CASWELL FILE
8-27-86

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

005352

MEMORANDUM

AUG 27 1986

OFFICE OF PESTICIDES AND TOXIC SUBSTANCES

SUBJECT: BANNER, Data Review; 4F3007; Action Code: 232; Accession # 073918-29; Record # 160801; Caswell # 323EE.

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

FROM: Alan C. Katz
Toxicologist, Review Section III
Toxicology Branch
HED (TS-769C)
7/24/86

THROUGH: Marcia Van Gemert, Ph.D.
Head, Review Section III
and
Theodore M. Farber, Ph.D.
Chief, Toxicology Branch
8.4.86
8/55 18
5/26/86

Action Requested:

Review toxicity data; response to previous EPA toxicology reviews.

A. Registrant's Response to EPA Review of the Two-Year Dietary Oncogenicity Study and Chronic Toxicity Study with CGA-64250 Technical in Rats (Accession #073918).

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.

Background:

The above cited study was reviewed by Tox Branch, and a Data Evaluation Record (DER) was issued on February 7, 1985 (Tox Document #004295). The study was classified as Core-Supplementary, pending submittal of additional data. A copy of the DER is attached (Appendix 1).

Review of Additional Information:

1. Dietary Preparation:

Diets were prepared without the use of a solvent during weeks 1-53. From week 54 until termination, the test material was dissolved in ethyl acetate (50% w/w) prior to incorporation into untreated basal diet. The control diet

did not contain ethyl acetate until week 65. From this time until termination the control diet was treated with ethyl acetate at the same concentration as that used in the high dose group.

2. Analyses of Stability and Homogeneity of Test Material in Diet:

Stability of the test material in rat diet pellets was reported as follows:

2 weeks at room temperature

100 ppm samples:	114/118%
1000 ppm samples:	96/96%
10,000 ppm samples:	72/69%

It is noted that this stability determination was performed "prior to initiation of the chronic feeding studies." There is no indication that the stability of the test material was determined in diet which contained ethyl acetate and was processed using a rotary evaporator. In the 2-year rat study, dietary concentrations were 100, 500 and 2500 ppm. Diet powder, rather than the pellet form used in the stability test, was administered. Fresh diet was administered weekly. However, the type of animal feed used in the stability analysis (Nafag 890) was not the same as that used in the chronic study (Spratt's Laboratory Diet No. 2). Because stability of the test substance was analyzed for incorporation in the pelleted form of a diet from a different manufacturer, and no data apply to diet in which ethyl acetate was incorporated as a vehicle, the stability results presented in support of this study are considered invalid. However, because analyses for concentration were performed on samples of diet (from batches actually used in the study) which were "aged" approximately 1 week week after blending, without refrigeration or other precautions to retard degradation, stability of the test compound may be qualitatively deemed adequate insofar as these concentrations were generally found to be near the nominal value and all diets were blended at weekly intervals.

Although blended diets were not analyzed for residual levels of ethyl acetate, use of this solvent is considered justifiable on the basis of improved homogeneity. Because the vehicle was incorporated in the control diet at a concentration equivalent to that used in the high dose diet (except during weeks 54 through 64) and, assuming adequate evaporative processing of blended feed (although, as noted, this was not appropriately demonstrated by analytical testing), the risk of introduction of a confounding toxicological variable through the use of this vehicle is considered minimal.

The rationale for changes in the analytical method, accompanied by data supporting the accuracy of all such methods used, was appropriately addressed by the Registrant.

3. Protocol, Deviations and Amendments:

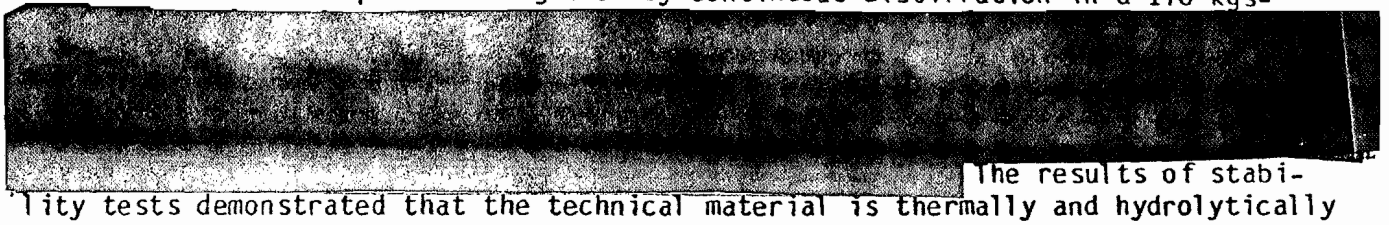
A cursory review of the protocol, deviations and amendments indicates general GLP compliance.

4. Statistical Evaluation:

The information provided by the Registrant in support of the statistical methods used in this study is considered generally acceptable. However, it is noted that "no set procedures are laid down for the exclusion of individual data points"; thus, there is no assurance that criteria regarding the omission of data points were consistently applied.

5. Test Substance Purity and Stability:

Data presented by the Registrant were adequate to establish purity and stability of the technical material. The test material, designated batch P. 4-6, was a pale brown viscous liquid. It was a composite of 3 separate batches which were mixed and then "purified together by continuous distillation in a 170 kgs-



The results of stability tests demonstrated that the technical material is thermally and hydrolytically stable.

6. Location of Dermal Fibromas:

The table in Appendix 2 was provided by the Registrant to clarify the location of dermal fibromas in male rats in this study.

7. Historical Control Data:

Historical control data were provided, using results of 9 studies which appear to meet several major criteria for comparative purposes. Background elements of these studies are outlined in the table in Appendix 3, as provided by the Registrant. It is not specified whether the controls in all studies were from the same supplier or whether all groups received the same diet, or if animals in all studies were housed 5 per cage as in the study in question; nevertheless, these studies are deemed generally useful for comparisons of incidence of "spontaneous" lesions.

a.) Incidence of Dermal Fibromas in Males

The historical control data for the occurrence of dermal fibromas in male Sprague-Dawley rats are presented in Appendix 4, as provided by the Registrant. Because there was no dose-related trend in the incidence of this lesion in males treated with CGA-64250 technical, and because the highest incidence (2500 ppm group: 8%) was within the historical range, the occurrence of dermal fibromas is not considered a treatment-related effect.

b.) Incidence of Thyroid Follicular Adenoma and Adenocarcinoma in Females

The historical control data for the occurrence of thyroid follicular adenomas/adenocarcinomas in female Sprague-Dawley rats are presented in Appendix 5, as provided by the Registrant. Although the adenocarcinomas among females in the present study were found only in the high dose group, the incidence (2 of 67 animals) was within that of the historical control range. The occurrence of thyroid follicular adenomas/carcinomas in this study is not considered a treatment-related effect.

c.) Incidence of Liver Lesions

The tabulation of data regarding the incidence of liver lesions in Sprague-Dawley rats, excerpted from the supplemental materials provided by the Registrant, is presented in Appendix 6. These tables provide summary data which are useful in comparing findings of the present 2-year study with those of historical controls in comparable studies. The data include incidences of vacuolation of hepatocytes, ballooned cells, and lipid deposition in the liver of male rats, as well as foci of enlarged hepatocytes in females.

