US EPA ARCHIVE DOCUMENT

Caswell File
004894

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

JUN 17 1985

MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

SUBJECT: Peer Review of Ronilan (Vinclozolin), Caswell No. 323C.

FROM: Reto Engler, Chief
Scientific Mission Support Staff
Toxicology Branch/HED (TS-769)

TO: Henry Jacoby, Product Manager
Fungicide Herbicide Branch
Registration Division (TS-769)

On April 4, 1985, a panel of Toxicology Branch personnel and Dr. Paynter, HED Senior Science Advisor met to discuss the data base on Ronilan, and the conclusion reached by independent reviewers of the data and lung slides of the mouse study.

A. Concurrence:

The following persons were in attendance; the signature indicates concurrence with the consensus report, unless otherwise indicated.

1. Peer Review Panel:

Orville E. Paynter, Ph.D.

William L. Burnam

Louis Kasza, D.V.M., Ph.D.

Laurence Chitlik, DABT

John Quest, Ph.D.

Bertram Litt

Reto Engler, Ph.D.

FYM3

2. Reviewer/Section Head, who presented the data for evaluation and consideration. Their signatures indicate the technical accuracy of the panel report. Given their work was reviewed, they are not considered part of the panel.

Roland Gessert, D.V.M. _____

Robert B. Jaeger _____

3. Dr. Farber was unable to attend the discussion; the signature indicates concurrence with the overall conclusion of the panel.

Theodore M. Farber, Ph.D. _____

B. Information Reviewed

The information reviewed consisted of several Toxicology Branch (TB) reviews dealing with the apparent oncogenic effects of Ronilan in mice. A Peer Review of the TB review by Dr. McConnell, NIEHS, a summary conclusion by Dr. Kasza based on an independent reevaluation of the lung histopathology, and one liners concerning other toxicity tests on Ronilan (package attached).

C. Summary Evaluation of the Evidence

1. Background:

a. The central issue of this latest evaluation as well as deliberations spanning several years was whether or not the lung adenomas observed in female (NMRI) mice were related to compound administration. The effects appeared to be dose related, however, within the range of historical controls.

b. Following the most conservative approach the Agency has "regulated Ronilan as an oncogen" while it continued to assess the scientific evidence as to whether such a conservative approach was, in fact, justified.

c. The Agency therefore took the initiative to have the lung slides re-evaluated by an independent pathologist.

2. Ancilliary Evidence:

The rat study on Ronilan did not show any oncogenic effects. The following mutagenic assays were negative (1) Chinese Hamster SCE, (2) Ames test with and without S-9 activation (3) host mediated assay with S. typhimurium, and (4) dominant lethal assay in mice.

While the mutagenicity test battery could be expanded nothing in these test points to an oncogenic potential.

3. The Bioassay in Mice:

The incidence of lung adenomas in female mice was reported by BASF as follows:

Dose (ppm)	0	162	486	1458	4374
Response	0%	2%	2%	8%	10%

The incidence of tumors showed a dose-related trend, but was within the historical range. For the laboratory which carried out the Ronilan study the historical incidence for female mice ranged from 0-9% in 5 studies (1974-78) with a mean of 5.6% and in other laboratories it was as high as 25.5% (1978-80).

The re-evaluation of the slides by the independent pathologist was consistent with this finding. However, one less tumor each was reported in the Agency sponsored evaluation at 162, 1458 and 4374 ppm changing the response rate to 0%, 0%, 2%, 6% and 8.6% for the dose groups listed above. These results still show a statistically significant response trend. However, the top dose incidence becomes more consistent with the historical control value of the laboratory which conducted the study.

D. Conclusion:

After consideration of all the facts before them the review committee members concluded that the mouse oncogenicity test and other studies i.e., the rat bioassay and the mutagenicity studies in particular did not support the finding that Ronilan was oncogenic. The primary reasons for this conclusion are:

1. No hyperplasia was observed at any dose level, hyperplastic changes are a strong indicator of oncogenic effects.

2.a) Lung adenomas in this strain of mice are common and their frequency shows a fairly wide variation (in the same laboratory, as well as in other historical control data).

2.b) The observed lung adenoma frequency was within the historical control limits for the same laboratory.

3. The Agency sponsored re-evaluation of slides also lead to the conclusion that the lung adenomas are not likely to be compound related, because of the low frequency of occurrence and absence of preneoplastic pathological changes.

4. The mutagenicity assays performed were all negative.

Several other factors listed below point out the lack of oncogenicity of Ronilan. Although the committee realizes that they are not in and of themselves sufficient to dismiss oncogenic effects, they should be considered together with the above rationale as weight of evidence.

1. The lung adenomas were only observed in one sex (female).

2. No progression to carcinomas was observed.

3. The long term feeding study in rats was negative; the dose levels (in ppm) for rats were the same as for mice and the MTD for rats seems to have been reached (body weight reductions).

E. Referral:

On an interim basis and before a final evaluation of Ronilan's oncogenic potential, Toxicology Branch has calculated dietary risks based on a Q_1^* of 1.1×10^{-2} (for mg/kg/day exposures). Based on the NOEL of 100 ppm in the 6-month dog study and a safety factor of 100X an ADI of 0.025 mg/kg/day was established for Ronilan. Since the oncogenic potential for Ronilan has been dismissed, the ADI should be used in the future as a basis for regulating food uses of this pesticide.

The chemical Ronilan is referred to the Herbicide Fungicide Branch PM 21 (Jacoby) for further action. As noted in this review the mutagenicity testing for Ronilan is insufficient and additional testing should be performed.

OPP:HED:TOX:R.ENGLER:sb 5/14/85 X77490 #1-D34
rev: 6/7/85

Engler

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

MAR 26 1985

MEMORANDUM

OFFICE OF PESTICIDES AND TOXIC SUBSTANCES

SUBJECT: Peer Review of Toxicity Data on Ronilan (Vinclozoline).

Caswell No. 323C

FROM: Reto Engler, Chief
Mission Support Staff
Toxicology Branch/HED (TS-769)

TO: Addressees

Attached for your review please find the evaluation of Ronilan's oncogenic effects data, spaning several years.*

Also included in the package is a memorandum (October 12, 1984) concerning an outside peer review on our findings on Ronilan. Based on this review, the lung slides were again evaluated for EPA under contract and the findings were evaluated and summarized by Dr. Kasza (memo Feb. 14, 1985).

In addition there is a listing of one-liners attached summarizing the other data on Ronilan, see also Jan. 4, 1985 memo.

*The memos in reverse chronological order are:

January 4, 1985	Rodriguez	to	Jacoby	(data summaries)
September 12, 1984	Gessert	to	Jaeger	
December 29, 1983	Gessert	to	Jacoby	(Attached BASF comments on April 15, 1983 memo)
September 7, 1983	Gessert	to	Jacoby	
May 5, 1983	Gessert and Kasza	to	Jacoby	

April 15, 1983	Gessert	to	Jacoby
May 24, 1982	Gessert	to	Jacoby
May 11, 1982	Gessert	to	Jacoby
April 17, 1982	Gessert	to	Special Registration Section

A meeting for addressing this issue is scheduled for Thursday, April 4, 1985 at 10:00 in Dr. Farber office.

ADDRESSEES:

Dr. Theodore M. Farber
William Burnam
Dr. Louis Kasza
Dr. Orville Paynter
Robert Jaeger
Dr. Roland Gessert (reviewer)
Laurence Chitlik
Dr. John Quest
Bertram Litt

cc: H. Jacoby
C. Gordon
R. Coberly/Caswell 323C

OPP:HED:TOX:R.ENGLER:sb 3/19/85 X77490 #1-D3

004894

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

February 14, 1985

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

TO: Reto Engler, Ph.D.
Scientific Mission Support Staff
Toxicology Branch, TS-769

FROM: Louis Kasza, D.V.M., Ph.D.
Pathologist
Toxicology Branch, TS-769

SUBJECT: Evaluation of Pathologic Findings in Mouse Lungs Exposed to Ronilan (Vinclozoline)

The male and female mice were divided into 5/5 groups, 50 animals in each group. The mice in these groups were fed with the test material at 0, 162, 486, 1,458 and 4,374 ppm, respectively. Based on Dr. Gessert's tabulation, there were differences in the incidences in lung adenomas between control and test groups in female mice (0%, 2%, 2%, 8%, and 10%). In five submitted "Historical Data from Onco Studies of NMRI Mice", lung tumors occurred in 11%, 4%, 5.7%, 0%, and 6% distribution. Based on the available data, the conclusion of the Memorandum of May 5, 1983, on page 10, was as follows:

"2) An apparent increase in the incidence of lung adenomas was seen in females on vinclozoline (one at 162 ppm, one at 486 ppm, 4 at 1,458 ppm, and 5 at 4,374 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 vinclozoline 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 vinclozoline 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."

An independent pathology laboratory, Experimental Pathology Laboratory, Inc. (EPL) re-evaluated the female lung histological sections and the results were submitted on January 31, 1985. For comparative reasons, we summarize the original (BASF Wyandotte Corp.), and the reviewer pathologist's findings, both the incidence number and, in parenthesis, the percentage:

GROUPS

	Control	162 ppm	486 ppm	1458 ppm	4374 ppm
BASF	0 (0%)	1 (2%)	1 (2%)	4 (8%)	5 (10%)
EPL	0 (0%)	0 (0%)	1 (2%)	3 (6%)	4 (8.6%)

The tumor incidences in the review pathologist's report are similar to the original evaluation. The differences are one less tumor at 162 ppm, 1458 ppm and 4374 ppm dose levels. Also, I would like to emphasize the lack of increased incidences of hyperplastic changes at all dose levels. I call your attention to this fact because the increased incidences of hyperplastic changes are regularly present when a compound is oncogenic. Considering all biological parameters, it is my conclusion that Ronilan is very likely not related to tumor induction in the lungs of female mice. I also support my interpretation by the Task Force of Past Presidents of the Society of Toxicology which gives us the following example of how historical data may be useful:

> "The following propositions may be taken as scientifically useful in the evaluation of a chemical carcinogenic response, with distinctions drawn between the use of concurrent control and historical control data. (1) If the incidence rate in the concurrent control group is lower than in the historical control groups, but the incidence rates in the treated groups are within the historical control range, the differences between treated and control groups are not biologically significant."

A similar conclusion was made by the EPL review pathologist:

"In conclusion, the results of this microscopic evaluation did not reveal a clear relationship between any of the microscopic findings and the dietary administration of Ronilan. The incidence of bronchiolar-alveolar adenomas in the 4,374 ppm treated female mice, even though greater than in the controls, is still small. This low incidence, coupled with the lack of preneoplastic hyperplastic changes or the progression to malignancy, indicates that this incidence is probably not related to the administration of Ronilan. The incidence of primary lung neoplasms in the Ronilan treated male mice is comparable to that seen in the male control mice. The incidence of other non-neoplastic microscopic observations in the Ronilan treated mice is comparable to that seen in the control mice."

cc: R. Gessert
R. Jaeger
W. Burnam

004894

OCT 12 [illegible]

Subject: Peer Reviews of Ronilan Mouse Oncogenicity Study
Caswell No. 323C

Study Identification:
Chronic Toxicity and Oncogenicity of Vinclozolin (Ronilan) in Mice. F. Leuschner, Laboratory for Pharm. & Toxicol, Hamburg, FRG. Dec. 15, 1977.

