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OFFICE OF PESTICIDES AND TOXIC SUBSTANCES

TO: Henry Jacoby (21)
Registration Division (TS-767)

THRU: William L. Burnam, Acting Chief
Toxicology Branch/HED (TS-769)

SUBJECT: Vinclozolin (RONILAN). Assessment of complete Mouse Oncogenicity Data. 7969-53
Accession#248264 CASWELL#323C

Registrant: BASF Wyandotte Corp.
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P.O. Box 181
Parsipany, New Jersey 07054

This study previously was submitted under Accession No. 096970. In the earlier submission individual animal data were not provided, and histopathological reports were not provided for mice in the three lower dose groups. The current report for the same study provides information on individual animals at all dose levels.

CONCLUSIONS:

1) An apparent increase in the incidence of leukemia/lymphoma type tumors in male mice appeared to result from administration of vinclozolin. However, the incidence in historical controls equaled or exceeded the incidence seen in this study, which would tend to indicate the apparent increase in leukemia/lymphoma incidence due to vinclozolin is not real.

2) An apparent increase in the incidence of lung adenomas was seen in females on vinclozolin (one at 162 ppm, one at 486 ppm, 4 at 1458 ppm, and 5 at 4374 ppm). These were within the range seen in some of the historical controls, but because of the apparent dose-relationship and the lack of tumors in the study controls, we concluded that vinclozolin was a weak and questionable oncogen for lung tumors. Because it produced a maximum 5/50 tumors of a benign nature in one species only (the mouse) at the high doses, and because a decrease in the latency of tumor appearance did not occur, we consider vinclozolin to be only weakly positive in the production of lung tumors. Nevertheless, a risk assessment was carried out using the multi-stage model and the positive findings in the lung.

3) An apparent production of liver adenoma was seen in 3/50 males receiving vinclozolin at the highest (4374 ppm) dose. This would tend to classify vinclozolin as a weak and questionable oncogen for liver tumors in the NMRI strain of mouse. Because of this extremely low incidence of benign tumors at the highest dose only, in one sex of the mouse only; and because a decrease in the latency of tumor appearance did not occur as a result of vinclozolin treatment, we consider vinclozolin to be of questionable oncogenic significance (weakly positive) in the production of liver tumors.

4) The data are equivalent to CORE Minimum.

CHRONIC TOXICITY AND ONCOGENICITY OF VINCLOZOLIN IN MICE. Study conducted by Professor Dr. F. Leuschner, Laboratorium fur Pharmakologie Und Toxicologie, Hamburg. December 15, 1977. Accession No. 248264.

This study previously was submitted under Accession No. 096970. In the earlier submission individual animal data were not provided, and histopathological reports were not provided for mice in the three lower dose groups. The current report for the same study provides information on individual animals at all dose levels.

PROCEDURE: See previous evaluation of May 17, 1982.

RESULTS:

PHYSICAL APPEARANCE, BEHAVIOR, AND MORTALITY: These parameters were not obviously affected by vinclozolin treatment. Mortality varied without regard to dose level, although the highest overall mortality after 112 weeks (males and females combined) was in the top treatment level (4374 ppm, or 656.1 mg/kg/day).

MORTALITY AT 112 WEEKS

DOSE	MALES	FEMALES	MALES AND FEMALES COMBINED
0	48%	80%	64%
162 ppm	62%	76%	69%
486 ppm	56%	64%	60%
1458 ppm	48%	68%	58%
4374 ppm	76%	78%	77%

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FOOD CONSUMPTION: No differences in food consumption can be detected in relation to treatment.

BODY WEIGHT: After 112 weeks, body weights were significantly less in males ($p \leq 0.01$) at the 1458 and 4374 ppm dose levels. Mean body weights were: (g ± S.D.)

	Control		162 ppm		486 ppm		1485 ppm		4374 ppm	
	M	F	M	F	M	F	M	F	M	F
Initial	20.6 0.6	18.6 0.5	20.5 0.6	18.5 0.5	20.6 0.6	18.5 0.5	20.7 0.6	18.5 0.5	20.5 0.6	18.5 0.5
122 wks.	44.8 4.1	36.0 3.3	45.4 5.0	38.3 5.0	42.6 3.4	35.9 4.9	41.8* 4.8	35.8 2.5	39.9* 4.4	34.5 3.6

HEMATOLOGY: Treatment had no effect on hemoglobin, erythrocyte count, leukocyte count, prothrombin time, or reticulocyte and platelet counts.

