dose was based primarily on the 3-month experiment where evidence of liver toxicity, lipid metabolism impairment, and possible hormone imbalance were seen at 5000 ppm with no effect on mortality. Two control groups were established for the main (18 month) study and the satellite (12 month) study. These groups were composed of 50 animals per sex in the main study and 10 animals per sex in the satellite study. The additional control groups were utilized to expand the data base for untreated C57BL mice.

2. Diet preparation and analysis

Diet was prepared by mixing a weighed amount of test substance with a small amount of food in a BOSCH household mixer. This mixture was then diluted with an appropriate amount of food to achieve the desired concentrations and then mixed for about 10 minutes in a GEBR. LÖDIGE laboratory mixer. The mixtures were prepared at intervals over which the chemical was proven to be stable (approximately 32 days), and were stored at room temperature. In a previous experiment, a total of six samples each of the 150 ppm and 4500 ppm diet mixtures were tested for homogeneity, and the stability of the compound in the mixture was tested for 32 days at room temperature. The pure compound was tested for stability at the end of the study. During the study, samples of treated food were analyzed at three month intervals for stability and concentration.

Results -

- a. Homogeneity analysis - The variation in the samples tested were found to be $\pm 0.9\%$ for the 150 ppm samples and $\pm 1.5\%$ for the 4500 ppm samples.
- b. Stability analysis - The vinclozolin-food mixture was found to contain 94% of the original concentration of chemical after storage for 32 days at room temperature. The purity of the stock vinclozolin was assayed to be 99.3% at the end of the experiment.

- c. Concentration analysis - The samples with a target concentration of 15 ppm varied on analysis between 85.7 and 100% of the theoretical value; the target concentration of 150 ppm varied between 86.5 and 97.3%; the target concentration of 3000 ppm varied between 90.1 and 97.2%; and the target concentration of 8000 ppm varied from 95.7 to 106.8% of the theoretical concentration.

3. Diet

Animals received food (ground Kliba maintenance diet rat/mouse/hamster 343 meal supplied by KLINGENTALMÜHLE AG, Kaiseraugst, Switzerland) and water *ad libitum*.

4. Statistics

A parametric one-way analysis of variance was performed utilizing the F-test (ANOVA) for body weights and body weight changes. If the results were significant ($p \leq 0.5$), comparisons of the control group with groups 2-5 were done simultaneously using Dunnett's test for the hypothesis of equal means. Both tests were performed two-sided. The two control groups were compared using the Student's t-test for the hypothesis of equal means. Statistical significance was listed as $p \leq 0.05$.

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

C. METHODS AND RESULTS

1. Observations

Animals were inspected twice daily on weekdays and once daily on weekends and holidays for signs of toxicity and mortality. Comprehensive clinical examinations were carried out at least once weekly.

Results - Increased mortality occurred after 18 months (60% males, 48% females) of treatment at 8000 ppm vinclozolin in the main study. The clinical findings reflect the increase seen in mortality with increased incidences of "reduced general state" and hypothermia. The deaths seen at the other doses were significant when compared to the combined control groups, but not significant in males when compared to the individual control group with the highest mortality. This indicates that the mortality seen at 3000 ppm and lower doses is near the normal variation. There was also an increase in the incidence of abdominal palpable masses in females at the high dose in the 18 month study. No other dose-related findings were reported. The distribution of premature deaths is shown in Table 2 for the 18 month main study and the 12 month satellite study combined. Two identically treated control groups were utilized for both the main and satellite studies to obtain more historical data on the C57BL/6/JICO mice. These control groups were combined in Tables 2.

TABLE 2. MORTALITY RATES AND COX OR GENERALIZED K/W TEST RESULTS IN THE MOUSE CARCINOGENICITY STUDY: 18-MONTH MAIN STUDY AND SATELLITE STUDY COMBINED.					
Treatment Period (Weeks)	Males, Dose (ppm)				
	0 n=120	15 n=60	150 n=60	3000 n=60	8000 n=60
0-26	0/120	2/60	1/60	0/60	7/60
27-52	5/120	5/58	4/59	9/60	20/53
53 ^a	19/115	9/53	9/55	9/51	5/33
53-67	3/96	3/44	5/46	0/42	7/28
68-80 ^b	3/93	2/41	2/41	3/42	1/21
Mortality rates⁺ (%)	11/101 (11%)**	12/51 (24%)*	12/51 (24%)*	12/51 (24%)*	35/55 (64%)**
Treatment Period (Weeks)	Females, Dose (ppm)				
	0 n=120	15 n=60	150 n=60	3000 n=60	8000 n=60
0-26	0/120	2/60	1/60	6/60	7/60
27-52	3/120	1/58	3/59	4/54	12/53
53 ^a	19/117	10/57	9/56	9/50	6/41
53-67	3/98	0/47	0/47	0/41	3/35
68-80 ^b	5/95	1/47	0/47	3/41	6/32
Mortality rates⁺ (%)	11/101 (11%)*	4/50 (8%)^c	4/51 (8%)^c	13/51 (25%)**	28/54 (52%)**

