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

004287

MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

SUBJECT: CGA 64 250; Banner; Tilt; Mouse Oncogenicity;
EPA Reg. #100-AUR; Caswell #323 EE;
Accession #250784 through 250786; 251237

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

THRU: Roger Gardner Roger Gardner 2-14-85
Acting Head, Review Section IV
Toxicology Branch
Hazard Evaluation Division (TS-769C)

Attached is the Toxicology Branch review of the 2-year oncogenicity study with CGA 64 250 (Banner/Tilt) in mice. The technical material was administered at dietary concentrations of 0, 100, 500 and 2500 ppm.

This study is classified CORE-Minimum. The test material was found to cause liver tumors in male mice at the high dose level. Based on an initial Toxicology Branch review of this study by William Dykstra, a risk assessment was performed. The risk assessment report will be issued separately by the Branch. Please note the following recommendations:

1) As indicated in the attached review of the mouse oncogenicity study, the registrant is required to submit results of analyses to demonstrate purity and stability of the test substance. Additionally, the registrant must submit a summary table of the clinical observations (including palpable tumors) for this study.

2) Toxicology Branch requests that EAB determine applicator exposure and exposure to persons (including 20 kg children) upon re-entry into treated areas.

3) Toxicology Branch requests a mouse metabolism study to identify metabolites resulting from a 100 ppm and a 2500 ppm feeding level. These results may elucidate the mechanism of oncogenicity in this species.

Toxicology Branch will retain the study report and supporting data until the additional summary data are received for review.

Alan Katz Alan Katz 2/14/85
Toxicologist
Toxicology Branch
Hazard Evaluation Division (TS-769C)

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1. CHEMICAL: CGA 64 250; Banner; Tilt; Propiconazole; 1-(2-(2,4-dichlorophenyl)-4-propyl-1,3-dioxolan-2-yl)methyl-1H-1,2,4-triazole.

2. TEST MATERIAL: Technical grade; pale brown viscous liquid; Batch No. P4-6; EPA Registration No. 100-AUR.

3. STUDY TYPE: Oncogenicity in mice.

4. STUDY IDENTIFICATION: "CGA 64 250; Long-term Feeding Study in Mice"; Huntingdon Research Centre, Huntingdon, Cambridgeshire, England; Final Report, No. CBG 196/81827; 11/4/82; Authors: B. Hunter, N. Slater, R. Heywood, A. Street, D. Prentice, W. Gibson, C. Gopinath; Sponsor: CIBA-GEIGY Limited, Basle, Switzerland; EPA Accession Nos. 250784 through 250786; 251237.

5. REVIEWED BY:
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6. APPROVED BY:
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7. CONCLUSIONS:

Core-Classification: Minimum. An oncogenic effect was found in the liver of male mice given CGA 64 250 Technical at the highest dietary concentration (2500 ppm). Non-neoplastic liver effects were also observed. With the exception of slightly reduced body weight gain among males during the first 3 months of the study, no adverse effects were apparent at the lowest concentration (100 ppm).

A risk assessment has been performed by the Toxicology Branch, based on the results of this study. The risk assessment is reported separately.

8. RECOMMENDATIONS:

The applicant is required to submit the following:

(1) Results of analyses for purity of the test material (including identification of all impurities) and stability of the test material in the diet.

(2) Clinical observations, summarized according to sex/dose group. A summary of palpable masses must be included.

9. BACKGROUND:

CGA 64 250 (Banner) is a fungicide for control of certain diseases in turf. The objective of this study, as stated by the study authors, was to "assess the potential tumorigenic effect of CGA 64 250 in the diet, when given to mice, and to evaluate its safety for extrapolation to man."

10. MATERIALS AND METHODS:

A detailed description of materials and methods, excerpted from the study report, is presented in Appendix 1. A summary is provided below.

The test material was stored at room temperature at the contract laboratory. Storage conditions at the sponsor's facility, prior to shipping, were not specified. The stability of the test material was also not reported.