Background: The mouse study has been submitted to OPP in support of registration of Ronilan and has been reviewed by the Toxicology Branch (several memos 1982-83). The reviews, inter alia, concluded that there was a trend showing increased incidences of lung adenomas. The registrant (BASF) contended in its submission of Feb. 9, 1984, that the lung adenomas observed were within the range of historical controls, and therefore not compound-related.

On August 30, 1984, Dr. Ernest E. McConnell, NIEHS, North Carolina, peformed a peer review of the findings, conclusions and rebuttals. He was provided with a copy of the mouse study, the reviews of the Toxicology Branch, and the rebuttal argument (historical control data) of the registrant.

After the reviews, Dr. McConnell, debriefed Dr. Reto Engler and Mr. Bruce Jaeger of the Toxicology Branch as follows:

Conclusions:

1. The mouse study is acceptable for evaluating oncogenic effects, in particular the MTD for the test compound has been reached (very significant liver weight increases).

2. Leukemias:

   1. Incidences in control groups are fortuitously low.
   2. Incidences in treated groups are well within the expected (historical) rate.
   3. No dose response trend was established.
   4. Therefore, the conclusion by Tox Branch that leukemias were not compound-related can be corroborated.

3.. Liver Tumors

BEST AVAILABLE COPY

   1. Show a low incidence, barely significant.
   2. They are, however, above historical controls.
   3. They are only seen at study termination.
   4. There are only benign tumors
   5. [illegible]

4. Lung Tumors

1. No effects in males were seen.
2. Adenomas (non-lethal) increased with dose in females, however with low incidence (10%).
3. Lung adenomas did not progress to carcinomas.
4. The historical range of lung adenomas is 4-11% in this mouse strain in studies conducted in the same laboratory. However, it is concluded that the incidence in the Ronilan mouse study are only exceeded by one historical data point and therefore cannot be considered "average." The trend of increases of lung adenoma, therefore, cannot be discounted based on the historical data alone. Furthermore, the historical control data, especially for pulmonary lesions, should only include those performed in the same laboratory and preferably by the same pathologist, and it is not clear that both these criteria are fulfilled for the data presented by BASF (i.e. same pathologist).

5. Dr. McConnell finally suggested that the study should be evaluated to define the incidence of alveolar hypyplasias (a presumed precursor stage of alveolar adenomas). This evaluation could significantly affect the weight of evidence (Tox Branch is now undertaking this effort).

Prepared by

Reto Engler, Ph.D.
Toxicology Branch
Hazard Evaluation Div.

Concurred by

Ernest E. McConnell, D.V.M.
Acting Director
Toxicology Research and Testing Program

cc: R. Jaeger
R. Gessert
W. Burnam
H. Jacoby

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

JAN 4 1985 004182

MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

SUBJECT: Ronilan Fungicide (Vinclozolin) - Amendment of PP#4E2998. CASWELL No. 323C

TO: Henry Jacoby, PM #21
Fungicide-Herbicide Branch
Registration Division (TS-767C)

FROM: Carlos A. Rodriguez C.A. Rodriguez 1/4/85
Review Section #6
Toxicology Branch/HED (TS-769)

THRU: Jane E. Harris, Ph.D., Section Head J.E. Harris 1/4/85
Review Section #6
Toxicology Branch/HED (TS-769)

Applicant: BASF Wyandotte Corporation
100 Cherry Hill Road
P.O. Box 181
Parsippany, N.J. 07054

Action Requested:

Established tolerance for residues of the fungicide Ronilan (vinclozolin), 3-(3-5-dichlorophenyl)-5-ethenyl-5-methyl-2, 4-oxazolidinedione and its metabolites 3,5-dichloroaniline moiety in/on peppers at 3.0 ppm.

Evaluation:

The 6-Month Dog Feeding Study with Ronilan (vinclozolin) showed an increased absolute and relative adrenal weight in both male and female dogs and decreased absolute kidney weight in male dogs at the dose level of 300 ppm (7.5 mg/kg/day). Based on these findings it appears that the dog is the more sensitive animal species to this compound. Therefore, Toxicology Branch recommends that the acceptable daily intake (ADI) should be based on the NOEL of 100 ppm (2.5 mg/kg/day) of the 6-month dog study rather than the two year rat NOEL of 486 ppm (24.3 mg/kg/day).

It should be noted that the effects reported for the rat at the LEL of 1458 ppm, namely body weight reduction and reduced serum bilirubin, represent a less serious toxicological profile than the effects reported at the LEL (300 ppm) of the 6-month dog study.

Conclusions:

A review of the existing toxicity data base indicates that following risks from both the existing and proposed uses:

1. The onocogenic lifetime dietary risk for both published and unpublished Toxicology Branch approved uses is 4.5×10^{-5}.

2. The incremental oncogenic lifetime dietary risk for peppers at 3.0 ppm is 10.1×10^{-7}.

3. The oncogenic dietary risk for previously approved and currently considered tolerances is 4.6×10^{-5}.

4. The current pepper tolerance will produce an absolute increase of 0.00552 mg/day in the TMRC which will result in a 2.25% increase of the total TMRC of all previously approved tolerances.

5. The toxicity data (exclusive of oncogenicity) used to calculate the ADI of 0.025 mg/kg/day is based on the 6-month dog study with a NOEL of 100 ppm (2.5 mg/kg). An increased adrenal weights in both sexes and increased kidney weights in male dogs at 300 ppm (7.5 mg/kg) were observed.

6. All previously approved and currently considered tolerances occupy 16.72% of the ADI.

Summary of Selected Toxicology Data Considered in Setting the Requested Tolerance.

a. Acute Oral LD_{50} (rat) = > 10,000 mg/kg (both sexes) Core-Classification: Minimum.

b. Acute Dermal LD_{50} (rat) = > 2,500 mg/kg (both sexes) Core-Classification: Minimum.

c. 90-Day Feeding Study (rat): NOEL = 450 ppm (22.2 mg/kg) highest dose level tested. Levels tested: 0, 150 and 450 ppm. Core- Classification: Minimum.

d. 90-Day Feeding Study (Dog): NOEL = 300 ppm (7.5 mg/kg/day). LEL = 1,000 ppm (25 mg/kg/day) increased platelet count, Howell-Jolly bodies in differential blood counts, cholestasia of liver, fatty deposits in renal tubules, cholestasia of kidneys. Levels tested: 0, 100, 300, 1000 and 2000 ppm. Core-Classification: Supplementary Study.

e. 6-Month Feeding Study (Dog): NOEL = 100 ppm (2.5 mg/kg/day). LEL = 300 ppm (7.5 mg/kg/day) increased absolute and relative adrenal weight (M/F); decreased absolute kidney weight (M). Levels tested: 0, 100, 300, 600 and 2000 ppm. Core-Classification: Minimum.

f. Mouse Teratology - Teratology NOEL = 6000 ppm (900 mg/kg/day) highest dose level tested. Maternal NOEL = 6000 ppm (900 mg/kg/day) highest dose level tested. Fetotoxic LEL 6000 ppm (900 mg/kg/day) - resorptions (highest dose level tested).

Levels tested: 0, 600 and 6000 ppm. Core-Classification: Minimum.

g. Rabbit Teratology - Teratogenic potential is not indicated in this study.

Maternal NOEL = > 300 mg/kg/day (900 ppm)
Fetotoxic NOEL = 80 mg/kg/day (2640 ppm)
Fetotoxic LEL = 300 mg/kg/day (9,900 ppm) - weight loss, post inplantation loss.
Levels tested: 0, 20, 80 and 300 mg/kg/day.
Core Classification: Minimum.

h. Mutagenic-host-mediated assay - In-vivo reverse mutation using T. typhimirium G46 was negative. There was no increase in mutation frequency in vinclozolin treated groups. Core-Classification: Acceptable.

i. Dominant Lethal Assay in Mice: Negative at 2000 mg/kg, only level tested.

j. 3-Generation Rat Reproduction: NOEL = 1458 ppm (72.9 mg/kg/day) - highest dose level tested.

k. Chronic Feeding/Oncogenicity Study in rats for 103 weeks: NOEL = 486 ppm (24.3 mg/kg)
LEL = 1458 ppm (72.9 mg/kg) body weight reduction, reduced serum bilirubin.
Oncogenic NOEL = > 4374 ppm (219 mg/kg) highest dose level tested.
Levels tested: 162, 486, 1458 and 4374 ppm.

l. 26-Month feeding/onco study (NMRI strain mice).
Systemic NOEL = 486 ppm (72.9 mg/kg)
Systemic LEL = 1458 ppm (218.7 mg/kg) decreased body weight in males.

Oncogenicity

1. An increase in leukemia/lymphoma was observed in males. However historical control data on this type of tumor equalled or exceeded the level observed in this study, indicating that the increase may not be real. (Accession No. 248264; Review dated May 5, 1983).

2. An apparent dose related increase in lung adenoma was noted in females. Historical data from 5 studies using the same strain (NMRI) indicated that in 4 of 5 studies the historical control incidence was significantly lower than that observed in the treated groups of this study. Along with considerations of the benign nature of the tumor and because latency did not decrease, it is concluded that the chemical is weakly positive in the production of lung tumors.

3. A low incidence of production of liver adenoma was observed in 3/50 male mice receiving vinclozolin at the high dose tested (4374 ppm) when compared with the control group 0/50 (0%). Based upon the findings that: the tumors were benign and occurred only at the highest dose in males, and the historical control data from this laboratory showed incidences of liver adenoma in males of 2 to 3% which are not significantly different from that observed in the vinclozolin-treated animals, and that a decrease in the latency of tumor appearance did not occur as a result of the treatment, we conclude that vinclozolin may be at most a weak and questionable oncogen for liver tumors in the NMRI strain of mice.

As indicated above (Summary of Tox. Data) the oncogenic potential of vinclozolin for lung adenomas is negative in the rat and weakly positive in the mouse. See "Assessment of Complete Oncogenicity Data", Accession #248267, review dated May 5, 1983. To obtain estimates of virtually safe dose relative to this action, a risk assessment was performed considering the mouse oncogenicity study for lung adenomas.

Toxicology Branch statistician Bert Litt performed a mutistage risk analysis for lung adenomas; and Q* value (lifetime dietary risk factor) of 0.0108 (or 1.1×10^{-2}) was obtained.