Data were taken from MRID No. 432547-04, Volume 1, Table 205, p. 0265 and Table 207, p. 0267 and a memorandum from Lori Brunsman of HED to David G Anderson of HED, dated 7/18/95, Vinclozolin Quantitative Risk Assessment Based on Wistar Rat (Chbb:THOM (SPF)) Rat and C57BL/JICO Mouse Dietary Studies. ⁺ = Number of animals that died during interval/Number of animals alive at the beginning of the interval. ^a = Interim sacrifice at week 53. ^b = Final sacrifice at week 79. ^c = Negative change from control. n = Total number of animals in a combined group. Significance of pair-wise comparison with control denoted a dose level; * = p < 0.05; ** = p < 0.01. Significance of trend denoted at control. Note: Time interval were selected for display purposes only.

2. Body weight

Animals were weighed each week for the first 14 weeks and at 4-week intervals thereafter until the end of the experiment.

Results - The group mean body weights at various times through the 18-month and 12-month studies compared to control group 0 are given in Tables 3a and 3b, respectively. The mean terminal body weights of both male and female mice were consistently and significantly ($p < 0.01$) decreased at 8000 ppm vinclozolin compared to controls in both the main and satellite studies. Slight decreases in weight gain were observable early in the experiment in both the main and satellite studies. The decrease in weight gain achieved statistical significance at various times in all dose groups in the 18-month study.

Treatment length (Days)	Males, Dose (ppm)					Females, Dose (ppm)				
	0	15	150	3000	8000	0	15	150	3000	8000
0	22.9	23.0	23.0	23.0	22.8	18.6	18.7	18.7	18.6	18.5
56	26.3	25.1*	24.8**	24.1**	22.8** (48) ^a	21.4	21.3 (49)	20.5**	21.1 (46)	19.7** (44)
182	32.4	30.6* (49)	31.1 (49)	28.4**	24.0** (44)	24.2	24.0 (48)	24.0 (49)	23.2* (44)	21.1** (43)
350	29.9 (49)	25.9** (44)	26.3** (47)	25.8** (42)	21.3** (31)	24.8 (48)	23.8 (48)	23.7 (47)	22.3** (41)	20.7** (36)
546	31.4 (43)	28.7** (40)	29.7* (39)	27.8** (39)	24.4** (20)	25.8 (44)	24.8 (46)	22.7** (47)	23.0** (38)	20.9** (26)
Total weight gain	8.5	5.7	6.7	4.8	1.6	7.2	6.1	4.0	4.4	2.4

Data taken from MRID No. 432547-04, Volume 1, Table 047-050, p. 0107-0110, and Table 071-074, pp. 0131-0134.

^a(No. animals if less than 50)

* $p < 0.05$; ** $p < 0.01$ significantly different from controls.

Treatment length (Days)	Males, Dose (ppm)					Females, Dose (ppm)				
	0	15	22.6	3000	8000	0	15	150	3000	8000
0	22.6	22.7	22.8	23.0	22.8	18.8	19.0	19.2	18.6	18.9
56	26.2	25.2	24.5	24.6	22.6**	21.6	21.4	20.9	21.0	19.8**
182	32.3	29.9 (9)	30.2	29.2*	23.9** (9)	24.5	23.8	23.6	22.6	20.7**
350	29.3 (9)	25.1 (9)	24.8* (9)	25.7 (9)	20.2** (6)	25.9	24.3	22.9** (9)	23.1* (9)	21.1** (6)

Data were taken from MRID No. 432547-04, Volume 1, Table 059-062, p. 0119-0122, and Table 083-086, p. 0143-0146.

* (No. animals if less than 10)

* p < 0.05; ** p < 0.01 significantly different from controls.

3. Food consumption and compound intake

Food consumption for each animal was determined weekly for the first 14 weeks and at 4-week intervals thereafter. Mean daily diet consumption was calculated as g food/kg body weight/day. Food efficiency (body weight gain, kg/food consumption, kg per unit time X 100) and compound intake (mg/kg/day) values were calculated as time-weighted averages from the food consumption and body weight gain data.

Results -

- a. Food consumption - The mice in the 8000 ppm groups consistently consumed less food than the control groups and the other treated groups. All treated groups showed slightly decreased food consumption compared with the control groups. This trend was evident from the beginning of the experiment, which maybe indicative of food palatability problems. The mean food consumption per mouse per day at various weeks through the main (18 month) study is given in Table 4. The same trend was evident in the satellite (12 month) experiment.

