004272

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

FEB 7 1985

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

SUBJECT: EPA Reg. #: 100-AUR; Banner; Fungicide for control of certain diseases in turf; Rat oncogenicity/chronic toxicity study
Caswell #: 323 EE
Accession #: 250787 through 250790

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

THRU: Roger Gardner Roger Gardner 2-6-85
Acting Head, Review Section IV
Toxicology Branch
Hazard Evaluation Division (TS-769C)

Attached is the review of the 2-year dietary oncogenicity and chronic toxicity study with CGA 64 250 in rats. The study was conducted at Huntingdon Research Centre (Huntingdon, Cambridgeshire, England) for the registrant/sponsor, CIBA-GEIGY Limited (Basle, Switzerland).

Conclusions and Recommendations:

This study is CORE Supplementary. No final conclusion regarding the study can be made until additional data are available for review. No NOEL was established for several histological effects. Dermal fibromas in males and thyroid follicular tumors in high dose females may be attributable to exposure to the test compound. Although this reviewer has described several apparent flaws in the conduct of this study, these deficiencies are not considered to have exerted a major impact on the overall integrity of this study. The CORE-classification may be upgraded following evaluation of the specific historical control data and other documents listed in the attached review.

The study report and supporting data will be retained within the Toxicology Branch for possible re-examination upon receipt of the requested additional information.

Alan Katz 2/6/85
Alan Katz
~~Toxicologist~~
Toxicology Branch
Hazard Evaluation Division (TS-769C)

1. CHEMICAL: Banner; CGA 64 250; 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 and chronic toxicity in rats.

4. STUDY IDENTIFICATION: "CGA 64 250, Potential Tumorigenic and Toxic Effects in Prolonged Dietary Administration to Rats"; Huntingdon Research Centre, Huntingdon, Cambridgeshire, England; Report No. CBG 193/8284; Test No. 789023; 9/30/82; Authors: B. Hunter, N. Slater, R. Heywood, A. Street, D. Prentice, W. Gibson, C. Gopinath; Sponsor: CIBA-GEIGY Limited, Basle, Switzerland; EPA Accession Nos. 250787 through 250790.

5. REVIEWED BY: Alan C. Katz, M.S., D.A.B.T. Signature: Alan Katz
Toxicologist
Toxicology Branch Date: 2/6/85
Hazard Evaluation Division (TS-769C)

6. APPROVED BY: Christine F. Chaisson, Ph.D. Signature: Christine Chaisson
Head, Review Section IV
Toxicology Branch Date: 2-6-85 CFC
Hazard Evaluation Division (TS-769C)

7. CONCLUSIONS:

Core-Classification: Supplementary. No NOEL was established for several histological effects. Additional data must be submitted in order to more fully assess the relationship of CGA 64250 treatment to dermal fibromas in male rats and thyroid follicular tumors in females. The core-classification may be upgraded, following review of the required data (specified below). Due to the higher incidence of ocular lesions in high dose animals compared to controls, ophthalmoscopic examinations should have been conducted for low and mid dose groups.

8. RECOMMENDATIONS:

The registrant is required to submit the following:

(1) A copy of the Standard Operating Procedures which were followed during the study, as well as documentation of any deviation(s) from such procedures, relating to diet preparation and storage conditions.

(2) A copy of the Protocol for this study, as well as documentation of any deviations from, additions to, or changes in, the Protocol.

(3) An explanation of the criteria used to identify statistical outliers (data excluded from calculations of group means and statistical analyses).

(4) A description of statistical methods used in organ weight analysis, including criteria for appropriate use of log transformation and covariate analysis.

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

(6) An explanation of the divergent results showing poor homogeneity and/or concentration for certain blends of test diet.

(7) Locations of the dermal fibromas found in the male rats.

(8) Historical control data (see Appendix 1 of this review) for dermal fibromas in male rats.

(9) Historical control data (see Appendix 1 of this review) for thyroid follicular tumors (i.e., adenoma and adenocarcinoma) in female rats.

(10) A summary of incidence data with respect to clinical signs (including those listed as "incidental" findings at the front of Appendix 11 of the study report), tabulated according to sex and dose group.