The lifetime dietary risk for previously approved uses (published and unpublished Toxicology Branch approved) is calculated using the total TMRC for the previously approved uses:

$$\frac{0.2452}{60} \times 1.1 \times 10^{-2} \text{ (mg/kg/day)} = 4.5 \times 10^{-5}$$

Using the TMRC for peppers, the lifetime dietary risk is:

$$\frac{0.00552}{60} \times 1.1 \times 10^{-2} \text{ (mg/kg/day)} = 10.1 \times 10^{-7}$$

The lifetime dietary risk for previously approved uses (published and unpublished Toxicology Branch approved) plus current action on peppers is calculated as follows:

$$\frac{0.2507}{60} \times 1.1 \times 10^{-2} \text{ (mg/kg/day)} = 4.6 \times 10^{-5}$$

m) Metabolism study (rat): Dosing at 40 mg/kg for 7 days. Six days after final dose - 47% eliminated in the urine; 52.8% eliminated in the feces; 0.7% retained in the gastrointestinal tract, 0.4% retained in liver. Core-Classification: Minimum.

Evaluation of the ADI:

The acceptable daily intake (ADI) based on the 6-month dog feeding study (NOEL 100 ppm or 2.5 mg/kg) and using a 100-fold safety factor, is calculated to be 0.0250 mg/kg/day. The maximum permitted intake (MPI) for a 60 kg human is calculated to be 1.5 mg/day. The theoretical maximum residue contribution (TMRC) from existing tolerances for a 1.5 kg diet is calculated to be 0.2452 mg/day. The current action will increase the TMRC by 0.0055 mg/day (2.24%) and utilize an additional 0.37% of the ADI. Please, refer to the attached printout.

Attachment

TS-769:RODRIGUEZ:sll:x73710:12/6/84 Card Mis. 1

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004182

CFR 180.380 Ronilan 11/28/84

File last updated 4/24/84

C.A. not needed

ACCEPTABLE DAILY INTAKE DATA

LOD mg/kg	NOEL ppm	S.F.	ADI mg/kg/day	MPI mg/day(60kg)
2.500	100.00	100	0.0250	1.5000

Noel change not rec.

DRAFT

Published Tolerances

CROP	Tolerance	Food Factor	mg/day(1.5kg)
Kiwi Fruit(204)	10.000	0.03	0.00450
Strawberries(152)	10.000	0.18	0.02759
Lettuce(84)	10.000	1.31	0.19622

MPI	TMRC	% ADI
1.5000 mg/day(60kg)	0.2283 mg/day(1.5kg)	15.22

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Unpublished, Lox Approved 3E2934

CROP	Tolerance	Food Factor	mg/day(1.5kg)
Lettuce(84)	0.000	1.31	0.00000
Onions(105)	1.000	0.83	0.01242
Raspberries(135)	10.000	0.03	0.00450

MPI	TMRC	% ADI
1.5000 mg/day(60kg)	0.2452 mg/day(1.5kg)	16.35

****

Current Action 4E2998

CROP	Tolerance	Food Factor	mg/day(1.5kg)
Peppers(120)	3.000	0.12	0.00552

MPI	TMRC	% ADI
1.5000 mg/day(60kg)	0.2507 mg/day(1.5kg)	16.72

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004894

SEP 12 1984

MEMORANDUM:

TO : Robert Bruce Jaeger
Acting Chief, Toxicology Branch

FROM : Roland A. Gessert, D.V.M.
Veterinary Medical Officer

SUBJECT: Vinclozolin. RONILAN®. BASF 2-Year Mouse Oncogenicity Study. Incidence of Lung Adenoma. Accession # 248264 Caswell # 323C

In order to further evaluate the significance of the incidence of lung adenomas in female mice, I again searched the histology reports for individual mice for the incidence of pulmonary alveolar hyperplasia as a precursor to lung adenomas. I was unable to find any lesions identified as alveolar hyperplasia.

However, I did find reported incidences of "pneumonia" and "initial pneumonia". (I wondered whether the "initial" pneumonia should have read "interstitial" pneumonia, although "initial" could be correct.)

One could conjecture that some of the "initial" pneumonia should have been reported as alveolar hyperplasia. The incidence of pneumonia in the mouse study is reported as follows:

	Control	162 ppm	486 ppm	1458 ppm	4374 ppm
Females	0	19	6	7	3
Males	5	9	9	6	3

The previously reported incidence of lung adenomas is as follows:

Females	0	1	1	4	5
Males	2	1	0	1	4

Benirschke, Garner, and Jones (PATHOLOGY OF LABORATORY ANIMALS), page 1081, also mentions Pulmonary Adenomatosis.

> "To be distinguished from the alveolar tumors in mice are adenomatoid lesions that occur spontaneously or that may be induced by certain chemicals or viruses. Adenomatosis is a hyperplasia of alveolar epithelium on existing stroma without new acinar or papillary formation. Often, the degree of hyperplasia will be so extensive as to obscure the alveolar architecture and differentiation from tumors may be difficult, particularly since both may develop in the same lung. One helpful criterion is the PAS-positive mucus in the cells and alveoli in adenomatosis, which is not seen in the true alveolar neoplasms."

RECOMMENDATION:

Because the incidence of alveolar hyperplasia may affect the evaluation of the role of vinclozolin in causing lung tumors in female mice, I recommend that the slides of lungs from this study be re-evaluated by the Toxicology Branch pathologist or some other pathologist designated by Toxicology Branch.

9/11/84

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

004894

DEC 29 1983

MEMORANDUM:

OFFICE OF PESTICIDES AND TOXIC SUBSTANCES

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

THROUGH: R. Bruce Jaeger, Section Head
Review Section # 1
Toxicology Branch/HED (TS-769)

SUBJECT: Vinclozolin. RONILAN. EPA Reg. No. 7969-53.
Oncogenicity Study in NMRI Mice. CASWELL No. 323C.
BASF Correspondence of August, 1983.

WJ Burns 12-29-83

REGISTRANT: BASF Wyandotte Corporation
Parsippany, New Jersey

The registrant submitted their arguments in rebuttal to our finding of oncogenicity of vinclozolin due to lung adenomas in NMRI mice.

The applicant asks, "Does the mouse lung tumor present any special need for more carefully designed analysis of both its biological and statistical significance as compared to other tumor types?"

Our Reply:

We did not subject the mouse tumor data to more detailed scrutiny because of any special concern regarding lung tumors. Indeed, our original special concern had been with the apparent increased incidence in leukemia/lymphoma in male mice, which without tumor data on the three low and intermediate levels showed an apparent increase in incidence between controls and high dose of from 4% (controls) to 20% (high dose). However, after examining leukemia/lymphoma data for all dose levels along with historical control data for the NMRI strain of mouse we noted that the incidence in the treated mice in this study did not exceed the leukemia/lymphoma incidence in many of the historical controls. Also, the incidence was not strictly dose related. Therefore, we believed we could be justified in concluding that it had not been demonstrated that vinclozolin was the cause of leukemia/lymphoma in male mice. However, the stepwise increase in lung adenomas on an apparent dose-related basis in female mice made it more difficult to dismiss lung tumors as resulting from vinclozolin treatment.

In their effort to demonstrate that the tumors in test mice are not the result of vinclozolin, the applicant points out some of the same observations we had made in our reviews:

1. Because of the weak statistical nature of lung adenomas in female mice only (no statistical significance in males, in rats, or in carcinomas), they believe a careful analysis of the biological significance of the lung adenoma lesion is essential.

2. They refer to Shimkin and Stoner regarding "criticisms that the mouse lung adenoma has no counterpart in 'human neoplastic pathology' and that positive results represent an acceleration process rather than true induction of tumors."

3. The applicant also points out that there generally are no substantial sex differences in tumor incidence. However, in the vinclozolin study, control females showed no lung adenomas and no lung carcinmas, while control males showed 4% lung adenomas and 4% lung carcinomas.

4. In the NMRI strain of mice the incidence of lung adenomas in control females (5 laboratories) ranged from 4.5% to 25.5%. The zero incidence in control females in the vinclozolin study is abnormally low; the incidence of 1/50 in the two low dose groups is also lower than controls in any of the other laboratories; and the incidence of 4/50 and 5/50 in the two high dose groups is within the range seen in the NMRI controls in the other laboratories. In their conclusion they argue that, "we have been able to find no data on vinclozolin that supports the biological significance of the hypothesis that it can induce lung adenoma in the NMRI mouse and would therefore argue that the significance of the trend test results from a single and possibly misleading statistical test in the absence of control tumors (normal average 5.6%) and this compound is not an oncogen. To take data on a non-carcinogen at a dose point without biologic or statistical significance and apply risk assessment methods designed to evaluate life-time carcinogenic findings is a procedure we cannot agree with."

5. To further demonstrate the absence of biological significance of the lung adenomas, the applicant points to the lack of differences in mortality of any of the groups, and the lack of differences in time of lung adenoma development.

6. The applicant points out that as the dose is increased nearly 10-fold from 162 ppm and 486 ppm to 4374 ppm, the incidence of lung adenomas increased from only the non-significant 2% to a slightly significant 10%, a rather flat slope. They state that "most real lung oncogens and carcinogens exhibit a much greater maximal response and very large slope of their dose response curves." They conclude that, "again this finding is consistent with the lack of biological significance of the statistical findings."

7. The applicant also observed that "the fact that no carcinomas were observed even in animals (females) surviving 26 months suggests that vinclozolin has not altered or influenced the normally naturally occuring tumorigenic process in the NMRI mouse which leads to lung adenoma/carcinoma formation. In contrast, for a biological relevant oncogen, it is to be expected that not only the incidence of lung adenoma is increased, but also the progression from adenoma to carcinoma accelerated"

Mutagenicity:

Mutagenicity testing conducted to date yielded positive results on yeasts and fungi. However, this would be expected of a fungicide. A single Ames test (Salmonella) produced a positive finding, but subsequent Ames tests have been negative. The submitted data for a Rec-Assay (Bacillus subtilis) conducted in Japan by Yasuhiko Shirasu, et al., are incomplete. A dominant lethal study in mice and a sister chromatid exchange study in Chinese Hamsters (DNA Repair) were negative. The mutagenicity evidence to data appears to support the non-oncogenicity of vinclozolin, although all the tests may not be adequately reported. Also, we do not have in our files the Ames tests of Prof. Oesch (1977), Chiesara et al., (1982), and BASF Toxicology Dept. (1983). The tests performed by I.E.T. in Japan are inadequately performed or reported.

Conclusions:

After reconsidering the evidence and arguments put forth by the applicant and the data on which these arguments are based, all of which we had recognized previously, we understand their point of view. However we cannot ignore the apparent dose related increased incidence of lung adenomas in vinclozolin treated mice. Even if we consider the expected incidence of lung adenomas in control NMRI mice to be 5.6%, the reliability of the zero (or near zero) incidence of the control mice in this particular test is supported by the near-zero incidence (1/50) in the two low-dosed groups (162 ppm and 486 ppm).

Vinclozolin is not oncogenic in the rat, and for the many reasons cited, is a weak oncogen in the mouse.

Roland A. Gessert

Roland Gessert, DVM
Review Section #1
Toxicology Branch/HED (TS-769)

PAGES 23 THROUGH 50 ARE NOT INCLUDED. THOSE PAGES CONSIST OF COMPANY-SUBMITTED REGISTRATION DATA.

