



UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

5-24-82
RCB Hummel
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File petition
PP# 86-2068

MAY 24 1982

MEMORANDUM

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OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

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

SUBJECT: Vinclozolin; (RONILAN). Further Assessment of Mouse
Oncogenicity Data. PP#8G2068; BAS 353F Fungicide
CASWELL#323C

In my memorandum of May 10-11, 1982 I stated a re-evaluation of Subject data would be forwarded to you within one week.

The re-evaluation follows:

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. 096970.

PROCEDURE:

Technical grade vinclozolin, batch 83,258, was administered to male and female NMRI mice at levels of 0, 162, 486, 1458, and 4374 ppm in the food. There were 50 males and 50 females per group per dosage level. The dosing was continued until a mortality of approximately 70% was reached in the control group - a period of 112 weeks, which may be rather long for a mouse "lifetime" study. The mice were about 26 days old at the beginning of the study and weighed 18 to 22 grams. The food was checked every three months for absence of aflatoxins (sensitivity of method was 2 ppb). The mice were housed singly in plastic cages at a room temperature of $24^{\circ} + 0.5^{\circ}\text{C}$ and relative humidity of $60\% + 3\%$. Cages were subjected to light and dark periods of 12 hours each, per day. Mice were observed daily for behavior, appearance, and fecal excretion. Food consumption was measured daily, and water intake was observed. Mice were weighed once a week.

Hematology was determined pre-treatment, and after 6, 13, 26, 52, and 78 weeks in 10 mice/group/sex, and the following determinations were made: hemoglobin, leucocytes, erythrocytes, differential count, and hematocrit. After 26, 52, and 78 weeks prothrombin time, platelets, and reticulocytes were determined.

Clinical biochemistry conducted pre-treatment, and after 6, 13, 26, 52, and 78 weeks in 10 mice/group/sex included glutamic pyruvic transaminase (SGPT), blood urea, glucose, and alkaline phosphatase. And also (except for week 6) sodium, potassium, calcium, and chloride. After 26, 52, and 78 weeks uric acid, bilirubin, total protein, and SGOT. Blood was drawn from the retrobulbar venus plexus under light ether anesthesia.

Tissues from the following organs of mice at 4374 ppm and of the control group were examined histologically after H & E staining: heart, liver, spleen, kidney, adrenal, thymus, pituitary, gonads, thyroid, brain, prostate/uterus, stomach, duodenum, jejunum, ileum, colon, rectum, parotid, eye, urinary bladder, bone marrow, trachea, aorta, esophagus, pancreas, lymph node, peripheral nerve, skeletal muscle, bone, mammae; also tumors and areas where tumors were suspected.

In addition, frozen sections of heart, liver, and kidney were made and stained with sudan. Bone marrow was sectioned following decalcification.

Organs which were weighed included: heart, liver, lungs, spleen, kidney, thymus, gonads, and brain.

Statistical evaluation was by analysis of variance and students' t-test. The limit for significance was $p =$ to or less than 0.01.

RESULTS:

Treatment had no effect on appearance, on behavior of the mice, nor on mortality.

Food consumption of males at 4374 ppm was slightly less than other groups from the 16th week on, and the weight gain tended to correspond, although body weight remained in the normal range until the 112th week, when it became statistically less than the controls.

Hematology: Treatment had no effect on blood count, hemoglobin, hematocrit, prothrombin time, reticulocyte or platelet count.

Clinical Biochemistry: Blood urea was significantly reduced at the 13th week only in females on the 486 ppm dietary level (5.1 mmol/L serum for treated; 6.5 mmol/L serum for controls. At the beginning of the study the BUN value for the controls was 5.8 mmol/L serum). Because of this isolated occurrence at the 13th week only, at the mid-dose only, this finding is not considered to be of toxicologic significance.

Total proteins in females at 4374 ppm were significantly reduced at the 52nd and 78th weeks (61.1 and 61.7 g/L serum in treated females; 64.7 and 62.1 g/L serum in control females.

The other biochemistry values all remain normal: glucose, bilirubin, sodium, potassium, calcium, SGPT, SGOT, and AP.

Urinalysis Results: 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. 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 was not adversely affected. These examinations were conducted prior to sacrifice.

Organ Weights: The testing laboratory lists individual and mean organ weights, and organ/body weight ratios. They minimize the toxicological significance of the comparisons because of the usual and normal high variations which occur in aged NMRI mice, control or treated.

Absolute liver, lung, and kidney weights in males at 1458 ppm were lower than controls. (The total body weight and heart weight also were reduced.)

At 4374 ppm the absolute mean body weight, heart, and left testicle weights were reduced significantly in the males. The relative organ/body weight ratios were all normal. The absolute mean liver weight in males were significantly increased over controls.

In the females at 4374 ppm, the absolute mean liver weight was significantly increased over the controls.