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 "determine the tumorigenicity and toxicity of CGA 64 250 in long term dietary administration to rats 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 2. A summary is provided below.

The test material, CGA 64 250 Technical (Batch No. P4-6), was stored at room temperature. Randomized Sprague-Dawley CD rats were used in the study. Each cage contained 5 rats of the same sex. The test material was administered in the diet ad libitum to 4 main groups (50 rats/sex/group) at dietary levels of 0, 100, 500 and 2500 ppm for the control, low, mid and high doses, respectively.

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, this reviewer notes 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, this reviewer is concerned 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. It is further noted that analytical methods for determination of the concentration of the test substance in diet were changed at least twice during the study. The rationale for these changes is not specified in the study report. The alterations in methodology for analytical chemistry do not appear to temporally correspond with the major change in procedures for diet preparation noted above. It is not clear to this reviewer whether multiple values presented (Appendix 3 of this review, excerpted from study report Addendum, Table 1) for concentration of the test material in diet (e.g., % nominal value of CGA 64250, low dose, days 281-287: top, 80/41/80/40; middle, 67/97/152/63; bottom, 33/71/101/35) represent results for separate blends of diet or replicate samples of the same batch. An explanation of these data, as well as a discussion of the poor homogeneity results apparent at one or more dose levels for blends prepared at other intervals (e.g., days 57-64 and 162-168), should be submitted to this Agency to enable it to more fully assess the quality of diet blending/analytical support for this study.

The experimental design, as taken from the report, was as follows:

"To each main group was attached a satellite group consisting of 20 males and 20 females; 10 males and 10 females were used for hematological investigation, and a different 10 males and 10 females were used for blood chemistry and urinalysis investigations. Since blood was withdrawn from the orbital sinus, these rats were not used for examination of potential ophthalmic effects. In addition, 10 males and 10 females were attached to each group for interim kill after 52 weeks. The rats were subjected to a detailed macroscopic examination with organ weight analysis; tissues were preserved and processed...

The rats were allocated to the 4 treatment groups as follows:

Group	Treatment	Main Group		Satellite Group				Interim Kill	
				Hematology		Blood Chem./ Urinalysis			
		M	F	M	F	M	F	M	F
1	Control(0)	1-50	321-370	51-60	371-380	61-70	381-390	71-80	391-400
2	100 ppm CGA 64250	81-130	401-450	131-140	451-460	141-150	461-470	151-160	471-480
3	500 ppm CGA 64250	161-210	481-530	211-220	531-540	221-230	541-550	231-240	551-560
4	2500 ppm CGA 64250	241-290	561-610	291-300	611-620	301-310	621-630	311-320	631-640

The cages constituting each group were dispersed so that environmental influences were equilibrated as far as possible for each treatment group. Each cage was identified by a colored label according to group, each label was uniquely numbered with cage and study number. Within the cage the rats were identified by ear mark."

Parameters observed during the study included clinical signs, mortality, food consumption, hematology (weeks 26, 52, 78 and 103), clinical chemistry (weeks 26, 52, 78 and 104), and urinalysis (weeks 24, 50, 76 and 102). Ophthalmoscopic and hearing examinations (main groups) were conducted prior to treatment and during weeks 25, 51, 77 and 103 in the control and high dose groups. The hearing tests were performed using a Galton whistle set at 10 KHz placed 1 meter from the rat's head. The total duration of treatment was 107 weeks for males and 109 weeks for females. Following sacrifice and gross necropsy at 52 weeks and termination, selected organs were weighed and tissues were examined microscopically.

Statistical evaluation of the data was performed. Analysis of variance and Student's 't' test were used to evaluate food consumption, body weight and clinical laboratory data for significant intergroup differences. Organ weight data were evaluated by analysis of covariance, Student's 't' test and Williams' test Some organ weights were adjusted for final body weight as covariate, or, "(w)here appropriate, organ weights were log transformed to stabilize variance." Tumor incidence data were evaluated by chi^2 analysis or exact probability calculations. Analysis of mortality data was performed using a log rank test. This reviewer is concerned that statistical tests on organ weight data may have been performed in an arbitrary manner. The registrant should indicate the rationale and criteria used to select which organ data to evaluate by analysis of covariance and which data is appropriate for application of log transformation.