**TABLE 4. GROUP MEAN FOOD CONSUMPTION
(G/ANIMAL/DAY) AT WEEKLY INTERVALS FROM THE MAIN STUDY**

Week of Study	Treatment Group/Exposure Level (ppm)									
	Males					Females				
	0	15	150	3000	8000	0	15	150	3000	8000
1	4.7	4.2	4.3	3.9	2.6	5.0	4.6	4.7	4.4	2.6
6	4.2	3.4	3.0	3.8	3.6	5.0	4.4	4.2	4.2	3.9
14	4.5	4.3	4.4	4.3	3.8	5.7	4.7	4.6	4.7	4.4
26	4.5	4.2	4.3	4.1	4.0	4.7	4.2	4.3	4.1	3.8
54	3.8	3.4	3.4	3.4	3.1	4.5	4.5	4.5	4.4	3.5
78	4.3	4.1	4.2	4.0	3.7	4.8	4.3	3.8	3.9	3.2
Total food consumed	2277.1	2092.3	2124.5	2099.3	1946.7	2637.6	2424.1	2414.1	2315.6	2008.3

Data taken from MRID No. 432547-04, Tables 005-008, pp. 0065-0068, and Tables 026-029, pp. 0086-0089.

- b. Compound consumption (time-weighted average) - The compound consumption calculated from the food consumption and body weights is given in Table 1. The study authors stressed that these values were estimates because of the approximate nature of the food consumption values due to spillage.
- c. Food efficiency - The authors calculated food efficiencies using weekly food intake and body weight measurements. These calculations resulted in large variations from week to week making the numbers difficult to evaluate. The food efficiencies for the entire experiment were calculated by the reviewer using the average weight gained and food consumed over the 18 month period. Utilizing the body weights taken on the first and last days of the study (MRID No. 432547-04, Tables 047 and 050, p. 0107 and 0110, respectively) the values are: 0.374, 0.272, 0.315, 0.229 and 0.082 for males at 0, 15, 150, 3000, and 8000 ppm vinclozolin, respectively. Females also showed decreased food efficiencies with treatment, especially with the high-dose. The calculated food efficiency values for females are: 0.273, 0.252, 0.166, 0.190, and 0.120 at 0, 15, 150, 3000, and 8000 ppm, respectively for body weights taken on day 0 and day 546 (MRID No. 432547-04, Tables 071 and 074, p. 0131 and 0134, respectively).

4. Ophthalmoscopic examination

An ophthalmoscopic examination was not performed.

5. Blood was collected from all surviving mice of the satellite groups following a 16 to 20 hour fasting period at the interim sacrifice (12 months), and from all surviving animals at the termination of the main study. Differential blood counts were performed on all samples from the 12 month study, and the control groups and high dose (3000 and 8000 ppm) group samples of the 18-month study. The CHECKED (X) parameters were examined.

a. Hematology

X	Hematocrit(HCT)*	X	Leukocyte differential count*
	Hemoglobin (HGB)*	X	Mean corpuscular HGB (MCH)
X	Leukocyte count (WBC)*		Mean corpusc. HGB conc.(MCHC)
	Erythrocyte count (RBC)*		Mean corpusc. volume (MCV)
	Platelet count*		Reticulocyte count
	Blood clotting measurements		
	(Thromboplastin time)		
	(Clotting time)		
	(Prothrombin time)		

* Required for subchronic and chronic studies.

Results - Vinclozolin treatment resulted in a dose-related increase in polymorphonuclear neutrophils in both sexes at the 12-month interim sacrifice (satellite study) and at the 18 month main study termination. The increase in these white blood cell parameters may be secondary to the stress caused by the stomach erosion and liver necrosis seen at these dose levels. A dose-related decrease in lymphocytes was also observed in both sexes at the same time points. A slight increase in monocytes was seen in both sexes in the 18 month study at the high dose. Although these were apparently dose-related effects, published hematology values for mice show wide variations in normal animals indicating all values are close to the normal range for inbred mice (see Charles River Laboratories Technical Bulletin, Base Line Hematology and Clinical Chemistry Values for CR Outbred Mice, Summer 1986). No dose-related morphological variations were reported in red or white blood cells. Results of the differential counts are summarized in Table 5.

b. Clinical chemistry

Clinical chemistry values are not required for a 83-2 oncogenicity study.