(1) Vacuolated Hepatocytes (Males)

The incidences of vacuolation of hepatocytes in males fed 100 or 500 ppm CGA 64250 technical material slightly exceeded the range established by the historical controls, but were comparable to the incidence in the concurrent control group. In the high dose (2500 ppm) group, however, the incidence of this lesion was found to be more than 50 percent greater than either the concurrent control or the highest historical control value. The data suggest that, at 2500 ppm in diet, CGA 64250 induced vacuolation of hepatocytes in the male rats.

(2) Ballooned Cells in the Liver (Males)

The incidences of ballooned liver cells in males fed diet containing the test material at concentrations of 0, 100, or 500 ppm were well within the historical control range. In the 2500 ppm group, however, the incidence of this lesion exceeded the concurrent control and the overall historical control values by 65-73%, thus suggesting a treatment-related effect.

(3) Lipid Deposition in the Liver (Males)

Historical control values appear to be of little use in the evaluation of lipid deposition in the liver as a toxic effect in male Sprague-Dawley rats. The incidence values reported for 7 studies range from 2 to 100 percent. Nevertheless, the data from the present study show a dose-related trend, and effects in the 500 and 2500 ppm groups may be considered treatment-related. The toxicological significance of this finding per se, however, is considered questionable since the incidences in the 500 and 2500 ppm male groups were slightly lower than the overall historical control value.

(4) Foci of Enlarged Hepatocytes (Females)

The incidences of foci of enlarged hepatocytes in the 0, 100 and 500 ppm groups were comparable and within the historical control range. However, the incidence of this finding in the 2500 ppm female group was substantially greater than the concurrent and historical control values. It may be concluded that CGA 64250 induced foci of enlarged hepatocytes in the high dose females.

d.) Incidence of Exocrine Atrophy in the Pancreas (Females)

The incidences of exocrine atrophy in the pancreas of female rats varied widely among the 10 historical control groups cited, ranging from 2 to 100 percent (Appendix 7). Nevertheless, the data from the present study show a dose-related trend, and effects in the 500 and 2500 ppm groups may be considered treatment-related. The toxicological significance of this finding is considered questionable, however, since the incidence in the 2500 ppm group was comparable to the overall historical control value.

e.) Luminal Dilatation of the Uterus

Incidences of luminal dilatation of the uterus varied moderately among the 10 historical control groups (Appendix 8). Although the incidence of this finding in the 2500 ppm group in the present study was within the range of values established by the historical controls, it moderately exceeded both the concurrent and overall historical control values. The increased incidence of luminal dilation of the uterus in females fed diet containing CGA 64250 technical at a concentration of 2500 ppm is considered treatment-related.

8. Summary of Incidence of Clinical Signs:

Clinical signs were tabulated by sex and group. Evaluation of these data indicates no apparent treatment-related change.

9. Pathology Addendum to Final Report:

A tabular summary of neoplastic findings in all satellite group rats was presented. No significant new data are noted by this reviewer.

10. Amended Pages to Final Report:

Amendments to the report, and reasons for these changes, were presented by the Registrant. These changes in the report did not alter any overall conclusions with respect to the toxicity of the test compound or the validity of this study.

11. Addendum to Final Report:

The addendum which was issued 6/25/85 presented no new data. The information presented in this addendum represents a recollation of previously reported data.

12. Core grade = Minimum MV 8.14.86

B. Registrant's Response to EPA Review of the 2-Year Dietary Oncogenicity Study With CGA 64250 Technical in Mice (Accession # 073919)

Study Identification: "CGA 64 250; Long-term Feeding Study in Mice"; Huntingdon Research Centre, Huntingdon, Cambridgeshire, England; 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.

Background:

The above cited study was reviewed by Tox Branch, and a Data Evaluation Record (DER) was issued on February 14, 1985 (Tox Document #004287). The study was classified as Core-Minimum. CGA 64250 was found oncogenic in the liver of male mice. Non-neoplastic liver effects were also observed. The NOEL for chronic toxicity was 100 ppm (the lowest dietary concentration of CGA 64250 tested). Additional data were requested. A copy of the DER is attached (Appendix 9).

Review of Additional Information:

1. Analyses of Stability and Homogeneity of Test Material in Diet:

Stability of the test material in rat diet pellets was reported as follows:

2 weeks at room temperature	
100 ppm samples:	114/118%
1000 ppm samples:	96/96%
10,000 ppm samples:	72/69%

It is noted that this stability determination was performed "prior to initiation of the chronic feeding studies." Additional, more recent (June, 1985) data were provided which show stability of CGA-64250 in "mouse diet" (composition and manufacturer not specified) stored for 28 days at 30° C. Concentrations tested were 5, 100 and 2500 ppm. There was no evidence of degradation at any of these concentrations. There is no indication that the stability of the test material was determined in diet which contained ethyl acetate and was processed using a rotary evaporator. In the 2-year mouse study, dietary concentrations were 100, 500 and 2500 ppm. Diet powder, rather than the pellet form used in the initial stability test, was administered. Fresh diet was administered weekly. However, the type of animal feed used in the initial stability analysis (Nafag 890) was not the same as that used in the chronic study (Spratt's Laboratory Diet No. 2). Because stability of the test substance was determined in the earlier assays using the pelleted form of a diet from a different manufacturer, the origin or composition of the powdered diet was not identified in the later assays, and no data apply to diet in which ethyl acetate was incorporated as a vehicle, the stability results presented in support of this study are considered invalid. However, because analyses for concentration were performed on samples of diet (from batches actually used in the study) which were "aged" approximately 1 week after blending, without refrigeration or other precautions to retard degradation, stability of the test compound may be qualitatively deemed adequate insofar as these concentrations were generally found to be near the nominal value, and because all diets were blended at weekly intervals during the study.

Although blended diets were not analyzed for residual levels of ethyl acetate, use of this solvent is considered justifiable on the basis of improved homogeneity. Because the vehicle was incorporated in the control diet at a concentration equivalent to that used in the high dose diet, and assuming adequate evaporative processing of blended feed, the risk of introduction of a confounding toxicological variable through the use of this vehicle is considered minimal.

The rationale for changes in the analytical method, accompanied by data supporting the accuracy of all such methods used, was appropriately addressed by the Registrant.

2. Analyses for Purity of the Test Material

The Registrant has satisfactorily addressed the question of purity of the test material. The test material used in the mouse oncogenicity study (Batch P.4-6) was identical to that used in the rat chronic toxicity/oncogenicity study, and the relevant data were discussed previously in this memorandum.

3. Diet Preparation

The Registrant provided adequate details of diet preparation, including use of ethyl acetate.

4. Summary of Incidence of Clinical Signs

Clinical signs were tabulated by sex and group. Evaluation of these data indicate no apparent change related to treatment with CGA 64250 technical.

5. Addendum to Final Report

The addendum which was issued 11/15/84 presented no new data. The information provided in this addendum represents a recollation of previously reported data.

C. Registrant's Response to EPA Review of Mutagenicity Studies with Propiconazole (CGA 64250) Technical (Accession #073920)

Background:

The Registrant submitted additional information for 8 mutagenicity studies, in response to DER's issued on February 8, 1985 (Tox Document #004276).

Review:

1. Supplement to L5178Y $TK^{+/-}$ Mouse Lymphoma Mutagenicity Test. Ciba-Geigy Test No. 811516. June 24, 1985.

Additional data were presented by the Registrant, including clarification of criteria for evaluation.