11. RESULTS:

Calculated levels of consumption of the test material over 104 weeks for low, mid and high dose males were 3.6, 18.1 and 96.4 mg/kg/day, respectively. Consumption of test material for females was 4.6, 23.3 and 10.6 mg/kg/day.

During weeks 29 and 30, the majority of animals in all groups showed signs of sialodacryoadenitis (viral infection) which caused dryness of the eyes, swelling around the face and throat, and associated weight loss. After week 30, the signs of infection disappeared.

The total number of unscheduled deaths occurring during the study were reported as follows:

Group	Males	Females
Control	30	42
100 ppm	31	36
500 ppm	32	36
2500 ppm	25	26

Survival was better among treated females than controls, as shown by the data above.

Food consumption was significantly lower for high dose females throughout the study and for high dose males from week 27 to termination. Low and mid dose male and female rats showed food consumption values comparable to those of the controls. Body weight gains of high dose male rats were significantly lower than control values during the first year, but not for the remainder of the study. High dose female rats showed reduced body weight gain throughout the study, while weight gain reduction occurred in mid dose females during the first 26 weeks of treatment. These decreases in body weight gain were compound-related. Food conversion ratios were increased (poor utilization) in high dose males and females during the first 26 weeks. Water consumption of high dose female rats was lower than that of the controls during the study. No effect on water consumption was evident for treated males.

No toxicologically significant treatment-related effects were noted on the basis of results of hematology, blood chemistry, urinalysis, ophthalmoscopy or hearing tests, although minimal and transient or sporadic differences occurred between treated and control groups with respect to several hematologic and blood chemistry parameters. Elevated blood urea levels were reported for high dose females in weeks 26, 33 and 52 and for high dose males in week 78, while lower glucose levels were found in mid and high dose females in weeks 26, 52 and 78, and mid and high dose males in week 52. Again, these treatment-related changes were not toxicologically significant. This reviewer notes that a slightly increased incidence of corneal lesions was found in high dose males at 77 weeks and 103 weeks and cataracts were found in 2 high dose females (but in none of the controls) at 103 weeks. These lesions are not considered likely to be related to treatment; however, it is unfortunate that the eyes of the animals in the low and mid dose groups were not examined prior to termination.

No macroscopic findings in rats sacrificed at 52 weeks were considered to be related to treatment. Liver weights were increased in high dose males and females at 52 weeks. The following histopathologic incidence data show that lipid deposition in liver cells was also increased in high dose males at 52 weeks:

	Control		100 ppm		500 ppm		2500 ppm	
	M	F	M	F	M	F	M	F
No. examined	10	10	10	10	10	9	10	9
Lipid deposition	2	0	2	1	0	1	6	0

Liver weights were also increased in high dose males and females at termination. Although the investigators reported that "(n)o [macroscopic] findings considered to be related to treatment were observed," this reviewer notes that necropsy observations showed an increased incidence of grossly enlarged livers among high dose males which died during the study or were sacrificed at termination. Also, an increased incidence of discolored foci or puncta were found in the lungs of high dose females.

	Control			100 ppm			500 ppm			2500 ppm		
	D*	I	T	D	I	T	D	I	T	D	I	T
Males												
No. examined	30	10	40	31	10	39	32	10	38	25	10	45
Enlarged liver	2	0	6	2	0	11	5	0	6	5	0	18
Lungs:Grey,green cream or white areas, foci or puncta	8	3	16	9	3	10	3	2	13	6	2	20
Females												
No. examined	42	10	28	36	10	34	36	9	35	26	9	45
Enlarged liver	4	0	12	5	0	13	7	0	16	2	0	19
Lungs: Pale, grey green, cream, brown or white areas, foci or puncta	4	1	4	9	0	5	6	1	7	8	3	17

*D= Decedents; I= Interim; T= Termination

An increased incidence of foci of enlarged liver cells in high dose females was reported. Livers of high dose males showed increased vacuolated hepatocytes and ballooned cells. A dose-related increase in liver cell lipid deposition in males was also apparent. These data are shown below.