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

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SEP 7 1983

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

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

THRU: R. Bruce Jaeger, Section Head
Review Section #1
Toxicology Branch
Hazard Evaluation Division (TS-769)

SUBJECT: Vinclozolin (RONILAN). EPA Registration No. 7969-53.
Accession 250113,4. Mutagenicity and Cytogenetic Data.
CASWELL #323C

Registrant: BASF Wyandotte Corp.
Parsippany, N.J.

In response to our requests for additional mutagenicity studies the registrant submitted a Sister Chromatid Exchange study conducted by their German Laboratories and a series of mutagenicity studies conducted by Yasuhiko Shirasu, et al., of the Institute of Environmental Toxicology in Japan. All these studies except the Rec Assay appear to have been conducted in a satisfactory manner and are negative for mutagenicity and genetic effects.

A dominant lethal study previously had been submitted for registration of vinclozolin and also was negative.

In the literature we located a report of a Greek study in which vinclozolin was found to increase the frequency of mitotic recombination in diploid colonies of Aspergillus nidulans. (Spyros G. Georgopoulos, et al. Mitotic Instability in Aspergillus nidulans Caused by the Fungicides Iprodione, Procymidone and Vinclozolin. Pestic. Sci. 1979. 10, 389-392.)

The reviews of the study reports for this submission are attached.

Roland A. Gessert

Roland A. Gessert, D.V.M.
Veterinary Medical Officer
Toxicology Branch/HED (TS-769)

RAG 9/6/83

Cytogenetic Investigations in Chinese Hamsters After a Single Oral Administration of Vinclozolin. Sister Chromatid Exchange. Project No. 16M0232/8013. By Drs. H.P. Gelbke and G. Engelhardt. BASF Gewerbehygiene und Toxikologie, Ludwigshafen, Germany. December, 1981. Accession No. 250,113.

Material Tested: Vinclozolin. Reg. No. 83,258. 3-(3,5-dichlorophenyl)-5-ethenyl-5-methyl-2,4-oxazolidine dione. Purity 98.1%.

Animals: Male and female Chinese hamsters, bred by BASF. Test animals weighed between 27 and 31 g.

Dose levels: 5620 mg/kg was the highest dose that could physically be administered. Since all animals survived this dose, 5620 mg/kg was chosen as the top dose in the study. The second dose chosen was 3830 mg/kg body weight.

Procedure:

The in vivo Sister Chromatid Exchange method chosen was that of J.W. Allen et al.; A simplified technique for in vivo analysis of sister chromatid exchange, using 5-bromodeoxyuridine tablets. Cytogenet. Cell Genet., 18, 231-237 (1977).

The animals' necks are shaved and anesthetized and a 50 mg Brd U tablet (5-bromodeoxyuridine) is implanted subcutaneously. Two hours later each hamster receives a single oral administration of vinclozolin suspended in an aqueous 0.5% CMC suspension. There are 4 animals/sex/dose.

Test Groups	Dose mg/kg	Number of animals*	
		Males	Females
1	Solvent control 20 ml of CMC/kg b.w.	4/4	
2	3830 mg vinclozolin/kg b.w. 20 ml/kg b.w.	4/4	
3	5620 mg vinclozolin/kg b.w. 20 ml/kg b.w.	4/4	
4	20 mg cyclophosphamide/kg b.w. 10 ml/kg b.w. (positive control)	4/4	

*As a rule, 5 male and 5 female animals are treated per test group in order to obtain the number of 4 male and 4 female animals necessary for analysis.

Following treatment the animals are observed for any clinical signs of toxicity.

The hamsters are sacrificed 24 hours following administration of the test substance. Two femora are removed for bone marrow preparation and the animals are necropsied and examined for gross pathological changes of the internal organs.

Bone marrow preparation: Two hours before sacrifice the hamsters are injected IP with 3.3 mg Colcemid/kg body weight to arrest mitosis in the metaphase.

After cutting off the epiphyses of the femora, the bone marrow is flushed into a centrifuge tube using a cannula filled with Hank's solution at 37°C (about 2 ml/femur). The suspension is thoroughly mixed and then centrifuged at 1500 rpm for 5 minutes, the supernatant is pipetted off except for a few drops, and the precipitate is resuspended.

For hypotonic treatment about 5 ml of a 1% sodium citrate solution at 37°C is added. The suspension is kept at 37°C in a water bath for 20 minutes and mixed thoroughly with a pipette every 4 or 5 minutes.

After recentrifugation at 1500 ppm for 5 minutes the supernatant is pipetted off except for one drop, and a 3:1 methanol/glacial acetic acid fixative is added to the sediment drop by drop while constantly shaking. After 30 minutes the fixative is replaced, the centrifuge tube is closed with Parafilm, and the suspension is kept overnight at 4°C.

After recentrifugation at 1500 rpm the supernatant is pipetted off except for one drop, and a suspension is prepared with fresh fixative.

3-5 drops of this suspension are dropped onto clean microscope slides and rapidly passed through a Bunsen burner flame, air dried, and subsequently stained. The preparations are stained by the FPG technique according to Perry, P. and S. Wolff. They are stained in Hoechst 33258 for 10 minutes, rinsed twice in buffer (pH 6.8), exposed to U.V. light (254 nm) for 25 minutes.

The preparations then are stored in a 2XSSC solution in a water bath at 60°C for 90 minutes, cooled, and stained in Giemsa, rinsed twice in distilled water, clarified in xylene, and embedded in Entellan.

Evaluation: Thirty differentially stained metaphases are evaluated from each male and female animal of the solvent control group, and of the two dose groups. Only 10 metaphases per animal are evaluated in the positive control group.

Statistical Evaluation: For differences in the SCE rate between the negative control group and the dose groups, the many-one rank test according to Steel (nonparametric, one-sided test; significance levels of 5% and 1%; multiple location comparison between independent random samples) is used.

Results:

Clinical Signs: The single oral administration of the CMC vehicle formulation, vinclozolin at 3830 mg/kg, and the positive control cyclophosphamide caused no signs of toxicity.

Hamsters receiving 5260 mg/kg vinclozolin demonstrated piloerection at 1 hour, which persisted until killed at 24 hours. After a few hours irregular respiration and atony also were observed.

There were no mortalities.

Necropsy: Yielded no changes in any of the organs attributable to treatments.

Sister Chromatid Exchange (SCE): The mean SCE rate for the high dose group (5620 mg/kg body weight) was 3.78 SCEs/Cell.

The mean SCE rate for the 3830 mg/kg dose group was 3.80 SCEs/Cell.

The mean SCE rate for the vehicle control group was 3.71 SCEs/cell.

This study demonstrates that vinclozolin does not induce sister chromatid exchange.

The mean SCE rate for the cyclophosphamide (positive control) was 29.55 SCEs/cell, demonstrating the expected cytogenetic effect.

Conclusions:

Under the test conditions reported, the in-vivo sister chromatid exchange (SCE) assay used for this study is adequate to generate valid results. The positive control, cyclophosphamide (20 mg/kg), apparently gave the expected positive response. The

test compound, Vinclozolin did not induce any SCE effects in the Chinese hamster at the dose levels tested (3830 and 5620 mg/kg). Because of the limited solubility of the test compound, the upper limit of the test concentration recommended in this study is acceptable.

Mutagenicity Testing on BAS-35204F in Microbial Systems. By Yasuhiko Shirasu, Masaaski Moriya, and Kimie Kato, Institute of Environmental Toxicology, Tokyo, Japan. February 20, 1978. Accession No. 250,114.

I. Rec-Assay (DNA Repair):

Test Material: BAS-35204F. Vinclozolin 3-(3,5-dichlorophenyl)-5-methyl-5-vinyl-1,3-oxazolidine-2,4-dione. Purity 92.8%.

Organisms used: Bacillus subtilis Strain H-17 (recombination-wild) and M-45 (deficient), to estimate the DNA damaging capability of the chemical.

Procedure:

Frozen cultures stored at -80°C were thawed and then were streaked onto the surface of a B-II agar plate, making certain they did not touch each other. A filter paper disk, 10 mm in diameter, was soaked with 0.02 ml of a solution of vinclozolin in DMSO and placed on the starting points of the bacterial streaks. After overnight incubation at 37°C the length of the inhibition zones were measured. Kanamycin was used as a negative control and Mitomycin C was used as a positive control.

Results:

Vinclozolin did not cause any difference in the diameter of the inhibition zones of B. subtilis strains M45 and H17.

Kanamycin, the negative control, induced similar diameter of inhibition zones (5 and 4.5 mm, respectively) in both strains.

Mitomycin C the positive control, induced an 8 mm inhibition zone in strain M45 and a 1 mm inhibition zone in strain H17, a difference of 7 mm.

Conclusions:

The results of this study appears to be inadequate to support the conclusion drawn in the report. The following inadequacies in performing the DNA-repair assay with Bacillus subtilis were noted:

1. Since the test compound at the maximum dose (2000 ug/disk) generated no interpretable results, the spot test should be repeated using higher concentration of the test compound per disk if possible.

2. If the spot test assay is apparently unable to evaluate the difference of growth inhibition between the two strains of B. subtilis, the test compound should be further evaluated by using the modified liquid suspension procedure described by Rozenkranz and his coworkers (1980). In this procedure, portions of the test compound are added to liquid cultures and survivors are enumerated.

3. Metabolic activation in this study is required. Bacteria should be exposed to the test substance both in the presence and absence of an appropriate metabolic activation system.

4. In evaluating the differential inhibition of these two bacterial strains, both of the bacterial cell suspensions of B. subtilis (M45 rec- and H 17) should be standardized to an equally desired density prior to testing. The sensitivity of the assay is largely dependent on the number of organisms seeded in the agar-overlay.

II. Reverse Mutation Tests with and without a Metabolic Activation System:

Organisms Used: Salmonella typhimurium, Ames Strains TA-1535, TA-1537, TA-1538, TA-98 and TA-100. All are histidine auxographs. Also E. coli WP2 hcr, requiring tryptophane.

Procedure:

Frozen cultures stored at -80°C were suspended in the same volume of 1/15 M phosphate buffer (pH 7). For the S. typhimurium strains a sterile solution of 0.5 mM L-histidine -0.5 mM D-biotin was added to molten soft agar (containing 0.6% agar and 0.6% NaCl) at the rate of 1/10 v/v, and for the E. coli strain a 0.5 mM L-tryptophane solution was added at the same rate. The prepared soft agar is termed "top agar".

For preparation of a metabolic activation system a male Sprague-Dawley rat (13 weeks old, 407 g) was given a single IP injection of 500 mg/kg Aroclor 1254, a polychlorinated biphenyl. On the evening of the 4th day after the injection the food was removed. On the 5th day the rat was killed by cervical dislocation and the liver immediately removed. The liver was perfused with an ice cold 0.15 M KCl solution and was homogenized in 3 volumes of the same solution (3 ml/g of the wet liver). The homogenate was centrifuged for 10 minutes at 700 g. The supernatant was recentrifuged at 9000 g for 10 minutes. The 9000 g supernatant was used in the experiment.