The results of a preliminary cytotoxicity test were not presented. According to the study report, "The concentration calculated to produce a 10% reduction in the cell number in comparison with the control is used as the second highest in the mutagenicity experiment together with four further concentrations corresponding to factors of 2.0, 0.5, 0.25 and 0.125." In the mutagenicity experiment itself, however, there was no evidence of cytotoxicity at the second-highest dose, based on the data presented for the numbers of viable clones in the assays with or without metabolic activation. In both the activated and non-activated systems, the positive control appeared to be more cytotoxic than the highest concentration of test material. Overall, the data suggest that the experiment should have been performed using a higher concentration of CGA 64250 technical. This test is considered unacceptable. The results are inconclusive and the test should be repeated.

2. Supplement to Saccharomyces Cerevisiae D7/Mammalian-Microsome Mutagenicity Test in Vitro. Ciba-Geigy Test No. 811558. May 7, 1985.

Additional information provided by the Registrant included data for individual plate counts, as well as a table of statistics for the assay with microsomal activation. These data were considered adequate to establish this study as acceptable. Under the experimental conditions of this study, CGA 64250 technical did not induce mutation in S. cerevisiae cells, with or without metabolic activation (rat liver S9mix).

3. Supplement to Point Mutation Assay with Mouse Lymphoma Cells; Host-Mediated Assay. Ciba-Geigy Test No. 811513. July 3, 1985.

Additional information provided by the Registrant included individual data and standard deviations, as well as the rationale for dose selection. Although no evidence of mutagenicity was found, the sensitivity of the assay is found to be unacceptable. No concurrent positive control was tested. Untreated control values for viability and mutant frequency fluctuated over a very wide range. Also, this test failed to demonstrate that the test substance (administered by gavage) or metabolite(s) was available for exposure to the ip-injected target cells.

4. Supplement to Chromosome Studies in Male Germinal Epithelium (Mouse Spermatogonia). Ciba-Geigy Test No. 811511. June 28, 1985.

Seven of the 15 animals in the high dose group died soon after administration of the first dose of CGA 64250 (498 mg/kg, via stomach tube). No additional animals died following administration of four subsequent doses. It is not clear whether deaths in this study may be attributable to toxicity or dosing accidents; the laboratory apparently did not attempt to gather any data which would be useful in making this distinction.

Reference (mitomycin C, 0.5 mg/kg ip) control data from a "parallel" test with 2 mice (100 metaphases/mouse examined) were not sufficient to demonstrate the reliability of the results of the experiment with CGA 64250.

The results of this study are considered inconclusive. Tox Branch further recommends that individual protocols be prepared for all future studies.

5. Supplement to Chromosome Studies in Male Germinal Epithelium (Mouse Spermatocytes). Ciba-Geigy Test No. 811512. June 25, 1985.

No individual protocol was prepared for this study. The study was carried out "in accordance to the standard operating procedure", a copy of which was not provided with the report or supplemental data. The Registrant noted that the high dose was set at 1/3 of the oral LD_{50} (ref: Laboratory Report GU 2.1; May 7, 1979).

Apparently, the animals used in this experiment were the same ones which were used in the study of spermatogonia (Test No. 811511, reviewed above). Seven of the 15 animals in the high dose group died soon after administration of the first dose of CGA 64250 (498 mg/kg, via stomach tube). No additional animals died following administration of four subsequent doses. It is not clear whether deaths in this study may be attributable to toxicity or dosing accidents; the laboratory apparently did not attempt to gather any data which would be useful in making this distinction. Slides from 1 of the 8 animals surviving to termination were not scored due to an insufficient number of "well spread metaphases."

The Registrant presented data from a "parallel" study using mitomycin C (0.5 mg/kg ip). Criteria for establishing parallelism were not specified. Although 12 mice were treated with mitomycin C, data were reported for only 8 of these animals. Five of the 8 mice showed 1 or more aberrations in metaphase I (100 metaphases examined/mouse). No aberrations were found in metaphase II in any of the animals. Overall, the reliability of the assay appears questionable. The results are therefore considered inconclusive. Tox Branch further recommends that individual protocols be prepared for all future studies.

6. Autoradiographic DNA Repair Test on Human Fibroblasts. Ciba-Geigy Test No. 811655. March 29, 1985. CGA 64250 Technical (90.7% a.i.), Batch No. op.103119.

All additional data requested by Tox Branch were provided by the Registrant. Therefore, the classification is upgraded to acceptable. The results indicate that, under the conditions of this experiment, CGA 64250 at concentrations up to and including 9.32 ug/ml showed no evidence of mutagenic activity.

7. Autoradiographic DNA Repair Test of Rat Hepatocytes. Ciba-Geigy Test No. 811514. March 29, 1985. CGA 64250 Technical (90.7% a.i.), Batch No. op.103119.

All additional data requested by Tox Branch were provided by the Registrant. Therefore, the classification is upgraded to acceptable. The results indicate

that, under the conditions of this experiment, CGA 64250 at concentrations up to and including 83.47 ug/ml showed no evidence of mutagenic activity.

8. Sister Chromatid Exchange. Ciba-Geigy Test No. 811515. June 26, 1985.

The Registrant noted that the high dose (1020 mg/kg) was set at 1/3 of the oral LD_{50} (ref: Laboratory Report: GU 2.1; August 27, 1982). Although the data show no evidence of SCE induction, the number of animals (2/sex/group) used in the study was less than that which we would generally consider acceptable. The rationale for not scoring slides which were prepared for an additional 2 animals/sex/group was not adequately established. This study is unacceptable. Tox Branch recommends that, for future studies, larger sample sizes should be used and all slides should be evaluated and scored whenever possible.

D. Registrant's Response to EPA Review of the 90-Day Feeding Study in Rats with Triazole Alanine (EPA Accession No. 073921).

Study Identification: "Triazolylalanine (THS 2212): Study for Subchronic Toxicity to Rats (Three-month feeding study);" Bayer AG Institute of Toxicology, Wuppertal-Elberfeld; Report No. 86476; Authors: Drs. D. Maruhn and E. Bomhard; EPA Accession No. 258416.

Background:

The above cited study was reviewed by Tox Branch, and deficiencies were noted in a memorandum from A. Katz to H. Jacoby, February 2, 1985 (Tox Document #004276). Additional items requested were presented by the Registrant.

Review of Additional Information:

1. Quality Assurance Statement:

A quality assurance statement was provided for Study No. T 7015713. The relevance of this statement is questionable, however, because other documents (e.g., diet analysis results) identify the subchronic study as No. T 9015049. The quality assurance statement is therefore considered unacceptable, pending clarification of this issue.

2. Summary of Clinical Observations of Male and Female Rats:

Summary tables were presented which appear sufficient to conclude that none of the clinical signs observed in this study appear to be treatment-related.

3. Individual Results from the Ophthalmological Examinations:

Results of ophthalmoscopic examinations were reported for 10 animals/sex in the control and high dose groups only. These data show no apparent treatment-related effect.

4. Analytical Chemistry (Diet Analysis, Homogeneity and Stability of Diet Admixtures, Certificate of Analysis):

The additional data for diet analyses were presented in a misleading fashion. These data were reviewed previously (see Appendix 10: memorandum from A. Katz to H. Jacoby, September 10, 1985). Homogeneity results are considered invalid, pending clarification. The data presented were generated in association with Study No. T 8015796; however, the subchronic rat study was identified as No. T 9015049. In order to evaluate the relevance of the homogeneity data, assurance must be provided that the methods and materials used in both studies were identical with respect to diet preparation. The data presented are considered adequate to establish the purity of the test substance (97.5%) as well as stability and concentration in the diet for this study.