CLINICAL BIOCHEMISTRY: The laboratory's data show that total proteins in females at the 4374 ppm dose level were significantly reduced ($p \leq 0.01$) at the 52nd week only (61.1 g/L serum in treated females; 64.7 g/L serum in control females). While the laboratory considers this to be a significant difference, we would not consider it toxicologically significant; the control value seems unusually high at this testing (64.7 g/L at 52 weeks, compared with 62.7 g/L at 26 and 78 weeks).

The mean serum uric acid levels appear lower in treated males at 486, 1458, and 4374 ppm than in controls at 78 weeks. (101.2, 97.5, 90.0, 82.7, and 85.9 umol/L for controls, 162, 486, 1458, and 4374 ppm, respectively. These differences are not statistically significant.

No treatment-related differences in biochemistry values are apparent for the other parameters measured: glucose, SGPT, blood urea, alkaline phosphatase, SGOT, and bilirubin.

URINALYSIS RESULTS: Composite urine samples were collected in a metabolic cage from 10 mice of each group pre-treatment, and after 6, 13, 26, 52, and 78 weeks on test. Observations or measurements were made for color, specific gravity, pH, protein, glucose, bilirubin, ketones, hemoglobin, and sediment. No changes were observed which could be attributed to treatment.

OPHTHALMOSCOPIC EVALUATION of the eyes did not reveal any abnormal findings. Also, HEARING of the mice, checked by a "simple noise test", was not adversely affected. These examinations were conducted prior to sacrifice.

ORGAN WEIGHTS: Mean relative liver weights were increased in both males and females on a dose-related basis, except for the low (162 ppm) dose in males, where the mean relative liver weight was less than controls. (Increases of 7.3%, 18.3%, and 53.8% for males at 3 high dose levels; increases in females of 28.6%, 29.3%, 80.6%, and 133.3% for 162, 486, 1458, and 4374 ppm dose levels, respectively.)

From the data it would appear no NOEL exists regarding liver weight in female mice, determined from mice surviving to the end of the experiment. However, the mean liver weight for control females is much lower than in test groups, being 1.66 grams. Only ten of the 50 control females survived to the end of the experiment, and the liver of one of these mice weighed only 0.19 grams, which is about 10% of the liver weights of the other control females. The mean liver weights of control females which died or were killed prematurely is 2.27 grams. By disregarding the mouse with the liver weight of 0.19 grams we derive a mean liver weight of 1.83 grams in control female mice surviving to the end of the experiment.

(This liver weight may have been a recording or typographical error, since a 0.19 gram liver would be most unusual.) We then determined the proportion of liver weight to body weight for the control and treated females surviving to the end of the test, as follows:

	Mean Body Weight	Mean Liver Weight	Percent Increase	Liver Percent of Body Weight	Percent Increase
Control	35.9 g	1.83 g	----	5.1	----
162 ppm	38.3 g	2.27 g	24.0	5.9	15.7
486 ppm	35.9 g	2.14 g	16.9	6.0	17.6
1458 ppm	35.8 g	2.98 g	62.8	8.9	74.5
4374 ppm	34.5 g	3.71 g	102.7	10.8	111.8

The registrant's laboratory claims statistically significant increases in liver weight in both males and females at the high treatment level, 4374 ppm. ($t = 2.760$ in males; $t = 3.122$ in females; student's t-test).

RELATIVE HEART WEIGHTS decreased in females on a dose-related basis. Decreases were 1.6%, 8.8%, 13.1%, and 19.3% for the four treatment levels (162, 486, 1458, and 4374 ppm, respectively).

Mean absolute heart weights were 0.26, 0.26, 0.25, 0.22, and 0.21 g for controls, 162, 486, 1458, and 4374 ppm respectively. This amounts to an absolute heart weight decrease of 0%, 3.8% 15.4%, and 19.2% for the 162 ppm, 486 ppm, 1458 ppm, and 4374 ppm groups, respectively.

RELATIVE SPLEEN WEIGHTS varied in males, but with no particular dose-related pattern. In the females the relative spleen weights of all treatment groups exceed those of controls by 149%, 65%, 125%, and 170% for the 162 ppm, 486 ppm, 1458 ppm, and 4374 ppm dose levels, respectively. The spleen weights of individual control females varied considerably among individual mice -- from 0.04 g to 1.98 g, or approximately 50-fold -- without any consistent associated causative or resultant pathology.

TESTES WEIGHTS (mean relative and absolute) were increased over controls at the two high dose levels (increased by 41.7 and 36.7% in males on 1458 and 4374 ppm dose levels, respectively).