1 ml of a reaction mixture (S-9 Mix) consisted of the following: 0.3 ml 9000 g supernatant, 8 mM $MgCl_2$, 33 mM KCl, 5 mM Glucose-6-phosphate, 4 mM $NADP^+$, 100 mM Sodium phosphate (pH 7.4).

One-tenth ml of the bacteria suspension, 0.1 ml of the vinclozolin solution, and 0.5 ml of the S-9 mix for metabolic activation were added to 2 ml of the molten top agar at 45°C. After thorough mixing this was poured onto the surface of a minimal agar plate with modified Vogel-Bonner E medium. After incubation at 37°C for 2 days the number of revertant colonies was counted. Compounds used as positive controls were: AF-2; 2-(2-furyl)-3-(5-nitro-2-furyl) acrylamide; B-propiolactone; 9-aminoacridine; 2-nitrofluorene; 2-aminoanthracene.

Results of Reverse Mutation Tests:

1) AF-2, B-propiolactone, 9-aminoacridine, and 2-nitrofluorene (the positive controls) induced a marked reverse mutation in each tester strain.

2) 2-aminoanthracene was activated by the S-9 mix and became strongly mutagenic for TA-1535, TA-100, TA-1537, TA-1538, and TA-98.

3) Vinclozolin induced no increase in the number of revertant colonies in any strains, compared with the level of spontaneous mutation, whether or not the S-9 mix was added.

III. Host-Mediated Assay:

Procedure:

Male ICR mice (7 weeks old, mean body weight 33.6 g) were divided into 4 groups:

Group	Compound	Dose mg/kg	Total Dose mg/kg	No. of mice
Control	5% Gum arabic			6
Test 1	Vinclozolin	200	400	6
Test 2	Vinclozolin	1000	2000	6
Positive Control	DMN*	50	50	6

*DMN: dimethylnitrosamine

The vinclozolin was administered to the mice in 2 equal doses during a 24 hour period by gastric intubation at the rate of 0.2 ml/10 g body weight. A single oral administration of 50 mg/kg dimethylnitrosamine was given as a positive control. Immediately after the second dosing, a 2 ml suspension (4.9×10^8 cells/ml) of S. typhimurium G46 (his^-) in logarithmic growth phase was inoculated IP. The mice in each group were killed by cervical dislocation 3 hours after the treatments, and 2 ml of

1/15 M phosphate buffer (pH 7) was injected into the peritoneal cavity. The fluid was removed from the peritoneal cavity using a sterile syringe with a needle. For determination of survivors, 0.1 ml of a 1/3 x 10^{-6} dilution of the removed fluid was added to the top agar not containing biotin, and the contents were poured on a minimal agar plate. For detection of revertants, 0.4 ml of the undiluted fluid was treated similarly. Triplicate samples of each were made. The revertants and survivors were counted after incubation at 37°C for 2 days.

The in vitro reverse mutation test using the strain G46 also was conducted.

Results:

The in vivo reverse mutation testing using the strain G46 was negative.

In the host-mediated assay, there was no increase in the mutation frequency in the vinclozolin treated groups as compared with the controls. The positive dimethylnitrosamine controls showed a significant ($p < 0.001$) increase in mutation frequency.

Conclusion:

Since the negative control and the strain specific positive controls used in the evaluation of the validity of the in-vitro reverse mutation assay (Ames Salmonella Plate Test) and the in-vivo reverse mutation assay (Host-Mediated Assay) were demonstrated within the acceptable range, the negative responses of the test compound, Vinclozolin, in the Ames Salmonella/Microsome Mutagenicity test (1 through 3000 ug/plate) as well as in the Host-Mediated Assay (200 X 2 and 1000 X 2 mg/kg) at the dose levels tested are acceptable. However, the individual numerical data for checking the Ames tester strain genotypes were missing in the report. The specific procedures used in this study to confirm the histidine requirement, deep-rough character, ultraviolet sensitivity as well as the presence of R factor conferring ampicillin resistance of tester strains should be submitted.

card 1

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

004894

MEMORANDUM MAY 5 1983

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.
100 Cherry Hill Road
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%

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	18.6	20.5	18.5	20.6	18.5	20.7	18.5	20.5	18.5
	0.6	0.5	0.6	0.5	0.6	0.5	0.6	0.5	0.6	0.5
122 wks.	44.8	36.0	45.4	38.3	42.6	35.9	41.8*	35.8	39.9*	34.5
	4.1	3.3	5.0	5.0	3.4	4.9	4.8	2.5	4.4	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 xamination (50 mice per sex per group):

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

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

- The above summaries tend to suggest that vinclozolin induces leukemia/ ymphoma in male mice, lung tumors in female mice, and perhaps liver tumors n males on the high dosage. However, it is recognized that the "normal" ncidence of some tumor types may be high. According to Benirschke, Garner, nd Jones (Pathology of Laboratory Animals, page 1054), leukemia incidence n mice varies from 19 to 100%, and may appear relatively early (at 8 to 10 onths of age). Hepatocellular tumors also are common, having an incidence f 26 to 99%, and appearing from 12 to 28 months of age. Pulmonary tumors ave 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 o the NMRI strain.

With this submission we received data on the incidence of leukemia/lymphom n control NMRI mice from 5 studies conducted in the same laboratory as onducted this study with vinclozolin. The studies were conducted during the ame time frame as the vinclozolin study. The incidence of leukemia/lymphoma n 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 experiment (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 historic 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.

Roland A. Gessert LDC 5/4/8

Roland A. Gessert, D.V.M.
Veterinary Medical Officer
Toxicology Branch/HED (TS-769)

I concur with the above presented pathology assessment.

Louis Kasza

Louis Kasza, D.V.M., Ph.D.
Pathologist
Toxicology Branch/HED (TS-769)

TS-769:th:TOX/HED:RAGessert:4-26-83:card 4

7969-EUP-13

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

004894

MEMORANDUM APR 15 1983

OFFICE OF PESTICIDES AND TOXIC SUBSTANCES

TO: Henry Jacoby
Registration Division (TS-767)

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

WLB

SUBJECT: Vinclozolin for Use on Stonefruit. Experimental Use Permit 7969-EUP-13. Temporary Tolerance PP#9G2204. CASWELL#323C

Action Requested:

The petitioner requests an experimental use permit for 14,616 pounds of RONILAN Fungicide 50-W, equivalent to 7,308 pounds active ingredient vinclozolin, to be used on stonefruit (apricots, cherries, nectarines, peaches, plums, and fresh prunes).

The petitioner also requests temporary tolerances of 25 ppm for residues of vinclozolin 3-(3,5-dichlorophenyl)-5,ethenyl-5-methyl-2,4-oxazolidinedione and its dichloroaniline-containing metabolites in or on the raw agricultural commodities, the aforementioned stonefruit.

Recommendation:

The oncogenic potential of vinclozolin is negative in the rat and may be questionable in the mouse. To obtain estimates of virtually safe dose levels relative to this action, a risk assessment was performed tentatively considering the mouse oncogenicity study as positive for lung tumors.

The NOEL for reproductive effects is negative in the rat at 1458 ppm. The teratogenic NOEL is 600 ppm in the mouse. Vinclozolin is not on the RPAR list.

The lifetime dietary risk is based upon findings of lung adenomas in NMRI mice at a dietary level of 1458 ppm. The multi-stage model provided a Q* of 0.011 $(mg/kg/day)^{-1}$. The lifetime risk for existing tolerances is 4.2×10^{-5}. The lifetime risk for stonefruits at a tolerance level of 25 ppm is 8.57×10^{-5} or twice that of existing tolerances. This assumes that exposure is for a lifetime and 100% of the crop is treated. Since exposure is only for a couple of years, the risks would be proportionately less ($8.57 \times 10^{-5} \times \frac{2}{70} = 2.45 \times 10^{-6}$). Since the EUP is for 7,308 pounds which would treated approximately 1000 acres, the per cent of crop is considerably less than 100%.

Discussion:

Lung Tumor and Liver Tumor data from the vinclozolin mouse oncogenicity study are shown below:

Group	No. Animals	% Lung Adenomas	% Lung Carcin.	% Lung Tumors	% Liver Tumors
0 ppm	50M	4	4	8	0
	50F	0	0	0	0
162 ppm	50 M	2	2	4	0
	50 F	2	0	2	0
486 ppm	50 M	0	6	6	0
	50 F	2	0	2	0
1458 ppm	50 M	2	4	6	0
	50 F	8	0	8	0
4374 ppm	50 M	8	2	10	6
	50 F	10	0	10	0

Lung and Liver Tumor data from NMRI controls of 5 other studies of the same duration conducted in the same laboratory at about the same time are given below. In addition, control data from NMRI mice in studies published in the literature (Green, Henschler, Haase) are shown following the 5 studies conducted concurrently with the vinclozolin study.

HISTORICAL DATA FROM ONCO STUDIES OF NMRI MICE

Study	No. of Animals and Sex	% Lung Adenomas	% Lung Carcinomas	% Lung Tumors	% Liver Tumors
	100 M	15	5	20	2
	100 F	9	2	11	0
II	50 M	8	0	8	0
	50 F	4	0	4	0
III	70 M	2.9	1.4	4.3	2.9
	70 F	5.7	0	5.7	1.4
IV	50 M	4	0	4	0
	50 F	0	0	0	0
V	50 M	2	0	2	0
	50 F	6	0	6	0
Green	14 M			50	0
	15 F			27	0
Henschler	30 M	3.3	16.7	20	6.7
	29 F	10.3	3.4	13.7	0
Haase	M & F	10			

Previously Reviewed Toxicity Data: Memo of 4/17/78 from R. Gessert. PP#8G2068.

1. Studies Conducted with Formulation, RONILAN:

   a) Rat Acute Oral LD_{50} > 16,000 mg/kg (both sexes)
   b) Rabbit Acute Dermal LD_{50} > 2,000 mg/kg (both sexes)
   c) Rat Acute Inhalation LD_{50} > 1.7 mg/L for 4 hours

2. Studies Conducted with Technical Chemical:

   a) Rat Acute Oral LD_{50} > 10,000 mg/kg (both sexes)
   b) Acute Dermal LD_{50} > 2,500 mg/kg (both sexes)
   c) 90-Day Rat Feeding: NOEL = 450 ppm
   d) 90-Day Dog Feeding: NOEL = 300 ppm
   e) Mouse Teratology: Negative at 600 ppm
   f) 3-Generation Rat Reproduction: NOEL = 1458 ppm
   g) Dominant Lethal Assay in Mice: Negative at 2000 mg/kg
   h) Chronic Feeding/Oncogenicity in Rats for 103 Weeks: Oncogenic Potential: Negative; NOEL = 486 ppm
   i) Chronic Feeding/Oncogenicity in NMRI Mice for 26 Months: Questionable oncogenic potential for benign lung and liver tumors
   h) Metabolism: Repeated oral dosing in rats

3. Further assessment of mouse oncogenicity data raised possible questions relating to leukemia/lymphoma, lung adenomas, and liver adenomas/hepatomas. Detailed appraisal of data of mice at all dosage levels together with data from control mice in 5 other studies conducted concurrently in the same laboratory under the same conditions and in the same mouse strain and in other studies reported in the literature, revealed an incidence of leukemia/lymphoma in the historical controls which equaled or exceeded the incidence seen in the vinclozolin study. These data indicate that vinclozolin presents no oncogenic risk for leukemia/lymphoma.