5. Signature Page:

A copy of the signature page (in German) for the study report was provided.

Classification:

This study is classified Core Minimum. Tox Branch requires the Registrant to provide an appropriate Quality Assurance Statement for this study. If pertinent homogeneity data are available, these should also be submitted for review.

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E. Additional Mutagenicity Data with Triazole Alanine (EPA Accession No. 073922).

Background:

Additional data were presented for mutagenicity tests with triazole alanine.

Review of Additional Information:

1. "Transformation/Liver Microsome Test." Ciba-Geigy Test No. 840324. September 12, 1984. Author: P. Bielstein.

   This study has been previously reviewed (See DER [Appendix 11] and memorandum from A. Katz to H. Jacoby, 5/20/86). Results of the cell transformation assay with triazolylalanine in mouse embryo fibroblasts BALB/3T3 were negative without activation and inconclusive with activation. Deficiencies are discussed in the DER ("Results/Discussion" section). The study is considered unacceptable.

2. Supplement to Bayer Report No. 11054 of August 9, 1982 (Micronucleus Test for Mutagenic Effect on Mice). Bayer Report No. 11054A. July 8, 1983. Author: B. Herbold.

   The original study report No. 11054 was reviewed previously by Tox Branch (See DER by A. Katz, Appendix 12A; Tox Document No. 004562, July 16, 1985). Subsequently, a separate DER was issued for this study; this included a review of the supplemental data provided in Report No. 11054A (see DER by G. Ghali, Appendix 12B; Tox Document No. 004766, November 15, 1985). Deficiencies in the assay were noted in the latter DER. It was concluded that "a weak positive response was indicated for 8000 mg/kg THS 2212 (triazolylalanine) induction of micronuclei in polychromatic erythrocytes in NMRI (Han) male and female mice." However, due to deficiencies in the design (i.e., lack of positive and negative controls at certain sampling periods), the study was classified unacceptable.

3. "Triazolylalanine (TA): Overview of Genotoxicity"

   (No Data)

F. Additional Studies With CGA-64250 Technical (EPA Accession Nos. 073923-29)

Background:

Reports of additonal studies with CGA-64250 were submitted for review.

Comments/Conclusions:

1. "Two-Generation Reproduction Study in Albino Rats with CGA-64250 Technical"; Toxigenics, Inc., Decatur, IL; Study No. 450-1202; March 12, 1985. Authors: C. Borders and C. Salamon. Sponsor: Ciba-Geigy Corporation, Agricultural Division, Greensboro, NC. EPA Accession Nos. 073923-27.

   The DER for this study is presented in Appendix 13. The study is considered Core-Minimum. Dietary concentrations of Banner (CGA 64250) were 0, 100, 500 and 2500 ppm. A NOEL for parental toxicity was not determined; the incidence of hepatic clear cell changes was increased in all CGA-64250-treated groups. Decreased body weights and increased incidence of hepatic cellular swelling also occurred in the 500 and 2500 ppm groups. The NOEL for developmental toxicity was 500 ppm, based on decreased offspring survival, body weight depression and an increased incidence of hepatic lesions at 2500 ppm (LOEL).

2. "One-Year Subchronic Oral Toxicity Study in Beagle Dogs with CGA-64250 Technical"; Food and Drug Research Laboratories, Inc., Waverly, NY. Study No. 7737; May 28, 1985. Authors: W. Johnson, S. Thompson and P. Becci. Sponsor: Ciba-Geigy Corporation, Agricultural Division, Greensboro, NC. EPA Accession No. 073928.

   The DER for this study is presented in Appendix 14. The study is classified Core-Minimum. Dietary concentrations of CGA 64250 were 0, 5, 50 and 250 ppm. The NOEL and LOEL were 50 and 250 ppm, respectively, based on evidence of mild irritation to the stomach mucosa in males in the high dose group.

3. "The effect of propiconazole on drug metabolizing enzymes in the livers of male rats and mice"; Ciba-Geigy Ltd., Basle, Switzerland; July, 1984. EPA Accession No. 073929.

   The DER for this special study is presented in Appendix 15. The study is considered acceptable; it provides supplementary data on hepatic induction effects of CGA 64250. Male rats (RAI strain) and mice (Mag strain) were used. The test material was administered via gavage to 6 rats or mice per group in 14 daily doses of 20, 80, 160 or 320 mg/kg body weight. Control groups of 8 animals each were similarly administered an equal volume (1 ml/kg) of the vehicle (2% carboxymethyl cellulose). Significant, dose-dependent increases in relative liver weights were found at all dose levels in both species. Hepatic DNA was increased in rats and mice. Protein content of the subcellular liver fractions was generally elevated in both species. The pattern of enzyme induction in both species was found to be similar to that of phenobarbital; however, no data were provided to indicate whether the test substance may also induce cytochrome P-448.

4. "Promotion Study With CGA 64250;" Ciba-Geigy Ltd., Basle, Switzerland; October 1, 1984. Accession No. 073929.

The DER for this special study is presented in Appendix 16. The study is considered acceptable, but inconclusive. CGA 64250 was found to act as a promoter of nonneoplastic and neoplastic proliferative changes in rat liver when administered to weanlings for up to 8 weeks at a dietary concentrationof 2000 ppm with or without pretreatment with an initiator (N-nitrosodiethylamine). A known promoter, phenobarbital, was used as a reference control. It has not been estalished, however, whether CGA 64 250 may also act as an initiator.

APPENDIX 1

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)

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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 C. Katz
Toxicologist
Toxicology Branch Date: 2/6/85
Hazard Evaluation Division (TS-769C)

6. APPROVED BY: Christine F. Chaisson, Ph.D. Signature: Christine F. Chaisson
Head, Review Section IV
Toxicology Branch Date: 2-6-85
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: Hematology		Satellite Group: Blood Chem./Urinalysis		Interim Kill	
		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.

Historical Control Data - Huntingdon Research Centre
Follicular Adenoma of the Thyroid
in Sprague-Dawley Female Rats
Animals Died on Test and Terminal Sacrifice

Study	Incidence No. with Lesion/ No. Examined	Percentage
D	0/99	0
G	0/49	0
J	0/50	0
K	0/99	0
L	0/61	0
M_a	0/50	0
M_b	2/50	4
N	0/100	0
O[1]	0/1	0
P	0/50	0
Overall	2/609	0.3

[1]All thyroids were not examined microscopically.

Lifetime Study in Rats with CGA-64250 Technical
Females

Dose	Incidence	Percentage
Control	1/64	2
100 ppm	0/66	0
500 ppm	0/65	0
2500 ppm	1/67	1

TILT CGA-64250 Reviews

The next 10 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.

APPENDIX 2

CGA-64250 Two Year Rat Study; CBG-193/8284
Location of Dermal Fibromas
(Males)

Dose Group (ppm)	Rat Number	Location
100	81	Dorsal
100	84	Dorsal
100	105	Shoulder
100	106	Lateral thoracic region
500	202	Dorsal
2,500	250	Shoulder
2,500	251	Dorsal
2,500	272	Shoulder
2,500	289	Shoulder
2,500	290	Dorsal, shoulder

APPENDIX 3

TILT CGA-64250 Reviews

The next 9 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.