CD-1 mice were randomly assigned to treatment groups. Each cage contained 4 mice of the same sex. The test material was administered in the diet ad libitum to 4 main groups (52 mice/sex/group) at dietary levels of 0, 100, 500 and 2500. A satellite group (12 mice/sex) was included at each concentration level, for sacrifice at one year.

	CGA 64 250 conc. (ppm)	No. of Mice Males	Females
1	0	52 (12)	52 (12)
2	100	52 (12)	52 (12)
3	500	52 (12)	52 (12)
4	2500	52 (12)	52 (12)

Diets were prepared weekly. During the first year of the study, the test material was ground directly into basal diet (Spratt's Laboratory Diet No. 2). During the second year (days 386-728), the test substance was dissolved in ethyl acetate prior to incorporation into the diet. Justification for this change is not presented in the study report; however, the reviewers note that analytical results for concentration and homogeneity during the second year appear to be generally superior to those of the first year. It should be noted that, for a properly designed toxicity/oncogenicity study using a single control group, the only variable in treatment between groups should be the dose of the test material administered. In the present study, however, it appears that an additional variable may have been introduced through the use of ethyl acetate as a solvent for the test material to facilitate incorporation into the feed. It is not clear from the study report whether (1) equal concentrations of ethyl acetate were used for each batch of test and control diet, or if (2) the blended diets were analyzed for residual levels of ethyl acetate.

Parameters observed during the study included clinical signs, mortality, food consumption, hematology and clinical chemistry (weeks 50 and 102), and urinalysis (weeks 51 and 102). All surviving mice in the satellite groups were sacrificed at 53 weeks, and all surviving mice in the main groups were sacrificed at 104 weeks. All animals were necropsied. Selected organs from mice sacrificed at the study midpoint or termination were weighed. All gross lesions and tissues were examined microscopically.

Statistical analyses of the data were performed. Group differences were considered significant where $p<0.05$.

11. RESULTS:

Calculated levels of consumption of the test material over 104 weeks for low, mid and high dose males were 10.0, 49.4 and 344.3 mg/kg/day, respectively. Consumption of test material for females was 10.8, 55.6 and 340.3 mg/kg/day.

There were no compound-related clinical signs as evidenced by the data in Appendix 1 of the study report. An increase in mortality was noted in males of the 2500 ppm group during the first 6 months. This finding is considered compound-related.

Survival at 104 weeks was as follows:

Group	Males	Females
Control	24/64	28/64
100 ppm	20/64	33/64
500 ppm	21/64	24/64
2500 ppm	14/64	32/64

No compound-related effect is evident in survival except in the high dose males. The number of mice killed for interim study at week 53 was as follows:

Group	Males	Females
Control	11	12
100 ppm	11	11
500 ppm	11	11.
2500 ppm	9	12

The compound-related effect on high dose male survival did not appreciably affect the ability to evaluate tumor expression, since a significant increase in liver tumors was found at this dosage.

Body weight gain of high dose males was reduced during the first 13 weeks, comparable to controls during weeks 13-52, and reduced again during the second year. Low and mid-dose male body weight gain was decreased during weeks 1-13 but comparable to controls for the remainder of the study. Body weight gain of high-dose female mice was reduced up to 78 weeks of treatment, but comparable to controls for the remainder of the study. Body weight gain of low and mid dose female mice during the study was comparable to that of the controls.

Food consumption was increased in high dose male mice during the study. Food consumption of other groups of male and female mice was comparable to that of the control groups during the study. Since food consumption was increased, the body weight decreases are due to the toxic effect of the test material.

A review of the individual clinical observations noted in Appendix 1 of the study report revealed no obvious treatment-related in-life signs. Since these observations were not summarized, however, this reviewer (AK) reserves a final conclusion with respect to the clinical signs associated with treatment until the required summary tables are provided.