   Toxicology Branch statistician Bert Litt performed a multi-stage risk analysis for lung adenomas; a Q* value of 0.010762 (or 0.011) was obtained. Subjecting the TMRC of stonefruit (0.4676 mg/day/1.5 kg) to this value:

   $\frac{0.4676}{60} \times 0.011\ (\text{mg/kg/day})^{-1} = 0.0077933 \times 0.011 = 8.57 \times 10^{-5} =$ lifetime dietary risk from stonefruit

   Applying the Q* value to the total TMRC for vinclozolin residue tolerances:

   $\frac{0.6959}{60} = 0.0115983;\ 0.0115983 \times 0.011 = 1.275 \times 10^{-4} =$ total lifetime dietary risk, all commodities

   Risk assessments were not performed for applicators; no exposure data are currently available.

4. Chronic feeding studies have been completed in the rat and mouse. The ADI is based on the rat. (Rat NOEL is 486 ppm, or 24.3 mg/kg). Based on the NOEL of 24.3 mg/day from the rat data and a safety factor of 100, the ADI is 0.2430 mg/kg/day and the maximum permissable intake is 14.58 mg/kg/day for a 60 kg person.

5. Residue Chemistry Branch concludes that residues will not exceed the 25 ppm level requested in the tolerance petition. (William L. Anthony and Robert Hummel, oral communications 2/24/83; followed by memo). The 25 ppm residue level far exceeds the levels that actually will be expected to exist when additional data are provided. (William Anthony, oral communication, 3/30/83).

RCB Review States:

a) Residue data on cherries following 1-6 applications at rates ranging from 0.75-3 lb. active ingredient (AI) show residues ranging from 0.87 ppm to 14.8 ppm at a 0-day PHI.

b) Peaches, nectarines, and apricots: In 15 studies, following 1-12 applications at rates of 0.25-1 lb. AI/A, residues ranged from < 0.05-27.5 ppm. The highest residue reflects 9 applications (vs the proposed 7) at the 1 pound AI/A rate and a 1-day (vs the proposed 3-day) PHI.

c) Plums, fresh prunes: In 6 studies following 1-4 applications at the rate of 0.25-1 pound AI/A, residues at a PHI of 5 days or longer were all < 0.90 ppm.

6. Published tolerances established under 40 CFR 180.380 utilize 0.22% of the ADI. Unpublished Toxicology Branch approved tolerances utilize the ADI to 1.57%. The current stonefruits action will utilize the ADI to 4.77%. (Computer printout attached).

7. Only one mutagenicity study was submitted by the registrant; a dominant lethal assay in the mouse was negative at a level of 2000 mg/kg.

The published literature also presents data demonstrating the ability of vinclozolin to induce mitotic recombination (positive against Aspergillus nidulans in mitotic recombination test). No other mutagenicity data are known to exist.

A complete battery of mutagenicity data will be required prior to granting any new permanent tolerances.

Published References Cited:

U. Green, et al. Comparative Study of the Carcinogenic Effect of BHP and BAP on NMRI Mice. Cancer Letters, 9 (1980) 257-261.

D. Henschler, et al. Carcinogenicity Study of Trichloroethylene by Longterm Inhalation in Three Animal Species. Arch. Toxicol. 43, 237-248 (1980).

P. Haase, et al. Evaluation of Dimethylhydrazine Induced Tumours in Mice as a Model System for Colorectal Cancer. Br. J. Cancer (1973) 28, 530.

Spyros G. Georgopoulos, et al. Mitotic Instability in Aspergillus nidulans Caused by the Fungicides Iprodione, Procymidone and Vinclozolin. Pestic. Sci. 1979. 10, 389-392.

Roland A. Gessert LDC 4/14/83

Roland A. Gessert, D.V.M.
Veterinary Medical Officer
Toxicology Branch/HED (TS-769)

TS-769:th:TOX/HED:RAGessert:3-31-83:card 4

MOUSE

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY 004894
WASHINGTON, D.C. 20460

MEMORANDUM

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° ± 0.5°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-treatement, 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

WRO for LDC 5/17/82
[illegible] 5/23/82

TS-769:th:TOX/HED:RGessert:5-17-82:card 2

Test for Significance of Differences Between Proportions 5/00489

Leukemia

[illegible]	[illegible]	[illegible]	[illegible] +/-2(S.D.)	One Tail P Statistic Fisher's
[illegible]	2	[illegible]	[illegible].00+/-(0.43)	
4374.000	10	[illegible]	20.00+/-(12.09)	0.014

BEST AVAILABLE COPY

HISTOPATHOLOGY INCIDENCE TABLE

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	P
Malignant Lymphoma																						
Granulocytic Leukemia																						
Angiosarcoma																						
Carcinoma, Metastatic																						
Sarcoma, Metastatic																						
Reticulum Cell Sarcoma																						
Hepatocholangiocarcinoma																						
Multifocal Hepatocellular 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	1		3		2	2	2			3		2			4
Multifocal Necrosis												2										2

Key: P = Present; 1 = Minimal; 2 = Slight; 3 = Moderate; 4 = Moderately Severe/High; N = No Section; I = Incomplete Section; A = Autolysis; X = Not Remarkable

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	Group 1	Group 5	Group 6	Group 7	Group 8
KIDNEY (NO. EXAMINED)	(77)	(44)	(45)	(48)	(45)
Tubular Carcinoma					1
Reticulum Cell Sarcoma	5	3			
Malignant Lymphoma	3	3			
Metastatic Alveolar Cell Carcinoma					
Metastatic Hepatocellular Carcinoma					
Mast Cell Tumor					
Tubular Adenoma					
Foci of Lymphocytes	15	1	1		
Chronic Nephritis	4	2	1	4	3
Hydronephrosis				2	
Suppurative Nephritis					
Cortical Cyst	3		1	6	1
Focal Mineralization				6	5
Tubular Necrosis					
Tubular Pigment					
Papillary Necrosis					
Infarction		1			
Amyloid					

MOUSE

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

004694

004894

MEMORANDUM

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
CASWELL#323C BAS 353F Fungicide.

We suggest that the registrant, BASF Wyandotte Corporation, be informed that upon further appraisal of the data from the mouse oncogenicity study of vinclozolin, it would appear vinclozolin at 4374 ppm may cause leukemia type tumors in male mice. In the treated mice we also noted 3 cases of adenoma in the liver; none were seen in the controls.

We recognize that such tumors are not rare in the mouse. We also acknowledge that, when comparing total numbers of tumors of all types in treated mice with total numbers of tumors in controls, oncogenicity is not evident. Therefore, in order to further elucidate the oncogenic potential of vinclozolin in the mouse, we request that tissues from the mid and low dose mice also be subjected to histological evaluation and that a report of the evaluation be submitted. In the report particular attention should be made to the leukemia and related diseases and to liver adenomas by animals and by organs in tabulating the results. An attempt also should be made at grading the lesions according to severity or deviation from average. Further, in order that risk assessments may be made, we request that applicator exposure assessments be provided for the various label uses of the pesticide.

A reevaluation of the study will be forwarded to you within one week.

Roland A. Gessert

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

TS-769:th:TOX/HED:5-10-82:RGessert:Rm. 816:X73710:card 1

INFORMATION CONCERNING PRODUCT IMPURITIES IS NOT INCLUDED

004894

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

3250

DATE: April 17, 1978

SUBJECT: BAS 352 F Fungicide. Vinclozolin. Temporary Tolerance & EUP for Use on Strawberries. Evaluation of Toxicity Data. BASF Wyandotte Corp., Parsippany, New Jersey

FROM: Roland A. Gessert, D.V.M., Toxicology Branch

TO: Special Registrations Section

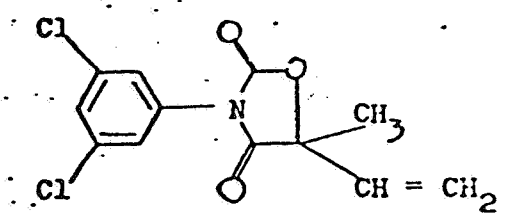
BASF Wyandotte Corporation requests an EUP and proposes establishment of a temporary tolerance for combined residues of Vinclozolin: 3-(3,5-dichlorophenyl)-5 methyl-5-vinyl-1,3-oxazolidine-2,4-dione in or on strawberries at 5 ppm.

CHEMICAL NAME: 3-(3,5-dichlorophenyl)-5-methyl-5-vinyl-1,3-oxazolidine-2,4-dione

Chemical Abstracts Usage (50471-44-8):

3-(3,5-dichlorophenyl)-5-ethenyl-5-methyl-2,4-oxazolidinedione

CHEMICAL STRUCTURE:



EMPIRICAL FORMULA: $C_{12}H_9NO_3Cl_2$

COMMON NAME: Vinclozolin (proposed)

SYNONYMS: BAS 352 F; 83 258

TRADE NAME: RONILAN (proposed); 50% by weight

PURITY OF TECHNICAL ACTIVE INGREDIENT: 93%, at least

IMPURITIES: [redacted]

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DECEMBER 5-2-78

EPA FORM 1320-6 (REV. 3-76)

Studies Conducted with Technical Material:

ACUTE ORAL TOXICITY, Male & Female Rats: LD_{50} greater than 10,000 mg/kg

ACUTE INTRAPERITONEAL TOXICITY, Male & Female Guinea Pigs: LD_{50} = 3,000 mg/kg

ACUTE INTRAPERITONEAL TOXICITY, Male & Female Mice: LD_{50} = 5,000 mg/kg

TOXIC SYMPTOMS: dyspnea, tremors, spasms, lethargy

POST MORTEM: hyperemia

ACUTE DERMAL TOXICITY, Male & Female Rats: LD_{50} greater than 2,500 mg/kg
No toxicity observed

PRIMARY SKIN IRRITATION, Male & Female Rabbits: Primary Skin Irritation Value = 1.3 (moderate)

PRIMARY EYE IRRITATION, Male & Female Rabbits: Primary Eye Irritation Value = 1.89; no keratitis

Studies Conducted with Formulation:

ACUTE ORAL TOXICITY, Male & Female Rats: LD_{50} greater than 16,000 mg/kg

ACUTE DERMAL TOXICITY, Male & Female Rabbits: LD_{50} greater than 2,000 mg/kg

PRIMARY EYE IRRITATION, Female Rabbits: Mean Primary Eye Irritation Score of 19.7; some corneal opacity and conjunctivitis; no iritis

ACUTE INHALATION TOXICITY IN RAT (dust): No mortality after 4-hour exposure to a dust concentration of 1.17 mg/l, equivalent to 0.59 mg/l active ingredient. The single, 4-hour inhalation caused slight mucosa irritations in some animals.