APPENDIX 4

Historical Control Data - Huntingdon Research Centre
Dermal Fibromas in Sprague-Dawley Male Rats
Animals Died on Test and Terminal Sacrifice

Study	Incidence No. with Lesion/ No. Examined	Percentage
D	8/100	8
G	0/50	0
J	0/50	0
K	5/100	5
L	6/62	10
M_a	0/50	0
M_b	2/50	4
N	6/100	6
O	1/50	2
P	1/50	2
Overall	29/662	4

Lifetime Study in Rats with CGA-64250 Technical
Males

Dose	Incidence	Percentage
Control	0/59	0
100 ppm	3/61	5
500 ppm	1/58	2
2500 ppm	5/61	8

APPENDIX 5

Historical Control Data - Huntingdon Research Centre
Follicular Adenocarcinoma of the Thyroid
in Sprague-Dawley Female Rats
Animals Died on Test and Terminal Sacrifice

Study	Incidence No. with Lesion/ No. Examined	Percentage
D	2/99	2
G	0/49	0
J	2/50	4
K	1/99	1
L	0/61	0
M_a	0/50	0
M_b	0/50	0
N	0/100	0
O[1]	0/1	0
P	2/50	4
Overall	7/609	1

[1]All thyroids were not examined microscopically.

Lifetime Study in Rats with CGA-64250 Technical
Females

Dose	Incidence	Percentage
Control	0/64	0
100 ppm	0/66	0
500 ppm	0/65	0
2500 ppm	2/67	3

APPENDIX 6

Historical Control Data - Huntingdon Research Centre
Vacuolated Hepatocytes in Sprague-Dawley Male Rats
Animals Died on Test and Terminal Sacrifice

Study	Incidence No. with Lesion/ No. Examined	Percentage
D	40/100	40
G	14/50	28
J[1]	0/50	0
K	26/100	26
L	22/62	35
M_a	13/50	26
M_b	14/50	28
N	41/100	41
O	14/42	33
P	18/50	36
Overall	202/654	31

[1]Lesion was not observed in study.

Lifetime Study in Rats with CGA-64250 Technical
Males

Dose	Incidence	Percentage
Control	26/64	41
100 ppm	31/67	46
500 ppm	29/66	44
2500 ppm	44/65	68

Historical Control Data - Huntingdon Research Centre
Ballooned Cells in the Liver of Sprague-Dawley Male Rats
Animals Died on Test and Terminal Sacrifice

Study	Incidence No. with Lesion/ No. Examined	Percentage
D	28/100	28
G	8/50	16
J	10/50	20
K	27/100	27
L	7/62	11
M_a	11/50	22
M_b	8/50	16
N	23/100	23
O	10/42	24
P	13/50	26
Overall	145/654	22

Lifetime Study in Rats with CGA-64250 Technical
Males

Dose	Incidence	Percentage
Control	15/64	23
100 ppm	8/67	12
500 ppm	13/66	20
2500 ppm	25/65	38

Historical Control Data - Huntingdon Research Centre
Lipid Deposition in the Liver of Sprague-Dawley Male Rats
Animals Died on Test and Terminal Sacrifice

Study	Incidence No. with Lesion/ No. Examined	Percentage
D[1]	-	-
G	50/50	100
J	33/50	66
K	7/100	7
L	3/62	5
M[1]	-	-
N	44/100	44
O[2]	1/42	2
P	19/50	38
Overall	157/454	35

[1]Livers were not examined with O.R.O. stain in study D and M.
[2]All livers were not examined with O.R.O. stain.

Lifetime Study in Rats with CGA-64250 Technical
Males

Dose	Incidence	Percentage
Control	4/60	7
100 ppm	7/61	11
500 ppm	15/59	25
2500 ppm	17/60	28

Historical Control Data - Huntingdon Research Centre
Foci of Enlarged Hepatocytes in Sprague-Dawley Female Rats
Animals Died on Test and Terminal Sacrifice

Study	Incidence No. with Lesion/ No. Examined	Percentage
D[1]	0/100	0
G	3/50	6
J	1/50	2
K[1]	0/100	0
L[1]	0/62	0
M_a	0/50	0
M_b	0/50	0
N[1]	0/100	0
O	1/45	2
P	0/50	0
Overall	5/657	0.8

[1]Lesion was not observed in study.

Lifetime Study in Rats with CGA-64250 Technical
Females

Dose	Incidence	Percentage
Control	1/67	1
100 ppm	2/69	3
500 ppm	2/67	3
2500 ppm	13/67	19

APPENDIX 7

Historical Control Data - Huntingdon Research Centre
Exocrine Atrophy in the Pancreas
of Sprague-Dawley Female Rats
Animals Died on Test and Terminal Sacrifice

Study	Incidence No. with Lesion/ No. Examined	Percentage
D	17/100	17
G	9/49	18
J	3/50	6
K	9/99	9
L[1]	3/15	20
M_a	5/50	10
M_b	1/50	2
N	9/98	9
O[1]	2/2	100
P	5/50	10
Overall	63/563	11

[1]All organs were not examined microscopically.

Lifetime Study in Rats with CGA-64250 Technical
Females

Dose	Incidence	Percentage
Control	1/59	2
100 ppm	3/61	5
500 ppm	6/62	10
2500 ppm	9/65	14

APPENDIX 8

Historical Control Data - Huntingdon Research Centre
Luminal Dilatation of the Uterus of Sprague-Dawley Rats
Animals Died on Test and Terminal Sacrifice

Study	Incidence No. with Lesion/ No. Examined	Percentage
D	0/100	0
G	7/50	14
J	0/50	0
K	27/99	27
L[1]	4/13	31
M_a	8/50	16
M_b	8/50	16
N	6/100	6
O[1]	4/8	50
P	4/50	8
Overall	68/570	12

[1]All organs were not examined microscopically.

Lifetime Study in Rats with CGA-64250 Technical
Females

Dose	Incidence	Percentage
Control	4/58	7
100 ppm	10/63	16
500 ppm	9/63	14
2500 ppm	17/65	26

APPENDIX 9

004287

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: Alan C. Katz, M.S., D.A.B.T. Signature: Alan C. Katz
Toxicologist
Toxicology Branch Date: 2/14/85
Hazard Evaluation Division (TS-769C)
and
William Dykstra, Ph.D. Signature: William Dykstra
Toxicologist
Toxicology Branch Date: 2/14/85
Hazard Evaluation Division (TS-769C)

6. APPROVED BY: Roger Gardner, M.S. Signature: Roger Gardner
Acting Head, Review Section IV
Toxicology Branch Date: 2/14/85
Hazard Evaluation Division (TS-769C)

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.

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)

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 8 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.

Appendix 2.

Number of Animals with Tumors/Number Examined

Time of Death	Males				Females			
	Control (0 ppm)	T-I (100 ppm)	T-II (500 ppm)	T-III (2500 ppm)	Control (0 ppm)	T-I (100 ppm)	T-II (500 ppm)	T-III (2500 ppm)
0-12 months	0/2	1/6[1]	2/5[1]	1/10[2]	1/4	1/4[1]	1/4[1]	1/1
Interim Sacrifice	3/11	0/11	4/11	5/9	1/12	0/11	0/11	2/12
13-24 months	22/27[3]	15/27	16/25	30/31	8/20	11/16	18/25	5/19
Terminal Sacrifice	21/24	8/20	17/21	14/14	12/28	15/33	13/24	17/32
Total/Dose Group	46/64	24/64	39/62	50/64	22/64	27/64	32/64	25/64

[1] Includes 1 animal designated for Interim Sacrifice

[2] Includes 3 animals designated for Interim Sacrifice

[3] Includes Animal #63 designated for Interim Sacrifice (died during week 53 while being bled).