TABLE 5. DIFFERENTIAL BLOOD COUNT % OF TOTAL										
CELL TYPE %	Treatment Group/Exposure Level (ppm)									
	Males					Females				
	0	15	150	3000	8000	0	15	150	3000	8000
EOS	1.11 ^a (0.70)	0.89	0.89	0.67 (0.54)	1.4 (0.20)	1.50 (1.14)	0.40	0.67	0.89 (1.03)	0.50 (0.50)
BASO	0.00 (0.00)	0.22	0.33	0.11 (0.13)	0.00 (0.00)	0.00 (0.02)	0.00	0.00	0.00 (0.05)	0.00 (0.00)
BAND	0.22 (0.07)	0.11	0.00	0.22 (0.82)	0.60 (0.00)	0.00 (0.09)	0.00	0.00	0.00 (0.32)	0.67 (0.19)
POLY	10.22 (18.77)	11.67	16.56	19.22 (19.90)	27.00 (25.95)	10.00 (17.30)	14.0 0	14.67	15.89 (21.63)	16.0 (29.69)
LYMP	85.33 (76.60)	82.33	77.22	74.78 (73.05)	67.20 (65.95)	85.00 (75.91)	80.5 0	79.78	77.33 (70.74)	77.33 (60.42)
MONO	3.11 (3.86)	4.78	5.00	5.00 (5.56)	3.80 (7.90)	3.50 (5.53)	5.10	4.89	5.89 (6.24)	5.50 (9.19)

Data taken from MRID No. 432547-04, Table 209, p. 0269; Table 212, p. 0272; Table 215, p. 0275; and Table 218, p. 0278.

^a%cell type from 12-month study(% cell type from 18 month study) Only the control (0) and high dose (3000, 8000 ppm) samples were examined at 18 months.

6. Urinalysis

Urinalysis is not required for an oncogenesis study.

7. Sacrifice and pathology

All animals that died and that were sacrificed on schedule by decapitation under CO₂ anesthesia were subject to gross pathological examination and the CHECKED (X) tissues were collected for histological examination. The (XX) organs, in addition, were weighed. The tissues were preserved for examination in 4% formaldehyde solution.

<u>X</u>	Digestive system	<u>X</u>	Cardiovasc./Hemat.	<u>X</u>	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	XX	Adrenal gland*
X	Cecum*	XX	Kidneys**		Lacrimal gland
X	Colon*	X	Urinary bladder*	X	Mammary gland*
X	Rectum*	XX	Testes**	X	Parathyroids*
XX	Liver**	X	Epididymides	X	Thyroids*
X	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	All gross lesions and masses*
	Nose				
	Pharynx				
	Larynx				

* Required for subchronic and chronic studies.

** Organ weight required in subchronic and chronic studies.

Results -

- a. **Organ weight** - The absolute and relative weights of the liver and adrenal glands were significantly increased at the high dose in both sexes. Decreases were observed in absolute weights of the brain and kidneys in both sexes and in the testes in the males in treated animals compared to the controls. The relative weight values, which remained constant with the testes and increased with kidney and brain while the absolute weights decreased, suggest that the weight changes in these organs reflect the smaller sizes of the mice in the higher dose groups. The organs that increased or decreased significantly in weight with vinclozolin treatment in the main (18 month) study are summarized in Table 6. The same trends were seen in the satellite (12 month) study.

TABLE 6. ORGAN WEIGHT CHANGES (mg) IN MICE TREATED WITH VINCLOZOLIN										
Organ or Tissue	Treatment Group/Exposure Level (ppm)									
	Males in mg (relative to body weight in %)					Females in mg (relative to body weight in %)				
	0	15	150	3000	8000	0	15	150	3000	8000
Liver	1252 (4.34) ^a	1119 (4.22)	1289 (4.76)	1468 (5.81) ^{**}	2295 ^{**} (10.49) ^{**}	1151 (4.99)	1139 (5.01)	1058 (5.04)	1390 ^{**} (6.55) ^{**}	2560 ^{**} (13.57) [*]
Kidneys	436 (1.51)	406 ^{**} (1.55)	392 ^{**} (1.46)	370 ^{**} (1.47)	348 ^{**} (1.59)	397 (1.73)	394 (1.73)	374 (1.79)	392 (1.86)	369 (1.96) ^{**}
Testes	188 (0.65)	179 (0.68)	187 (0.69)	185 (0.74) ^{**}	152 ^{**} (0.69)	—	—	—	—	—
Brain	484 (1.69)	481 (1.85) ^{**}	482 (1.79) [*]	465 ^{**} (1.86) ^{**}	433 ^{**} (1.99) ^{**}	491 (2.15)	494 (2.19)	484 (2.34) ^{**}	457 ^{**} (2.18)	417 ^{**} (2.23)
Adrenals	3.674 (0.013)	4.474 [*] (0.017) [*]	4.395 (0.017) [*]	8.154 ^{**} (0.033) ^{**}	8.550 ^{**} (0.040) ^{**}	9.326 (0.041)	8.761 (0.039)	9.149 (0.044)	12.053 ^{**} (0.057) ^{**}	12.346 ^{**} (0.066) [*]

Data taken from MRID No. 432547-04, Pathology Report, pp. 0812, 0813, 0820, and 0821; Tables 3 and 4, p. 0778-0779.