Histology/Tumors: In summarizing necropsy findings other than tumors, no differences were noted between controls and mice on the high dose level (4374 ppm).

The laboratory's tumor summary chart totals tumors by their location. In total numbers of tumors it would not be clear whether or not vinclozolin is an oncogen. However, in tallying the tumors by type into groups by treatment and sex, and by eliminating duplication (in 2 or more organs), it would appear vinclozolin treatment at 4374 ppm may cause leukemia in male mice, but probably not in females. Most of the tumors reported in the liver and kidney were also of the leukemia grouping. The tallies, adjusted to eliminate duplication, for leukemia are as follows:

Control males: 2/50 cases	Control females: 11/50 cases
Treated males: 10/50 cases	Treated females: 13/50 cases

In the treated males were also encountered 3/50 cases of adenoma in the liver; none were seen in the controls.

Using the analysis of variance and students' t-test the incidence is not significant at the 0.01 level. Using the Fisher's one-tail statistical method, the level of significance is 0.014., which is below the level of 0.05. This is the level of significance usually accepted by the Agency as being evidence of an oncogenic effect. With data on the intermediate treatment levels we may be better able to determine the oncogenic potential.

We recognize that such tumors are not rare in the mouse. Nevertheless, in order to further elucidate the oncogenic potential of vinclozolin in the mouse, we request that tissues from the mid-dose and low-dose animals also be subjected to histological evaluation, and that a report of the evaluation be submitted. Particular attention should be made to the leukemia and related diseases and to liver adenomas by animals and by organs in tabulating the results.

Non-Neoplastic Lesions:

The laboratory does not list separately gross lesions or abnormalities detected on necropsy. They list conditions found on microscopic examination, and probably many of the tissues were sectioned at a particular locus because gross examination showed a lesion to be present there.

Generally, it does not appear that treatment had any adverse pathological non-neoplastic effects. A possible, but questionable, effect might have been seen in the testes. Testicular atrophy occurred in 26 treated males and in 18 control males. Prostate atrophy occurred in 4 treated males, but it could be presumed this is related to testicular atrophy. On the other hand, these are very old mice and the testicular effects might be related to senility, even though occurrence is greater in those treated.

Although the laboratory adequately defines the various lesions observed they should tabulate the lesions according to incidence and grade them according to severity. Samples of a Summary Incidence Table and Histopathology Incidence Table are attached which should be used as examples in summarizing the lesions and grading their severity.

Conclusions:

Oncogenicity: Vinclozolin may cause leukemia in male mice, but probably not in females. We also view with suspicion the 3 cases of liver adenoma in treated males.

Chronic Toxicity: The reduced absolute mean body weights at 4374 ppm and 1458 ppm in males possibly may be a result of treatment with the chemical, as well as the increased mean liver weights in males and females at the 4374 ppm level. Since no histopathological examinations were conducted at lower treatment levels, a NOEL cannot be set for chronic toxicity.

Roland A. Gessert

Roland A. Gessert, D.V.M.
Toxicology Branch
Hazard Evaluation Division (TS-769)

Attachment

WAO for LDC 5/17/82

Test for Significance of Differences Between Proportions 5/17/82

Leukemia

ppm	#	RESP	Total	%	+/-2(S.D.)	One Tail P	Statistic
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Fisher's

0.000	2	50	4.00+/- (6.43)				
4374.000	10	50	20.00+/- (12.09)	0.014			

Female Mice

Group IV - Sacrificed

ANIMAL NUMBER	800	807	815	817	818	819	822	824	828	831	833	834	836	844	845	856	857	860	862	863	865	866
LIVER																						
Hepatocellular Carcinoma																					P	
Hepatocellular Adenoma	P	P	P	P	P	P	P	P	P		P	P				P	P	P	P		P	
Malignant Lymphoma																						
Granulocytic Leukemia																						
Angiosarcoma																						
Carcinoma, Metastatic																						
Sarcoma, Metastatic																						
Reticulum Cell Sarcoma																						
Hepatocholangiocarcinoma																						
Multifocal Hepatoceellular																						
Degeneration																						
Basophilic Foci		2																				
Mononuclear Cell Infiltration	1				2						3	1	1	2	1					1		
Foci of Mononuclear Cells																						
Angiectasis						2								3								
Focus of Cellular Change															3							
Multifocal Hepatitis	2	2	4	3	3	3	3	1	3			2	2	2			3		2		4	
Multifocal Necrosis												2									2	

Key: P = Present
 1 = Minimal
 5 = Severe/High

N = No Section
 2 = Slight
 1 = Incomplete Section

A = Autolysis
 3 = Moderate
 4 = Moderately Severe/High

X = Not Remarkable
 4 = Moderately Severe/High

SUMMARY INCIDENCE TABLE

[illegible]