APPENDIX 10

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

005094

SEP 10 1985

MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

SUBJECT: Triazolylalanine (THS 2212); Diet Analysis for Subchronic Feeding Study in Rats

EPA Reg. #: 3125-320; Record #: 155160
Accession #: 258662
Caswell #: 862 B
Data Submitted By: Mobay Chemical Corporation
(Mobay Report No. 86476)

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

FROM: Alan C. Katz, M.S., D.A.B.T. 9/3/85
Toxicology Branch
Hazard Evaluation Division (TS-769C)

THRU: Robert P. Zendzian, Ph.D. 9/4/85
Acting Head, Review Section IV

Action Requested:

Review captioned data (Mobay Report No. 86476: "Summary of Diet Analysis Results"; dated April 15, 1985). This data was not included in the original submission of data pertaining to a 90-day rat feeding study with triazolylalanine, and was requested by the Toxicology Branch (see attached memo, ACK to HMJ, 2/8/85).

Discussion:

Homogenicity results require additional clarification. The data presented were generated in association with Study Number T8015 796; however, the subchronic rat study under primary review is identified as Study No. T9015 049. In order to evaluate the relevance of these data, it must be demonstrated that the methods and materials used in both studies were identical with respect to diet preparation. Also, it is not stated whether the 3 samples tested at each of the 2 concentrations were taken from the same batch of blended feed. Further, we note that 2 values are presented for each sample tested; it is not clear whether these individual values represent determinations on "replicate" portions from each sample, or duplicate determinations on the same sample. The registrant should address the issues of sensitivity of the method used, and the reasons for any apparent intra-sample variability. Methods used in diet preparation and sampling should be more fully explained.

Conclusions:

The data presented are considered adequate to establish purity of the test substance (97.5%) as well as stability and concentration in the diet for this study. Homogenicity data could not be evaluated, and is therefore considered unacceptable. The Toxicology Branch, however, does not find this deficiency alone to be sufficient cause to consider this particular study invalid, and will complete its evaluation based on the merits of other data provided.

APPENDIX 11

DATA EVALUATION REPORT

A. Study Type: Mutagenicity; BALB/3T3 Cell Transformation

B. Compound: Triazolyl alanine; CGA 131 013 Technical, Batch no. TLB 1207 (Purity not disclosed)

C. Study Report Citation: Beilstein, P.(9/12/84);Transformation/Liver-Microsome Test (In vitro test for transformation-inducing properties in mammalian fibroblasts); Testing facility: Ciba-Geigy Limited, Basle, Switzerland; Test No. 840324; Submitted by Mobay Chemical Corporation, Kansas City, MO. EPA ID#: 3125-320; Action Code: 400; Caswell #862B; Accession #257997.

D. Reviewed by: Alan C. Katz, M.S., D.A.B.T.
Toxicologist
Toxicology Branch
Hazard Evaluation Division (TS-769C)
(Signature)
5/16/86
(Date)

E. Secondary Review by: Irving Mauer, Ph.D.
Geneticist
Toxicology Branch (TS-769C)
(Signature)
05/16/86
(Date)

F. Procedures:

See Appendix 1, attached (excerpt from study report).

G. Results/Discussion:

In the preliminary assay for cytotoxicity, the highest concentration of triazolyl alanine (1000 ug/ml) caused no reduction in the number of colonies formed with the non-activated system when compared with the negative solvent control. In the cytotoxicity test with activation (rat S9 mix), this concentration of test substance reportedly caused a reduction of approximately 20 percent; however, data substantiating this finding were not presented. Also, since it is noted in the study report (p.7) that "(t)he best suited concentration of the test substance as the highest for the transformation assay is that causing a 50% reduction in colony-forming ability in comparison with the negative (solvent) control," the rationale for selection of this concentration as the highest level for use in the transformation assay should be explained.

Results of the transformation assays, as excerpted from the study report, are presented in Tables 1 through 4. These data show that triazolyl alanine, at levels up to and including 1000 ug/ml, did not induce transformation of cells under the conditions of the assay without metabolic activation.

Data from an initial assay using the S9 activation system were not reported, due to "a high background of transformed colonies in the negative and positive controls and at the five concentrations of the test substance." Results of a repeat test with activation are shown in Table 3 and statistically analyzed in Table 4. Although the study report author stated that "(s)tatistical comparison of the results from the solvent control and all five treatment groups revealed no significant difference (confidence limit = 5 %)", this

evaluation is subject to question. The statistical comparison which was applied to the data did not take into account the normalized values for the proportion of viable cells which were transformed. As shown in Table 3, the transformation frequency values for the test preparations, in order of increasing concentration, were: 0.49, 1.71, 1.83, 2.34 and 4.10, while values for the solvent and untreated controls were 1.15 and 1.09, respectively. These data suggest a slight treatment-related effect.

Mean viability control values for the solvent and untreated control groups were reported in the range of 20.7-26.1 percent. Individual or historical viability control values were not presented.

Cell transformation was clearly demonstrated in the positive control assays using methylcholanthrene (without activation) and 2-acetylaminofluorene (with activation), although cell viability levels were substantially reduced with both of these agents. Likewise, triazolyl alanine should have been tested at a higher concentration so as to cause greater cytotoxicity (or to a limit dose of 5000 ug/ml).

H. Conclusions:

Under the conditions of the assay without metabolic activation, triazolyl alanine at concentrations up to and including 1000 ug/ml did not appear to cause transformation of BALB/3T3 cells. In the presence of a metabolic (S9) activation system, however, there was evidence of a dose-related increase in transformation frequency.

I. Classification/Recommendations:

This study is classified as unacceptable. The assay should be repeated, and should include higher concentrations of the test substance.

TILT CGA-64250 Reviews

The next 8 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

X 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.

APPENDIX 12A

DATA EVALUATION REPORT

A. Compound:

Triazolylalanine

B. Compound Number:

THS 2212

C. Study Report Citation:

Title: "THS 2212; Triazolylalanine; Micronucleus Test for Mutagenic Effect on Mice"

Author: Dr. B. Herbold

Laboratory: Bayer AG Toxicological Institute

Study Number: T4011615; Report No. 11054

Date: 8/9/82

D. Reviewed By: Alan C. Katz, M.S., D.A.B.T. _____ (Signature)
Toxicologist
Toxicology Branch
Hazard Evaluation Division(TS-769C) 7/15/85 (Date)

E. Secondary Review: Robert Zendzian, Ph.D. _____
Acting Head, Review Sec.IV (Signature)

_____ (Date)

F. Classification:

Acceptable.

G. Conclusion:

Under the conditions of this test, triazolylalanine (THS 2212) caused slight induction of micronuclei in bone marrow PCE stem cells, which was apparent 24 hours after an oral dose of 8000 mg/kg. No effect was seen at 48 or 72 hours. The data provided in this study are considered acceptable.

H. Materials and Methods:

A micronucleus test was performed in male and female mice in order to evaluate the test compound for clastogenic activity and interference with normal mitotic cell division in polychromatic erythrocyte (PCE) stem cells in mammalian bone marrow in vivo. A single dose of the test compound (Lot No. E238099) was administered by gavage (20 ml/kg) to Bor:NMRI (SPF) mice at 8000 mg/kg. The vehicle (0.5% cremophor, 20 ml/kg) was used as a negative control. Endoxan, 87 mg/kg (60 mg/kg cyclophosphamide; 10 ml/kg) served as a positive control. Five animals/sex/group were killed at 24, 48 and 72 hours. Bone marrow smears were prepared and PCEs were examined for induction of micronuclei. Two thousand PCEs were scored for each animal in the THS 2212-treated and negative control groups; 1000 PCEs were scored for each animal in the positive control group.