ACUTE INHALATION TOXICITY IN RAT (aqueous spray of 1% suspension): No mortality after 4-hour exposure to a spray concentration of 0.2 mg/liter of air, corresponding to an active ingredient concentration of 0.1 mg/liter of air. The single, 4-hour inhalation of the spray caused slight irritations of the mucous membrane.

PRIMARY SKIN IRRITATION, Male & Female Rabbits: Primary Skin Irritation Value of formulation = 2.75

SUBCHRONIC (90 day) TOXICITY IN MALE & FEMALE RATS: No influence at 150 or 450 ppm in food on behavior, external appearance, food or water consumption, body weight gain, hematology, clinical biochemistry, urine composition, eyes, hearing, dentition, gross appearance of organs & tissues, organ weights, nor on histological examination of liver, adrenal, and pituitary. No rats died prematurely. The lowest toxic dose exceeds 450 ppm.

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SUBCHRONIC (90 day) TOXICITY IN MALE & FEMALE DOGS: At 100, 300, 1000, or 2000 ppm in food over a period of 3 months, all doses were tolerated well without externally recognizable symptoms of intoxication. Repeated ophthalmological examinations revealed no changes in the refractory media or in the fundus of the eye. Feed acceptance was variable in one male at 2000 ppm and in all females, including the control group, but no adverse effect was observed on weight gain. Clinical chemistry studies and urine analyses revealed no biologically relevant differences between the trial and control groups.

Hematograms revealed increased platelet counts in females receiving 1000 and 2000 ppm, and Howell-Jolly bodies were found in differential blood counts of both males and females at these pesticide levels.

Postmortem examination revealed no macroscopic changes in the organs due to the compound, but increases in the relative liver and adrenal weights in females receiving 2000 ppm were observed.

TERATOLOGY (PRENATAL TOXICITY) IN MICE: The compound was administered in food at levels of 0, 600, 6000, and 60,000 ppm during the entire gestation period. Caesareans were performed on the 18th day post coitum. All fetuses were examined for any externally recognizable symptoms of toxicity; 2/3 of the fetuses of each litter for deformities, variations, and retardations of the skeleton; and 1/3 of the fetuses of each litter for deformities, variations, and retardations of the organs.

600 ppm were tolerated without any clinically recognizable symptoms of toxicity. No changes were observed in the average amount of food consumed or in the gain in body weight of the pregnant animals. The examination of the fetuses did not reveal any indication of a compound-induced effect.

Animals which received 6000 ppm in their diet did not exhibit any clinically recognizable symptoms of toxicity. One animal died on the 10th day post coitum. In the initial days of the test the animals consumed less food than the animals which were administered 0 or 600 ppm in their diet. There was no gain in weight corresponding to the gestation. However, the animals did not lose any weight, either.

When the animals were sacrificed on the 18th day post coitum, no implantation sites were discovered. The fertilized egg cells had died before nidation.

60,000 ppm were not consumed by the animals. From the first day of the test the animals ate practically none of the food. This caused a decrease in the average body weight. All the animals died within 9 days. No nidation sites were detected in the animals which died after the 6th day post coitum.

The pesticide is not a teratogen.

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3-GENERATION REPRODUCTION STUDY IN RATS: The compound was tested in different stages of development in the rat, and the examinations covered the pre- and postnatal development. Two breeding studies each were performed with 3 successive generations with oral administration of the test compound. In all, the experimental period lasted approximately 2 years. Particular attention was paid to possible teratogenic properties of the compound.

The compound was fed at levels of 162, 486, and 1458 ppm. Treatment began for the 1st (P-) generation 7 weeks before the 1st breeding test and was continued without interruption in all following generations until the end of the study. A corresponding untreated group served as control.

Fertility and breeding capacity were not definitely influenced at any of the concentrations in the examined animals (P-, F_1-, and F_2- generation): copulation, duration of pregnancy, litter size, birth weight, and breeding rate of the pups lay within the normal range. The indices of fertility, pregnancy, lactation, and viability corresponded in experimental and control animals.

Examinations of the general tolerance indicated no intolerance phenomena. Behavior, external appearance, feces, food consumption, intake of drinking water, and body weight gain were normal at all concentrations (162, 486, and 1458 ppm in the food) in the parent animals (P-, F_1-, and F_2- generation). None of these rats died prematurely. The animals of the F_3- generation developed normally, also. Specific checks of external criteria and sensory functions revealed no impairment.

On necropsy, macroscopic inspection revealed no pathological changes in parent or young animals. The comparison of organ weights and the histological examinations in the F_3-generation after 9 weeks of life disclosed no changes attributable to the test compound.

Under the test conditions the lowest toxic concentration can be said to lie above 1458 ppm in the food. The compound did not show teratogenic properties in this study.

MUTAGENIC EFFECT ON THE MALE MOUSE, USING THE DOMINANT LETHAL TEST: 2000 mg/kg was administered on 5 successive days via stomach tube in 20 ml/kg 0.5% aqueous CMC formulation. Control animals received only the CMC formulation. There were no clinically recognizable symptoms of toxicity, and no evidence of any effect on conception rate, average number of implants, percentage of viable fetuses, or percentage of dead implants. Under conditions of the test, the compound could not be shown to have any mutagenic effect on male mice.

METABOLISM; REPEATED ORAL DOSING IN RATS:

The disposition and metabolic fate of 3-(3,5-dichlorophenyl)-5-methyl-1,3-oxazolidin-2,4-dione, vinclozolin, in rats (bodyweight ca 200g) was studied after daily oral administration of the ^{14}C-labelled pesticide for seven days. All experiments were performed at a nominal dose level of 40 mg/kg bodyweight per day.

After daily oral administration of ^{14}C-vinclozolin to rats, excretion of radioactivity was fairly rapid and was similar in both male and female rats. Approximately 43% and 50% of the daily administered dose were excreted in the urine and feces, respectively, during each day of the dosing period. Six days after the final dose (12 days after the first dose) means of 47% and 54% of the total dose had been excreted in urine and feces, respectively, and means of 0.1% and 0.04% of the total dose were retained in the gastrointestinal tract and liver, respectively. No radioactivity was detected in the remainder of the body at six days after the final dose, nor was any detectable radioactivity excreted in the expired air during 24 hours after the final dose.

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After single oral doses of ^{14}C-vinclozolin to male rats with cannulated bile ducts, a mean of 65% of the dose was excreted in the bile during two days. During the same time means of 19% and 15% were excreted in urine and feces, respectively.

After the first oral dose of ^{14}C-vinclozolin to rats, mean peak concentrations of radioactivity in plasma were reached at one hour in both males (12.8 ug equivalents/ml) and females (10.1ug equivalents/ml). During the dosing period, predose concentrations of radioactivity in plasma increased gradually and were slightly higher in female rats than in males. After the final dose, mean peak plasma concentrations of radioactivity were reached at one hour in males (14.2 ug equivalents/ml) and at 7.5 hours in females (15.0 ug equivalents/ml) and declined with a half-life of about 20 hours in both cases.

After daily oral dosing of ^{14}C-vinclozolin to rats for seven days, concentrations of radioactivity were generally higher in the tissues of female rats than in those of males. Concentrations of radioactivity significantly higher than those in plasma were found at most times in liver, kidneys, gastrointestinal tract, fat, adrenals and ovaries. Radioactivity still could be detected in nearly all tissues examined at 96 hours after the final dose, but by this time concentrations were similar to those in plasma, except in liver, kidneys, gastrointestinal tract, and female fat.

Whole-body autoradiography showed an extensive distribution of radioactivity with concentrations being highest in the gastrointestinal tract, bile ducts, bladder, liver, and kidneys. Concentrations were lower in one rat sacrificed 24 hours after a single dose than in a rat killed 24 hours after the last of seven daily doses. However, at four days after the last of seven doses, radioactivity was only detectable in the gastrointestinal tract and the liver, and these concentrations were low.

A major metabolite in fecal extracts was tentatively identified by mass spectrometry as being N-(3,5-dichlorophenyl)-2-methyl-2,3,4-trihydroxybutyramide. It was also shown that the glucuronide conjugate of this metabolite was the major radioactive component excreted in urine and bile. Unchanged vinclozolin was found in fecal extracts, but not in urine.

CHRONIC TOXICITY AND ONCOGENIC STUDIES IN RATS:

Vinclozolin was given in concentrations of 162, 486, 1458, and 4374 ppm in the food for 130 weeks, when a mortality rate of approximately 70% was reached in the control group. There were 100 rats per group (50 males & 50 females).

The lowest mortality rate was found for the highest vinclozolin concentration; the highest mortality rate, in the untreated control group. Prelethal symptoms and cause of death were similar in experimental and control animals. The tumors did not disclose, in respect to their nature and extent, an influence of the test compound; at the highest treatment the tumor rate was 55% as against 63% in the control rats.

Behavior, external appearance, feces, intake of drinking water, hematology, examination of sight, hearing, and dentition, as well as macroscopic and microscopic inspection did not indicate definite intolerance phenomena. Differing organ weights should be interpreted as a typical high deviation of aged rats, and in connection with the loss of body weight at the higher concentrations. The weights of prostate and seminal vesicles were always within the normal range.

The following effects of the test compound were seen: The body weight was significantly reduced at 1458 and 4374 ppm. The food consumption was, in relative terms, initially increased at 4374 ppm; otherwise it corresponded to body weight. At both 1458 and 4374 ppm the serum level of total bilirubin tended towards reduction. In the urine the content of 17-ketosteroid and ascorbic acid was increased, the maximum being found after 8 test weeks. After 104 test weeks at the latest these symptoms had disappeared. No further changes were revealed by clinical biochemistry or urinalysis.

Under the test conditions, the no-effect level can be said to fall between 486 and 1458 ppm in the food.

In the study, vinclozolin showed no carcinogenic properties.

CHRONIC TOXICITY AND ONCOGENIC STUDY IN MICE:

Vinclozolin was given in concentrations of 162, 486, 1458, and 4374 ppm in the food for 26 months, when a mortality rate of approximately 70% was reached in the control group. In this study special attention was paid to possible carcinogenic properties of the test compound.

None of the tested concentrations (162, 486, 1458, and 4374 ppm in the food) led to definite intolerance phenomena during the 26 months of administration. Only body weight gain of the males at 4374 ppm was slightly, but significantly inhibited, the food consumption being reduced in parallel. This finding, however, might still be incidental.

The mortality rates of experimental and control animals were nearly the same, as were the prelethal symptoms and cause of death. Neither did the tumors disclose, in respect to their nature and extent, an influence of the test compound: at the highest concentration the tumor rate amounted to 46% as against 51% in the control group. Differing organ weights should be interpreted as typical high deviation of aged mice.

Behavior, external appearance, feces, water consumption, hematology, clinical biochemistry, urinalysis, and also examination of sight, hearing, and dentition, remained unchanged even at the highest concentration (4374 ppm).