HISTOLOGY, NON-NEOPLASTIC LESIONS: In summarizing necropsy findings other than tumors, no differences were noted between controls and treated mice at any dose level.

NEOPLASMS: The following tumor incidence was the subject of further examination (50 mice per sex per group):

		162 ppm	486 ppm	1458 ppm	4374 ppm
Leukemia/lymphoma - Males	2 (4%)	9 (18%)	9 (18%)	8 (16%)	10 (20%)
- Females	11 (22%)	9 (18%)	9 (18%)	7 (14%)	13 (26%)
Lung Adenoma - Males	2 (4%)	1 (2%)	0	1 (2%)	4 (8%)
- Females	0	1 (2%)	1 (2%)	4 (8%)	5 (10%)
Lung Carcinoma - Males	2 (4%)	1 (2%)	3 (6%)	2 (4%)	1 (2%)
Females	0	0	0	0	0
Liver Adenoma - Males	0	0	0	0	3 (6%)
- Females	0	0	0	0	0

NOTE: Lung adenomas were not found in mice having lung carcinomas; lung carcinomas were not found in mice having lung adenomas.

The above summaries tend to suggest that vinclozolin induces leukemia/lymphoma in male mice, lung tumors in female mice, and perhaps liver tumors in males on the high dosage. However, it is recognized that the "normal" incidence of some tumor types may be high. According to Benirschke, Garner, and Jones (Pathology of Laboratory Animals, page 1054), leukemia incidence in mice varies from 19 to 100%, and may appear relatively early (at 8 to 10 months of age). Hepatocellular tumors also are common, having an incidence of 26 to 99%, and appearing from 12 to 28 months of age. Pulmonary tumors have a recorded incidence of 15 to 90%, and appear later in the mouse's life (12 to 18 months, or later). However, we did not have specific data related to the NMRI strain.

With this submission we received data on the incidence of leukemia/lymphoma on control NMRI mice from 5 studies conducted in the same laboratory as conducted this study with vinclozolin. The studies were conducted during the same time frame as the vinclozolin study. The incidence of leukemia/lymphoma in these studies is shown below:

NMRI Historical Control Leukemia/Lymphoma Incidence

Study Number	Sex	Percent Leukemia/Lymphoma
I	Male	21
	Female	46
II	Male	16
	Female	22
III	Male	7.1
	Female	18.6
IV	Male	10
	Female	18
V	Male	22
	Female	18
Controls, This study	Male	4
	Female	22
4374 ppm This Study	Male	20
	Female	26

It is evident from the foregoing data that the leukemia/lymphoma incidence of vinclozolin-treated mice in this study falls well within the range for control NMRI mice in 5 other studies conducted in the same laboratory.

The registrant had also beeen requested to provide data on the incidence of liver tumors and of lung tumors on controls from these same 5 studies in NMRI mice, and also the dates the studies were conducted. This information has now been received. The historical data are as follows:

TABLE 1

HISTORICAL DATA FROM ONCO STUDIES OF NMRI MICE

Study	No. of Animals and Sex	Lung Adenomas	Lung Carcinomas	Lung Tumors	Liver Tumors
I	100 M	15 (15%)	5 (5%)	20 (20%)	2 (2%)
	100 F	9 (9%)	2 (2%)	11 (11%)	0
II	50 M	4 (8%)	0	4 (8%)	0
	50 F	2 (4%)	0	2 (4%)	0
III	70 M	2 (2.9%)	1 (1.4%)	3 (4.3%)	2 (2.9%)
	70 F	4 (5.7%)	0	4 (5.7%)	1 (1.4%)
IV	50 M	2 (4%)	0	2 (4%)	0
	50 F	0	0	0 0	0
V	50 M	1 (2%)	0	1 (2%)	0
	50 F	3 (6%)	0	3 (6%)	0

TABLE 2

VINCLOZOLIN MOUSE ONCOGENICITY STUDY

Group	No. of Animals and Sex	Lung Adenomas	Lung Carcinomas	Lung Tumors	Liver Tumors
0 ppm	50 M	2 (4%)	2 (4%)	4 (8%)	0
	50 F	0	0	0	0
162 ppm	50 M	1 (2%)	1 (2%)	2 (4%)	0
	50 F	1 (2%)	0	1 (2%)	0
486 ppm	50 M	0	3 (6%)	3 (6%)	0
	50 F	1 (2%)	0	1 (2%)	0
1458 ppm	50 M	1 (2%)	2 (4%)	3 (6%)	0
	50 F	4 (8%)	0	4 (8%)	0
4374 ppm	50 M	4 (8%)	1 (2%)	5 (10%)	6 (3/50)
	50 F	5 (10%)	0	5 (10%)	0

NOTE: Mice which had lung adenomas did not show lung carcinomas; mice having lung carcinomas did not show lung adenomas.