I. Results:

No clinical or cytotoxic effects were apparent in animals given the test compound. The results for males and females were evaluated on a combined basis, since no sex-related differences were found. These results may be summarized as follows:

Table 8. Micronucleus Test Results - THS 2212
Number of Cells Containing Micronuclei / 1000

Group	Normochromatic Erythrocytes	Polychromatic Erythrocytes
Negative Control (Vehicle)	1.2	1.4
THS 2212		
24 hrs.	1.8	3.1*
48 hrs.	1.2	1.6
72 hrs.	1.0	1.2
Endoxan (Positive Control)	1.8	23.4**

* $p < 0.05$ (Wilcoxon signed rank test)
** $p < 0.01$ (Wilcoxon signed rank test)

J. Discussion:

As shown in Table 8, a slight but statistically significant increased micronuclei count was found in THS 2212 at 24 hours. The study report states that this finding is "biologically insignificant, since it lies within the biological fluctuation range of the system and thus represents no increase which might indicate evidence of any chromosome-breaking effect by THS 2212." However, this interpretation does not appear to be supported on the basis of data included in the report.

APPENDIX 12B

DATA EVALUATION RECORD

Mutagenicity (Micronucleus)

Herbold, B. THS 2212 Triazolylalanine: Micronucleus test for mutagenic effect on mice. An unpublished Report (Bayer No. 11054; Mobay Agchem No. 84005) prepared by Bayer AG for Mobay Chemical Corporation. Dated August 9, 1982.

ACCESSION NUMBER: 252132.

LABORATORY: Bayer AG Institut fuer Toxikologie, Wuppertal, Federal Republic of Germany.

TEST MATERIAL: The test material was identified as THS 2212, batch no. E238099 (triazolylalanine), which is a degradation product of Bayleton^{R}. Chemically, it is described as 2-amino-3-(1,2,4-triazolyl-1-yl)-propanoic acid, and its empirical formula is $C_5H_8N_4O_2$. The purity of the test material was not stated in this report; however, in another report (report No. 11491) the same material with the same batch number was said to be "analytically pure."

METHODS:

Animal Test Species and Husbandry: Male and female Bor: NMRI (SPF Han) mice, supplied by F. Winkelman, Borchen, Federal Republic of Germany were used in the study. They were 8 to 12 weeks old and weighed 25 to 35 g when the assay was initiated. Animals were separated by sex and treatment group, caged in type II Makrolon cages (5 per cage), and identified by cage number and picric acid individual markings.

Animals were held at 23° C, average relative humidity of 68 to 79 percent with alternating 12 hour light/dark cycles. Food (Altromin 1324, Altromin GmbH, Lage, Federal Republic of Germany) and tapwater were supplied ad libitum.

Preparation of Test Material: The test material, THS 2212, was emulsified in 0.5 percent Cremophor and administered by oral intubation (p.o.) A single dose level of 8000 mg/kg was chosen because preliminary testing showed that this dosage could be "tolerated without symptoms".

Controls: The positive control, Endoxan[1], was dissolved in water and 10 ml/kg were administered p.o. at a dosage of 87 mg/kg. The vehicle (negative) control was also administered p.o. and was given at a volume of 20 ml/kg.

Bone Marrow and Slide Preparation: The method for preparing bone marrow slides was that of Schmid[2].

Evaluation of Slides: For each animal, 1000 polychromatic erythrocytes (PCEs) were counted, scoring the micronuclei (MN) and calculating their frequency of occurrence. Since this first evaluation did not yield conclusive results, an additional 1000 PCEs in the negative control and THS 2212 - treatment groups were evaluated. In addition, the PCE to normochronic erythrocyte (NCE) ratio was determined to identify animals with pathology leading to bone marrow depression that was unrelated to compound administration and to identify general activity of the test compound as it relates to erythropoiesis. If an individual animal had an NCE:PCE ratio of more than 3:1 without seeing or anticipating this response among other dosed animals, a pathological process unrelated to the test compound was recognized and these animals were excluded from further evaluation. If, however, the PCE:NCE ratio in dosed groups was substantially below the concurrent negative control group, the presence of a generalized erythropoietic effect could be inferred.

Statistics: The Wilcoxon rank sum test was applied only to the positive control and to the highest values obtained in THS2212-treatment groups. If the probability of error in the difference was less than 5 percent then the result was considered to be statistically significant.

RESULTS:

Animals Effects: At a p.o. dosage of 8,000 mg/kg THS 2212 caused no toxic symptoms in the mice. Their behavior, feeding habits, external appearance and motor activity did not show compound-related effects, and there were no test compound-related mortalities reported.

Slide Evaluation: In the negative control mice at 24 hr, the average ratio of PCE to NCE was 1.6 to 1 (2000/1246); MN per 1000 NCE was 1.2 and MN per 1000 PCE was 1.4. In the mice dosed at 8000 mg/kg at 24 hr, the average ratio of PCE to NCE was 1.16 to 1 (2000/1724); MN per 1000 NCE was 1.8 and MN per 1000 PCEs was 3.1. At 48 hr the mice dosed at 8000 mg/kg averaged 1.16 for PCE/NCE (2000:1724); MN per 1000 NCE was 1.2 and MN per 1000 PCE was 1.6. Seventy-two hours after treatment with 8,000 mg/kg of

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[1] Cyclophosphamide, the a.i., was calculated to be a 60 mg/kg dose.

[2] Schmid, W. 1975. Mutation Res. 31:9-15; ibid 1975. Mitteilung III. pp. 53-61.

the test material, the average ratio of PCE to NCE was 1.14 to 1 (2000/1760). Twenty four hours post-treatment with 87 mg Endoxan/kg per os gave a PCE/NCE of 0.93 (1000/1079); 1.8 MN per 1000 NCE and 23.4 MN per 1000 PCE. By the Wilcoxin test, the number of NCEs/1000 PCEs and MN/ 1000 PCEs were statistically different from the negative control ($p < 0.01$) in the Endoxan treated mice; only the number of MN/1000 PCE at 24 hr after dosing with 8000 mg THS 2212/kg were elevated at a statistically significant level ($p < 0.05$).

The authors submitted a supplemental report in which mice were treated per os with 8,000 mg THS 2212 per kg because "the high value of one individual female resulted in a micronucleus frequency of 3.1/1000 for the THS 2212 group that had been sacrificed and prepared 24 hours after treatment." Hence, they increased the number of PCEs to be evaluated to 2,000 and the evaluation was performed with two different series using different slides. The summary of these results follows:

In the negative control animals MN per 1000 NCE and per 1000 PCE averaged 1.15 and 1.4, respectively, for 2 series; THS 2212-treated animals at 24 hr averaged 1.85 MN per 1000 NCE and 2.95 MN per 1000 PCE; THS 2212-treated animals at 48 hr averaged 1.15 MN per 1000 NCE and 1.5 MN per 1000 PCE; THS 2212-treated animals at 72 hr averaged 1.0 MN per 1000 NCE and 1.5 MN per 1000 PCE; THS 2212-treated animals at 72 hr averaged 1.0 MN per 1000 NCE and 1.3 MN per 1000 PCE. No positive control data, e.g., Endoxan-treated animals, were reported for this supplemental experiment. The authors stated this test compound did not produce significant differences any at sampling times using the Wilcoxon rank sum test.