	Control		100 ppm		500 ppm		2500 ppm	
	M	F	M	F	M	F	M	F
No. examined	64	67	67	69	66	67	65	67
Foci of enlarged liver cells	2	1	0	2	2	2	5	13
Vacuolated hepatocytes	26	28	31	34	29	39	44	23
Ballooned cells	15	2	8	1	13	2	25	2
No. examined, Oil Red "O"	60	59	61	60	59	62	60	65
Lipid deposition	4	9	7	15	15	17	17	4

No NOEL for liver effects is apparent. Additionally, the pancreas showed a dose-related effect in exocrine atrophy in female rats as shown below:

	Control		100 ppm		500 ppm		2500 ppm	
	M	F	M	F	M	F	M	F
No. examined	60	59	61	61	61	62	62	65
Exocrine atrophy	3	1	5	3	1	6	3	9

No NOEL for pancreatic effects is apparent. Luminal dilatation of the uterus also appeared to be a dose-related effect, with a NOEL not established.

	Control	100 ppm	500 ppm	2500 ppm
No. examined	58	63	63	65
Luminal dilatation, uterus	4	10	9	17

The numbers of tumor-bearing rats and rats with malignant tumors in comparison to the total number of rats examined are shown below:

	Control		100 ppm		500 ppm		2500 ppm	
	M	F	M	F	M	F	M	F
No. examined	60	60	60	60	60	60	60	60
No. with tumors	25	43	41	47	42	48	34	40
No. with malignant tumors	5	15	19	14	17	25	15	15

No defined treatment-related effect is noted in the data. Although the numbers of tumor-bearing and malignant tumor-bearing males were increased in the treated groups compared to the controls, there were no apparent dose relationships.

The incidence of dermal fibroma was increased in the high dose males. The location of the dermal fibromas was not specified. Both location and historical data on this tumor type are necessary to complete the evaluation. The possible relationship of thyroid follicular adenocarcinoma to treatment must also be considered. Historical control data on thyroid follicular tumors in females are required for this evaluation.

	Control		100 ppm		500 ppm		2500 ppm	
	M	F	M	F	M	F	M	F
No. examined	59	64	61	66	58	65	61	67
Dermal fibroma	0	0	3	0	1	0	5	0
Thyroid follicular								
adenoma	0	1	2	0	1	0	1	1
adenocarcinoma	0	0	0	0	2	0	0	2
total	0	1	2	0	3	0	1	3

APPENDIX 1.
Recommendations for Submission of Historical Control Data*

The best historical control data are obtained using the same species and strain, from the same supplier, maintained under the same general conditions in the same laboratory which generated the study data being evaluated. The data should be from control animals on recent (no more than 5 years before initiation or after termination of the study being evaluated) consecutive, long term oncogenicity/toxicity studies. If there is not a sufficient data base meeting all of these criteria, data should be presented for control groups most closely fitting these conditions. Additional information should be provided for each set of control group incidence values presented, as follows: (1) identification of species, strain, name of supplier and geographical location; (2) name of the laboratory in which the study was performed, and when; (3) description of general conditions under which the animals were maintained, including the type or brand of diet and type of bedding, if possible; (4) the approximate age of the control animals at the beginning of the study and at the time of sacrifice or death; (5) description of the control group mortality pattern observed during or at the end of the study and of any other pertinent observations (e.g., diseases, infections, etc.); (6) name of the pathology laboratory and examining pathologist responsible for gathering and interpreting the pathological data from the study; and (7) what lesions may have been combined to produce any of the incidence data. The historical control data should be presented as discrete control group incidences, segregated by sex.

* Adapted from OPTS EP, 8/9/84. Paynter, Orville E., Oncogenic Potential: Guidance for Analysis and Evaluation of Long Term Rodent Studies.

TILT CGA-64250 Reviews

The next // 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

✓ Detailed methods and results of a registrant submission.

___ Duplicate pages.

The information not included generally is considered confidential by product registrants. If you wish to obtain the information deleted, please contact the individual who prepared this response to your request.