^a(Relative weight as % of body weight)

* p < 0.05; ** p < 0.01; *** p < 0.001 significantly different from controls (control group 0).

- b. Gross pathology - The incidences of gross lesions that changed with the dose of vinclozolin are summarized in Table 7. Lesions associated with the liver, adrenal gland, and stomach and reduced size of the testis, epididymis and prostate are notable with the 3000 and 8000 ppm doses. The increase seen in the incidence of discolored foci of the glandular stomach is primarily due to the incidence in the premature decedents. This was the most commonly found lesion in animals that died or were sacrificed in extremis. It was found in 50% of the males and 54% of females. About 15% of males and 8% of surviving females were found to have discolored foci of the glandular stomach. Trends toward decreased ovarian cysts and sternum deformations were seen with increasing doses of vinclozolin.

TABLE 7. INCIDENCE OF GROSS LESIONS IN MICE FED VINCLOZOLIN IN THEIR DIET FOR 18 MONTHS (50 MICE/GROUP INCLUDING DECEDENTS)

Affected Organ or Tissue/ Gross Lesion	Treatment Group/Exposure Level (ppm)									
	Males					Females				
	0	15	150	3000	8000	0	15	150	3000	8000
Glandular stomach/ discolored foci	4	8	7	8	18 ^{***}	3	3	3	9 [*]	14 ^{**}
Liver/enlarged	1	0	1	3	6	0	0	0	1	2
Liver/discolored/foci	1	3	3	7 [*]	27 ^{***}	2	1	1	4	33 ^{***}
Testes/reduced size	1	1	0	3	4	—	—	—	—	—
Epididymides/ reduced size	0	0	0	11 ^{***}	12 ^{***}	—	—	—	—	—
Seminal vesicle/ reduced size	1	3	3	36 ^{***}	42 ^{***}	—	—	—	—	—
Prostate/reduced size	0	2	0	27 ^{***}	28 ^{***}	—	—	—	—	—
Ovary/cyst	—	—	—	—	—	9	4	8	2	0
Ovary/enlarged	—	—	—	—	—	0	0	0	0	16 ^{***}
Adrenal cortex/ enlarged	0	1	0	19 ^{***}	17 ^{***}	2	1	0	20 ^{***}	14 ^{***}
Adrenal cortex/ discoloration	0	0	0	1	4	0	0	0	1	0
Sternum/deformation	15	14	15	6	5	1	1	1	4	1

Data taken from MRID No. 432547-04, Pathology Report, pp. 0831-0836.

* p < 0.05, ** p < 0.01, *** p < 0.001 significantly different from controls. Fisher exact test performed by reviewer.

c. Microscopic pathology -

- 1) Non-neoplastic - A summary of the relative changes obtained by microscopic examination of tissues and organs from treated animals compared to control animals in the main (18 month) study is given in Table 8. Several histological findings in the liver were reported at the 3000 and 8000 ppm doses in both sexes. Treatment at the two highest doses also resulted in hyperplasia of the Leydig cells in the testes and atrophy of the seminal vesicles and coagulation glands in males, and uterine atrophy in females. The 8000 ppm dose also resulted in ovarian stromal hyperplasia, a decrease in ovarian cysts and the absence of follicles in the ovaries and uterine cystic hyperplasia. Treatment related increases in adrenal cortex lipoidosis was seen in both sexes. Increased incidences of foam cells and eosinophilic crystals in

TABLE 8. INCIDENCE OF NON-NEOPLASTIC LESIONS IN MICE FED VINCLOZOLIN IN THEIR DIET FOR 18 MONTHS 50 MICE/GROUP INCLUDING DECEDENTS