Necropsy observations at the termination of the study indicated a treatment-related increase in liver lesions among mid and high dose males and in high dose females. Selected gross changes are shown below:

Liver Lesions Observed at Terminal Necropsy

	Control	100 ppm	500 ppm	2500 ppm	Control	100 ppm	500 ppm	2500 ppm
Mass(es)/raised areas/ swellings/nodular areas	10	9	15	14	3	3	0	11
Enlarged	1	3	3	5	2	6	2	9
Lobular markings accentuated	0	0	1	1	0	0	0	4
Surface irregular/pitted	1	1	0	1	2	0	1	5
Number of mice examined	24	20	21	14	28	33	24	32

Food conversion ratios were increased for high dose male and female mice during weeks 1-24. Other treatment groups had food conversion ratios comparable to control values.

No compound-related hematological effects were noted. SGPT and SGOT were significantly increased in high dose males and females at 52 weeks and in high dose males at 100 weeks. SAP was increased in high dose males at week 100. These changes are considered indicative of liver damage. Urinalysis results did not reveal any treatment-related effects.

Increased liver weight was noted in high and mid dose males and in high dose females at both interim and terminal sacrifice. There was good correlation between gross and microscopic findings. Enlarged livers containing gross pathological changes were seen in high dose animals. Non-neoplastic changes in high dose males and females consisted of hepatocyte enlargement, vacuolation and fat deposition. Livers of low and mid dose mice were comparable to those of controls.

Amyloidosis occurred more frequently in treated animals compared to controls, but was not dose-related.

The number of tumor-bearing animals in comparison to the total number of animals examined is presented in Appendix 2, as taken from the report. No treatment-related effect is evident. The incidence of liver cell tumors, as taken from the report, are presented in the following table:

Incidence of Liver Cell Tumors

D= Died on study
I= Interim sacrifice
T= Terminal sacrifice

	Control			100 ppm			500 ppm			2500 ppm		
	D	I	T	D	I	T	D	I	T	D	I	T
Male mice with:												
Benign liver cell tumors	7	1	5	2	-	5	3	2	5	10	1	11
Malignant liver cell(a) tumors	8	-	7	5	-	2	7	1	7	20*	3	3
Number of mice examined	29	11	24	33	11	20	30	11	21	41	9	14
Female mice with:												
Benign liver cell tumors	1	-	3	-	-	-	-	-	2	-	-	5
Malignant liver cell(a) tumors	-	-	1	-	-	1	-	-	-	-	-	3
Number of mice examined	24	12	28	20	11	33	29	11	24	20	12	32

* 1 metastasising liver cell tumor
(a) Mice with one or more tumors, at least one of which is malignant

CGA 64 250 treatment was associated with early expression of malignant liver cell tumors in male mice. The preceding table shows that, at interim sacrifice after 1 year, malignant liver cell tumors were found in 0/11, 0/11, 1/11, and 3/9 control, low, mid and high dose males, respectively. No liver cell tumors were found in any of the female mice sacrificed at the 1-year interim.

The total incidences of liver cell tumors, benign and/or malignant, are shown below:

Group	Males	Females
Control	28/64	5/64
100 ppm	14/64	1/64
500 ppm	25/62	2/64
2500 ppm	48/64*	8/64

*$p < 0.001$ (Fisher's one-tail)

The incidence of liver tumors is significant at the high dose level for males. These liver effects are considered compound-related.

Other non-neoplastic and neoplastic lesions in treated mice of both sexes were comparable to those of controls..

TILT CGA-64250 Reviews

The next 6 page(s) is/are not included in this copy of the TILT reviews.

The material not included contains the following type of information:

___ Identity of product inert ingredients

___ Identity of product impurities

___ Description of the product manufacturing process

___ Description of product quality control procedures

___ Identity of the source of product ingredients

___ Sales or other commerical/financial information

___ A draft product label

___ The product confidential statement of formula

___ Information about a pending registration action

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