LUNG TUMORS: The apparent dose-response relationship for lung tumors in the vinclozolin-treated females must be noted. However, lung adenoma is not a rare tumor in mice and there are data indicating that an increase in the spontaneous incidence of lung tumors in old mice frequently occurs. Benirschke, Garner, and Jones (Pathology of Laboratory Animals, page 1055) give a spontaneous incidence of 15-90% pulmonary tumors in various strains of mice (usually 25% or higher). Historical data for this tumor in the NMRI Strain from 5 experiments as presented in Table 1 reports an incidence of 11% in the females of Study I, which is close to the 10% and 8% incidences seen in females (Table 2) in the treated high dose groups. But in the other 4 experiments (Table 1) the increase in the historical control groups is significantly lower than in the treated high dose groups.

Of the five female mice with lung tumors which received the 4374 ppm vinclozolin dosage, 3 were killed at the termination of the study at week 112; one died at week 108 of the study (25 months); and one died at week 102 (two weeks short of 2 years). It therefore is evident that a decrease in the latency of tumor appearance did not occur as a result of vinclozolin treatment.

Based on these and other data presented below, we conclude that vinclozolin may be a weak and questionable oncogen for lung tumors as seen in a single species (mouse), one strain (NMRI), one sex (females), and producing only a relatively low incidence of benign tumors.

LIVER TUMORS: Table 2 on the vinclozolin mouse study shows that there is a 6% increased incidence (3/50) in liver tumors (adenomas) in the male highest dose group, compared with the control, 162 ppm, 486 ppm, and 1458 ppm dose groups. The historical control data in the NMRI strain show an incidence in the males of 2% and 2.9% in Experiments I and III, respectively (Table 1). There were no liver adenomas/hepatomas in Experiments II, IV, and V of the historical control data.

It is known that mouse liver neoplasms are spontaneously present in several strains of mice. For example, Benirschke, Garner, and Jones (Pathology of Laboratory Animals, page 1054), give a spontaneous incidence of 40.7%-99% liver tumors in various strains of aged male mice; most show an incidence of 72-99%.

The three male mice on the highest vinclozolin treatment level and which had liver tumors died on weeks 105, 103, and 96 -- all close to two years. These were the only mice in the study with liver tumors. It therefore is evident that a decrease in the latency of tumor appearance did not occur as a result of vinclozolin treatment.

Therefore, since we have a low incidence (3/50, or 6%) of benign liver tumors at the highest dose only in a single species (mouse), of the NMRI strain, in one sex (male), we conclude that vinclozolin may be at most a weak and questionable oncogen for liver tumors in the NMRI strain of mice.

CONCLUSIONS:

1) An apparent increase in the incidence of leukemia/lymphoma type tumors in male mice appeared to result from administration of vinclozolin. However, the incidence in historical controls equaled or exceeded the incidence seen in this study, which would tend to indicate the apparent increase in leukemia/lymphoma incidence due to vinclozolin is not real.

2) An apparent increase in the incidence of lung adenomas was seen in females on vinclozolin (one at 162 ppm, one at 486 ppm, 4 at 1458 ppm, and 5 at 4347 ppm). These were within the range seen in some of the historical controls, but because of the apparent dose-relationship and the lack of tumors in the study controls, we concluded that vinclozolin was a weak and questionable oncogen for lung tumors. Because it produced a maximum 5/50 tumors of a benign nature in one species only (the mouse) at the high doses, and because a decrease in the latency of tumor appearance did not occur, we consider vinclozolin to be only weakly positive in the production of lung tumors. Nevertheless, a risk assessment was carried out using the multi-stage model and the positive findings in the lung.

3) An apparent production of liver adenoma was seen in 3/50 males receiving vinclozolin at the highest (4347 ppm) dose. This would tend to classify vinclozolin as a weak and questionable oncogen for liver tumors in the NMRI strain of mouse. Because of this extremely low incidence of benign tumors at the highest dose only, in one sex of the mouse only; and because a decrease in the latency of tumor appearance did not occur as a result of vinclozolin treatment, we consider vinclozolin to be of questionable oncogenic significance (weakly positive) in the production of liver tumors.

The data are equivalent to CORE Minimum.

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I concur with the above presented pathology assessment.

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