Affected Organ or Tissue/Lesion	Treatment Group/Exposure Level (ppm)									
	Males					Females				
	0	15	150	3000	8000	0	15	150	3000	8000
Liver/ centralobular fatty infiltration	3 6% ^a	5 10%	4 8%	3 6%	23 ^{***} 46%	4 8%	3 6%	11 [*] 22%	0	16 ^{**} 32%
Liver/ focal necrosis	1 2%	5 10%	3 6%	15 ^{***} 30%	21 ^{***} 42%	9 18%	3 6%	8 16%	12 24%	23 ^{**} 46%
Liver/ pigment storage	0	1 2%	0	6 [*] 12%	44 ^{***} 88%	0	3 6%	5 [*] 10%	17 ^{**} 34%	43 ^{***} 86%
Liver/ diffuse hypertrophy	0	0	0	1 2%	47 ^{***} 94%	0	0	0	2 4%	32 ^{***} 64%
Liver/ bile duct proliferation	0	1 2%	0	8 ^{**} 16%	44 ^{***} 88%	1 2%	2 4%	4 8%	31 ^{**} 62%	44 ^{***} 88%
Liver/ cellular alterations	2 4%	0	0	1 2%	18 ^{***} 36%	0	0	0	1 2%	28 ^{***} 56%
Liver/ basophilic foci	2 4%	0	0	0	15 ^{***} 30%	0	0	0	1 2%	19 ^{***} 38%
Liver/ focal hyperplasia	0	0	0	0	7 ^{**} 14%	0	0	0	2 4%	12 ^{***} 24%
Lunga/ foam cells	1 2%	1 2%	2 4%	2 4%	11 ^{**} 22%	4 8%	1 2%	0	0	2 4%
Lunga/ eosinoph. crystals	1 2%	0	1 2%	2 4%	10 ^{**} 20%	4 8%	1 2%	0	0	3 6%
Testes/ diffuse Leydig cell hyperplasia	4 8%	2 4%	0	6 12%	22 ^{***} 44%	—	—	—	—	—
Seminal vesicle/ atrophy	1 2%	2 4%	2 4%	42 ^{***} 86%	45 ^{***} 94%	—	—	—	—	—
Coagulation glands/ atrophy	0	0	0	4 9%	17 ^{***} 59%	—	—	—	—	—
Ovaries/ follicle absence	—	—	—	—	—	19 38%	14 28%	20 40%	23 46%	35 ^{***} 70%
Ovaries/ stromal hyperplasia	—	—	—	—	—	0	1 2%	0	1 2%	11 ^{***} 22%
Uterus/ atrophy	—	—	—	—	—	2 4%	5 10%	5 10%	9 [*] 18%	46 ^{***} 92%
Thymus/ lymphocyte. depletion	1 2%	0	0	2 4%	11 ^{**} 22%	1 2%	0	0	0	2 4%
Adrenal cortex/ lipoidosis	4 8%	1 2%	1 2%	11 [*] 22%	46 ^{***} 92%	14 28%	19 38%	21 42%	39 ^{**} 78%	47 ^{***} 94%
Continued on the next page										

TABLE 8. INCIDENCE OF NON-NEOPLASTIC LESIONS IN MICE FED VINCLOZOLIN IN THEIR DIET FOR 18 MONTHS 50 MICE/GROUP INCLUDING DECEDENTS

Affected Organ or Tissue/Lesion	Treatment Group/Exposure Level (ppm)									
	Males					Females				
	0	15	150	3000	8000	0	15	150	3000	8000
Kidney/basophilic tubules	8	7	12	2	1	9	1	0	0	1
Kidney/chronic nephropathy	11+	9	8	2	1	18	11	5	5	2
Kidney/lymphoid infiltration	20	10	13	18	7	29	33	26	24	7
Ovaries/cysts	-	-	-	-	-	18	6	10	4	1
Uterus/cystic hyperplasia	-	-	-	-	-	45	33	21	19	0

Data taken from MRID No. 432547-04, Pathology Report, pp. 0855-0864 (The data in Table 8 on this page was added after the Fisher exact test was determined. *Percent occurrence in animals examined [(incidence/no. examined) x 100].

* p < 0.05, ** p < 0.01, *** p < 0.001 significantly different from controls. Fisher exact test performed by reviewer.

+ Average of the 2 control groups.

the lungs and lymphocyte depletion in the thymus were seen at the 8000 ppm dose in males. The same trends were seen with the 12-month study and in the decedents. An increased incidence of erosion and ulcers in the glandular stomach was seen in premature decedents from all experimental and control groups when compared to the survivors of the other groups. The ulcers appeared in the control animals as well as the treated, however, the incidence was slightly higher in the high dose animals than in the controls.

In addition, there were apparent dose related decreases in several histological findings that should be noted. Only part of the following findings are presented in Table 8. Although, the mechanism of interaction of vinclozolin with these lesions is unknown, the possibility that they may be related to the antiandrogenicity and/or the lipid metabolism/storage/adrenal effects of vinclozolin should not be excluded. In males, decreases occurred in kidney basophilic tubules (14% in combined control vs. 2% at 8000 ppm), kidney lymphoid infiltration (37% combined controls vs. 14% at 8000 ppm), kidney chronic nephropathy (21% in combined controls vs. 2% at 8000 ppm) and pituitary cysts (37% in combined controls vs. 4% at 8000 ppm). In females, decreases occurred in kidney basophilic tubules (11% in combined control vs. 2% at 8000 ppm), kidney lymphoid infiltration (61% in combined controls vs. 14% at 8000 ppm), kidney chronic nephropathy (38% in combined controls vs. 4% at 8000 ppm), brain calcification (41% in combined controls vs. 2% at 8000 ppm), ovarian follicles absent (40% in combined controls vs. 70% at 8000 ppm), uterine cystic hyperplasia (87% in combined controls vs. 0% at 8000 ppm), pituitary hyperplasia of the pars dist. (38% in combined controls vs. 2% at 8000 ppm) fibro-osseous lesions of the sternum (47% in combined controls vs. 6% at 8000 ppm) and of the femur (58% in combined controls vs. 8% at 8000 ppm).