Under the test conditions the lowest toxic concentration can be expected around or slightly above 4374 ppm. Vinclozolin did not show carcinogenic properties.

These studies all meet core minimum standards.

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RECOMMENDATION: In one rat test, the no effect level appears to be between 436 and 1458 ppm. In the 3-generation rat reproduction study, the no effect level is above 1458 ppm. Other studies show vinclozolin to be exceptionally non-toxic. 1458 ppm, therefore, appears to be close to the no effect level. Using a 100-fold safety factor, a daily acceptable daily intake of 14 ppm appears to offer adequate protection. The 5 ppm tolerance requested for strawberries (which represent 0.18% of the total diet) therefore can easily be granted. Vinclozolin is a new compound for which other tolerances have not yet been requested.

Roland A. Gessert, D.V.M.
Toxicology Branch
4/26/78

J.E.W. 5/1/78

Tox Chem No. 323C Ronilan

File Last Updated 8/28/84

Study/Lab/Study #/Date	Material	EPA Accession No.	Results: LD_{50}, LC_{50}, PIS, NOEL, LEL	TOX Category	CORE Grade/ Doc. No.
Teratology - mice; BASF Med. Biol. Res. Lab; 2/18/75	Technical		Teratogenic NOEL = 600 ppm LEL = 6000 ppm (resorptions) Levels tested: 0, 600, 6000, 60,000 ppm.		000244
Teratology - rabbit; Huntingdon Research Centre; ID #80/232; 9/4/82	Technical	070400	NOEL > 300 mg/kg/day (HDT) A teratogenic potential is not indicated. Fetotoxic NOEL = 80 mg/kg/day.		Minimum 002409
3 Generation reproduction - rat; Laboratorium for Pharmakologic and Toxikologic; 12/9/77	Technical		NOEL > 1458ppm (HDT) Teratogenic NOEL >1458 ppm(HDT) Levels tested 162,486 and 1458 ppm		000244
90 Day feeding - dog; BASF Med. Biol. Res. Lab; 4/28/75	Technical		NOEL = 300ppm LEL = 1000ppm (increased plalet counts Howell-Jolly bodies in differential blood counts cholestasia of liver - fatty deposits in renal tubules, cholestasis of kidneys.) Levels tested = 100, 300, 1000, 2000 ppm		Supplementary 000244 000245
90 Day feeding - rat; Laboratorium for Pharmakologic and Toxikologic ; 5/26/75	Technical		NOEL > 450ppm (HDT).		000244
6-Month feeding - dog; BASF; #he-ru/ro; 8/16/82	Technical Vinclozolin (>98.1% pure)	248123 248124	NOEL = 100 ppm LEL = 300 ppm. [Increased absolute and relative adrenal weight (M/F), decreased relative pituitary wt(F)] Levels tested in beagles: 0, 100, 300, 600, and 2000 ppm.		Minimum 002214

004894

Tox Chem No. 323C RONILAN

Study/Lab/Study #/Date	Material	EPA Accession No.	Results: LD_{50}, LC_{50}, PIS, NOEL, LEL	TOX Category	CORE Grade/ Doc. No.
130 Week feeding/oncogenic- rat; Laboratorium for Pharmakologic and Toxikologic ; 12/15/77	Technical		NOEL = 486ppm LEL = 1458ppm (body wt.reduction, reduced serm bilirubin) Oncogenic NOEL > 4374 ppm (HDT) Levels tested:162,486,1458 & 4374ppm		000244
26 Month feeding/onco.- mice; Laboratorium for Pharmakologic and Toxicologic; 12/15/77	Technical Batch #83258	096970	Systemic NOEL = 486 ppm Systemic LEL = 1458 ppm (decreased body weight in males). Oncogenicity 1. An increase in leukemia/lymphoma was observed in males. However historical control data on this type of tumor equalled or exceeded the level observed in this study, indicating that the increase may not be real. 2. An apparent dose related increase in lung adenoma was noted in females. Historical data from 5 studies using the same strain(NMRI) indicated that in 4 of 5 studies the historical control incidence was significantly lower than that observed in the treated groups of this study. Along with considerations of the benign nature of the and because latency did not decrease, it is concluded that the chemical is weakly positive in the production of lung tumors. 3. A low but increased incidence of liver adenoma was noted at the HDT in males. Because the tumors were benign & latency was not decreased it is concluded that the chemical is weakly positive in the production of liver tumors. Doses tested: 0, 162, 486, 1458, 4374 ppm - NMRI strain.		000244 001885 002717 002669 Minimum

Tox Chem No. 323C - RONILAN

Study/Lab/Study #/Date	Material	EPA Accession No.	Results: LD_{50}, LC_{50}, PIS, NOEL, LEL	TOX Category	CORE Grade/ Doc. No.
Metabolism - rat; 7 day feeding	^{14}C labelled Technical		Dosing at 40 mg/kg for 7 days Six days after final dose: 47% eliminated in urine 52.86% eliminated in feces 0.1% retained in gastrointestinal tract 0.04% retained in liver (These are mean values) Major metabolite in fecal extracts was N-(3,5-dichlorophenyl)-2-methyl-2,3,4-trihydroxybutyramide. Glucoronide conjugate of this was major metabolite in urine and bile.		000244 Minimum
Mutagenic - dominant lethal - mice; BASF Med. Biol. Res. Lab.	Technical		NOEL > 2000 mg/kg (only level tested)		000244
Mutagenic - chinese hamster sister chromatid exchange; BASF;#16M0232/8013; 12/81	Technical Vinclozolin (98.1%)	250113	Mean SCE rate for high dose (5620 mg/kg) = 3.78 SCEs/cell Mean SCE rate for control = 3.71 SCEs/cell Vinclozolin does not induce SCE. (At 5620 mg/kg - piloerection, irregular respiration and atony were observed). Doses tested: 3830 and 5620 mg/kg		Acceptable 003181
Mutagenic - rec-assay (DNA Repair); Yasuhiko Shirasu, et al.,Inst. of Environmental Tox.; 2/78	Technical Vinclozolin (92.8%)	250114			Inadequate 003181
Mutagenic - reverse mutation	Technical Vinclozolin (92.8%)	250114	No increase in number of revertant colonies in any strains with or without the S-9 activation mix. Strains tested: Ames - TA 1535, TA 1537, TA 1538, TA 98, TA 100. Also E. coli WP2 hcr.		003181

Tox Chem No. 323C

Study/Lab/Study #/Date	Material	EPA Accession No.	Results: LD_{50}, LC_{50}, PIS, NOEL, LEL	TOX Category	CORE Grade/ Doc. No.
Mutagenic - host-mediated assay	Technical Vinclozolin (92.8%)	250114	In vivo reverse mutation using S.Typhimirium G46 was negative. There was no increase in mutation frequency in vinclozolin treated groups.		Acceptable 003181
Dermal sensitization-#79/661; 4/29/81	Vinclozolin (50%)		Non sensitizing Doses tested: 0.1 ml/8 cm^2 of a 2, 6, 20, or 60% concentration of the formulation.		Minimum 002040
Dermal sensitization-guinea pig; BASF; #79/89; 9/7/79	Vinlozolin (50%)		Potential sensitizer		002040 Supplementary 002799 Guideline
Acute oral LD_{50} - rat; 2/20/73	Technical		LD_{50} > 10 g/kg (HDT)	IV	000244 Minimum
Acute IP LD_{50} - guinea pig	Technical		LD_{50} = 3000 mg/kg		000244 Minimum
Acute IP LD_{50} - mice; 2/20/73	Technical		LD_{50} = 5000 mg/kg (dyspnea, tremors, spasms, lethargy)		000244 Minimum
Acute dermal LD_{50} - rat; BASF Med. Biol.Res.Lab; 11/9/77	Technical		LD_{50} > 2,500 mg/kg (HDT)	III	000244 Minimum
Primary dermal irritation - rabbit; BASF Med. Biol. Res. Lab; 11/9/77	Technical		PIS = 1.3/8.0 (moderately irritating)	III	000244 Minimum
Primary eye irritation-rabbit; BASF Med. Biol. Res. Lab; 11/9/77	Technical		PIS = 1.89/8.0 (no keratitis)	IV	000244 Minimum

Tox Chem No. 323C

Study/Lab/Study #/Date	Material	EPA Accession No.	Results: LD_{50}, LC_{50}, PIS, NOEL, LEL	TOX Category	CORE Grade/ Doc. No.
Acute inhalation LC_{50} - rat; BASF; 3 to 4/78	EPA Reg.7969-53 Vinclozolin (50%)	244529	LC_{50} > 29.1 mg/l (actual conc.-HDT; (No mortalities or toxic signs) Doses tested: Actual conc. - 1.2, 11.0, 29.1mg/L-Sprague-Dawley strain	IV	Guideline 000803
Acute oral LD_{50} - rat; Huntingdon Res. Centre; 3/22/76	Formulation (Ronilan or BAS 352 04F)		LD_{50} >16 g/kg	IV	Minimum 000244
Acute dermal LD_{50}- rabbit; Huntingdon Res; 3/5/76	Formulation		LD_{50} >2.0 g/kg	III	Minimum 000244
Acute inhalation LC_{50}- rat; BASF Med. Biol.Res. Lab; 11/4/76	Formulation		LC_{50} > 0.59 mg a.i./L/4 hrs. (Only level tested - no mortality, slight mucosa irritations)	III	Minimum 000244
Acute inhalation LC_{50}- rat; BASF Med. Biol.Res. Lab; 11/4/76	1% aqueous suspension of formulation		LC_{50} > 0.1mg active ingredient/L/4hr (slight mucosa irritations)	II	Minimum 000244
Primary eye irritation- rabbit; BASF Med. Biol. Res.Lab; 3/78	Formulation		Conjunitivitis continuing through day 8.		Minimum 000245
Primary eye irritation- rabbit; BASF med. Biol. Res. Lab; 11/4/76	Formulation		PIS = 19.7/110 (some corneal opacity and conjunctivitis, no iritis).	III	Minimum 000244
Primary dermal irritation - rabbit; BASF Med. Biol. Res. Lab; 11/4/76	Formulation		PIS = 2.75/8.0	III	Minimum 000244

004894

File Base Updated: _____ Current Date _____

Study/Lab/Study #/Date	Material	EPA Accession No.	Results: LD_{50}, LC_{50}, PIS, NOEL, LEL	TOX Category	CORE Grade/ Doc. No.
Ronilan 323C ceptable Daily Intake - RA/ORP/HED/Tox;			ADI = 0.025 mg/kg/day MPI = 1.5 mg/day (60kg) Safety factor = 100 Dated [illegible] Updated: Study: 6 month dog ~~[illegible]~~ Study NOEL: 100ppm Lab.: BASF Study #: he-ru/ro Study date: 8/16/82 Doc. No.: 002214 Comments: dog most sensitive; NOEL in rat 2y. study 486ppm. Presumption of oncogenicity dismissed by peer review committee June 17 1985.		Doc. # is ADI updated

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004894

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