- 2) Neoplastic - A significantly increased incidence of hepatocellular carcinoma was seen in female mice at the 8000 ppm dose compared to either control group. The incidence of hepatocellular carcinoma in control group 1 was 2/50 (MRID 432547-04, p. 884). No other treatment related changes in the incidences of neoplastic lesions were reported. A summary of the incidences of liver tumors and other more commonly occurring neoplastic lesions seen in treated and control group 0 mice in the main (18 month) study is given in Table 9. The liver tumors primarily occurred in older animals that completed the study. Hepatocellular carcinomas were found in 17 out of 26 (65%) female survivors and in 3 out of 20 (15%) male survivors. The incidence of liver carcinomas in male mice reached statistical significance ($p < 0.05$) only if the comparison was limited to the surviving animals in the treated and control groups. Only 1 hepatocellular carcinoma was found in a female mouse at 8000 ppm in the 12-month satellite study animals, and only 5 were reported in the female premature decedents at the 8000 ppm dose in the 18-month study. None were found in male premature decedents at the 8000 ppm dose, however, one hepatocellular carcinoma in a male mouse was reported at the 3000 ppm dose in the 18-month study.

TABLE 9. INCIDENCE OF NEOPLASTIC LESIONS IN MICE FED VINCLOZOLIN IN THEIR DIET FOR 18 MONTHS (50 MICE/GROUP INCLUDING DECEDENTS)

Affected Organ or Tissue/ Lesion	Treatment Group/Exposure Level (ppm)									
	Males					Females				
	0	15	150	3000	8000	0	15	150	3000	8000
Liver/ hepatocellular carcinoma	0	0	0	1 (2%) ^a	3 (6%)	0	0	0	0	22 ^{***} (44%)
Liver/ hemangioma	0	0	1 (2%)	1 (2%)	2 (4%)	0	0	0	0	0
Other sites/hemangioma	0	1 (2%)	0	1 (2%)	0	0	0	0	0	0
Liver/ hemangiosarcoma	0	0	1 (2%)	2 (4%)	1 (2%)	0	0	0	0	2 (4%)
Other sites/hemangiosarcoma	0	1 (2%)	0	1 (2%)	0	0	0	1 (2%)	1 (2%)	0
Lungs/ adenoma	8 (16%)	1 [°] (2%)	2 [°] (4%)	1 [°] (2%)	0 ^{**}	1 (2%)	3 (6%)	1 (2%)	1 (2%)	1 (2%)
Lymphoreticular system/ lymphoma	8 (16%)	2 [°] (4%)	2 [°] (4%)	3 (6%)	1 [°] (2%)	8 (16%)	5 (10%)	7 (14%)	6 (12%)	3 (6%)

Data taken from MRID No. 432547-04, Pathology Report, pp. 0882-0886.

^aPercent occurrence in animals examined [(incidence/no. examined) x 100].

[°] $p < 0.05$, ^{**} $p < 0.01$, ^{***} $p < 0.001$ significantly different from controls. Fisher exact test performed by reviewer.

D. DISCUSSION

Groups of 50 male and 50 female C57BL/6/JICO mice were fed diets containing 0, 15, 150, 3000, or 8000 ppm vinclozolin for 18 months. Groups of 10 males and 10 females were fed the same diets in a satellite experiment for 12 months to provide for interim blood and pathology examinations. Two control (0 ppm) groups containing 50 animals per sex were maintained to help expand the data base for normal variations in this mouse strain. Comparisons were made in the study to each of the control groups and, in some cases, to the combined control groups. There were no biologically significant differences in the control groups. The reported comparisons were made with control group "0" unless otherwise indicated.

Treatment of female C57BL/6 mice for 18 months at 8000 ppm vinclozolin in the diet resulted in a significant increase in the incidence of hepatocellular carcinomas. A total of 22 (44%) of the females in the 8000 ppm dose group, including premature decedents, and 17 (65%) of the 18-month survivors were found to have hepatocellular carcinomas. The average intake of vinclozolin by females in the 8000 ppm dose group was calculated by the authors to be about 1411 mg/kg/day. A total of 3 (6%) of the males at 8000 ppm developed hepatocellular carcinomas. These affected animals were among the 20 18-month male survivors. Liver carcinoma was found in one male mouse at 3000 ppm that died prematurely in the 18-month study, and in one female at 8000 ppm in the 12 month study. No other treatment-related neoplastic lesions were reported.

Mortality was increased to 60 and 48% for males and females respectively at 8000 ppm compared to about 10% mortality for the combined control groups in the 18-month experiment. Mortality for males treated for 15 months was greater than 50% at 8000 ppm. Lesions in the glandular stomach (erosion, ulcers) were commonly found in all groups of animals that died before the end of the study, but were not commonly seen in the survivors. A number of lesions were identified upon histological examination of livers from animals treated at 3000 and 8000 ppm. Increases in diffuse hypertrophy, basophilic foci, and focal hyperplasia of the liver were primarily seen at 8000 ppm. Secondary targets were reproductive tissues and the adrenal cortical tissue of both sexes. Increases in the incidence of foam cells and eosinophilic crystals in the lungs and lymphocyte depletion in the thymus were seen in males at the 8000 ppm dose. Significant increases in absolute and relative weights of the livers and adrenals of animals at 3000 and 8000 ppm were consistent with the microscopic findings. The decrease in weight gain seen in both sexes at 3000 and 8000 ppm was considered to be substance-related by the study author.

The evaluation of the decreased weight gain seen in all treated groups from the first month of the experiment was complicated by possible unpalatability of the diet, which resulted in decreased food intake and food scattering. The decreases in weight gain seen at 15, 150, and 3000 ppm were sporadically significant through the course of the experiment, but were not dose-related and were accompanied by decreased food intake. The weight decreases at 15 and 150 ppm were not considered related to compound intake by the study author. However, the mean terminal weights of the female mice at 150 ppm was about 12% less than the mean weights of the control animals in both the main and satellite studies. The mean terminal weight decreases in the male mice compared to the controls were less than 10% at 15 and 150 ppm in the main study, but in the satellite study, the mean terminal weight at 150 ppm was significantly ($p < 0.05$) decreased and was 15.5% less than the mean

satellite control body weight. However, there was an overall decrease in relative efficiency of food utilization that may have been caused by the androgen receptor inhibition, systemic toxicity and/or food scattering, especially at the 2 highest dose levels.

The increases in absolute adrenal weights seen in males at 15 and 150 ppm did not show dose dependency and were not accompanied by any adverse gross or microscopic findings at these low dose levels. The changes in absolute and relative kidney weights and in relative brain weights were not accompanied by adverse gross or microscopic findings at any dose of vinclozolin, and may reflect the decreased weight gain seen in treated mice. A decrease in terminal body weight of greater than 10% was considered to be an adverse effect, especially when the changes were accompanied by slight decreases in overall food efficiencies. Whether this effect can be attributed to the compound intake or to the unpalatability of the diet cannot be definitely determined.

There were several lesions and effects in the kidneys and pituitary in males and of the kidney, brain, pituitary, bone, ovary and uterus in females that were higher in controls than at 8000 ppm. Although a relationship among these effects and the antiandrogenicity and/or lipid metabolism/storage/adrenal effects of vinclozolin is unknown, the possibility of a relationship should not be dismissed.

A No-Effect-Level (NOEL) of 15 ppm (2.1 mg/kg/day for males, 2.8 mg/kg/day for females) was identified, and a Lowest-Effect-Level (LOEL) of 150 ppm (20.6 mg/kg/day for males, 28.5 mg/kg/day for females) was determined based on decreased weight gain resulting in greater than 10% decrease in mean terminal weights compared to mean control weights.

Some members of the RfD/QA Committee objected to the NOEL of 50 ppm for males (2.1 mg/kg/day) and females (2.8 mg/kg/day) and considered that the NOEL should be 150 ppm (20.6 and 28.5 mg/kg/day for males and females, respectively) with the LEL being 3000 ppm (432 and 557 mg/kg/day for males and females, respectively) based on the failure of a good dose response at 0, 50 and 150 ppm in the body weight and body weight gain data and efficiency of food utilization. However, after acknowledging that mouse body weight gain and food efficiency data in a mouse feeding study are sometimes difficult to interpret, the committee considered that since the NOEL of 1.2 mg/kg/day from the rat studies was lower and acceptable, the NOEL in the mouse study was mute. The committee considered the matter no further.

E. STUDY DEFICIENCIES

The survival of male mice in the 8000 ppm dose group was below the guideline requirement for 15 month survival in an oncogenic study. The severe toxic effects incurred at this dose indicate that a dietary level of 8000 ppm was above the maximum tolerated dose. This experiment, however, had 3 additional dose levels that fulfill the requirements.

The NOEL and LOEL can not be precisely defined due to the large differences in the dose concentrations (150 ppm to 3000 ppm).