DISCUSSION:

The authors concluded that there was "no indication of mutagenic effect of THS 2212 at a dose level of 8000 mg/kg per os in the micronucleus test on mice, i.e., in a somatic mutagenicity test in vivo" after the first assay. However, treatment at this dosage gave 3.1 MN/per 1000 PCE at the 24 hour sampling period (significant difference at $p < 0.05$ in the non-parametric rank sum test of Wilcoxon). In the supplementary assay, the THS 2212 treated groups averaged 3.1 and 2.8 MN per 1000 PCEs (2 series). This value was approximately twice that of the negative control group or other treatment groups sampled at different intervals, however, the standard deviation (1s) was equal or approximately equal to the mean value and the authors concluded that these results were of no biological relevance.

Our assessment is that micronuclei induction by THS 2212 did not exceed the negative control at 48 or 72 hr, if the 24 hour negative control value was used for comparison. However, negative control values were not reported at the 48 and 72 hours sampling periods in either the first assay or supplementary assays. Also, there were 3/5 males and 1/5 females with MN/1000 PCE values at 24 hours ranging from 3.5 to 7.5 in the first assay

where a statistically significant ($p < 0.05$) mean value was obtained at 24 hr. The positive control with Endoxan indicated that the assay was capable of a positive response.

In the supplemental assays, there were no negative control groups for the MN assay at 48 and 72 hr and no positive control group was included. The results of 24 hour sampling after treatment with 8000 mg THS 2212/kg appeared to be higher than the results presented for the negative control group and the other THS 2212 treatment groups assayed at 48 and 72 hr, although the differences were not found to be statistically significant ($p < 0.05$). We therefore assess that the supplemental assays were unacceptable because there was no positive control group and the required negative control groups at 48 and 72 hr were not included.

CONCLUSIONS:

Because the 24-hour-dosed mice had a statistically significant ($p < 0.05$) increase in the number of micronuclei per 1000 PCE when compared to the 24-hour-negative-control group in the first bioassay, a weak positive response was indicated for 8000 mg/kg THS 2212 (Triazolylaline) induction of micronuclei in polychromatic erythrocytes in NMRI (Han) male and female mice. However, the lack of negative controls at 48 and 72 hours precluded evaluation at these sampling times. Also the supplementary assays lacked a positive control for each sampling period and negative controls at 48 and 72 hr samplings, and hence cannot be evaluated.

CLASSIFICATION: Unacceptable. Critical data on negative and positive control groups were absent from the studies and the purity of the test compound was not stated.

APPENDIX 13

EPA: 68-02-4225
DYNAMAC No. 1-073A
July 1, 1986

DATA EVALUATION RECORD

BANNER (CGA-64250)

Two-Generation Reproduction Study in Rats

STUDY IDENTIFICATION: Borders, C. K. and Salamon, C. M. Two-generation reproduction study in albino rats with CGA-64250 technical. (Unpublished study No. 450-1202 by Toxigenics, Inc., Decatur, IL, for CIBA-GEIGY Corporation, Greensboro, NC; dated March 12, 1985.) Accession Nos. 073923-7.

APPROVED BY:

I. Cecil Felkner, Ph.D.
Department Manager
Dynamac Corporation

Signature: I. Cecil Felkner

Date: 7-1-86

1. CHEMICAL: Banner, CGA-64250.

2. TEST MATERIAL: CGA-64250 technical, 89.7%, FL-830377.

3. STUDY/ACTION TYPE: Two-generation reproduction study in rats.

4. STUDY IDENTIFICATION: Borders, C. K. and Salamon, C. M. Two-generation reproduction study in albino rats with CGA-64250 technical. (Unpublished study No. 450-1202 by Toxigenics, Inc., Decatur, IL, for CIBA-GEIGY Corporation, Greensboro, NC; dated March 12, 1985.) Accession Nos. 073923-7.

5. REVIEWED BY:

Robin B. Phipps, B.S.
Principal Reviewer
Dynamac Corporation

Signature: Robin B. Phipps
Date: June 30, 1986

Michael Narotsky, B.A.
Independent Reviewer
Dynamac Corporation

Signature: Michael Narotsky
Date: June 30, 1986

6. APPROVED BY:

Guillermo Millicovsky, Ph.D.
Teratogenicity and Reproductive Effects
Technical Quality Control
Dynamac Corporation

Signature: G Millicovsky
Date: JUNE 30 1986

Alan Katz, M.S., D.A.B.T.
EPA Reviewer

Signature: Alan Katz
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7. CONCLUSIONS:

A. The LOEL for parental toxicity of Banner (CGA-64250) is assessed at 100 ppm based on an increased incidence of hepatic clear-cell change at all dose levels, decreased body weights and increased incidence of hepatic cellular swelling at 500 and 2500 ppm and decreased food consumption at 2500 ppm. An NOEL for parental toxicity was not determined. The LOEL and NOEL for developmental toxicity are assessed at 2500 ppm and 500 ppm, respectively, based on decreased offspring survival and body weights and an increased incidence of hepatic lesions at 2500 ppm.

B. This study is classified Core Minimum.

Items 8-10--see footnote 1.

11. MATERIALS AND METHODS (PROTOCOLS):

A. Materials and Methods: (See Appendix A for details.)

1. Test Material: The test material was identified as CGA-64250 technical, 89.7%, FL-830377. At weekly intervals, the test material was mixed with the basal diet to achieve active ingredient concentrations (w/w) of 0, 100, 500, and 2500 ppm. Test diets were administered ad libitum throughout the study. Homogeneity and stability of prepared test diets were assayed prior to the start of the study. Concentrations of the test material in the test diets were determined at monthly intervals.

2. Test Animals and Experimental Design: Male and female Charles River CD albino rats, 22 days of age, were supplied by Charles River Breeding Laboratories, Inc., Portage, MI. Following a 13-day quarantine, 15 males and 30 females, 35 days old, were randomly assigned to each of the four treatment groups and designated as F_0 parental animals. After a 12-week premating period, females were bred 2:1 with males of the same group to produce F_{1a} litters. Mating was confirmed by the observation of a copulatory plug or the presence of sperm in the vaginal smears. The resulting F_{1a} pups were weaned on lactation day 21. Two weeks later, F_0 females were bred to different males of the same group to produce F_{1b} litters. Females which failed to conceive 'a' litters were not rebred. At the end of lactation, the F_1 parental animals were randomly selected from the F_{1b} progeny, and were maintained and bred as described above to produce the F_{2a} and F_{2b} litters.

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[1] Only items appropriate to this DER have been included.

3. Measurements and Observations: All parental animals were observed daily for mortality, morbidity, and clinical signs of toxicity. In addition, each animal was given a thorough physical examination at least once each week.

   Body weights and food consumption of all F_0 and F_1 parental animals were recorded weekly during the premating period. All males and those females that did not conceive or maintain live pups throughout lactation were then weighed monthly until terminal sacrifice. Body weights and food intake were not recorded for mated females during the gestation or lactation periods.

   During gestation, conception was confirmed by observation of a vascular membrane in the vagina and/or the detection of progeny by palpation.

   Gross necropsies and histopathological examinations were conducted on all parental animals. The weights of brains, ovaries, and testes were recorded for animals that were sacrificed at termination. Corresponding organ-to-body weight and organ-to-brain weight ratios were calculated.

   On the day of parturition, the number, sex, and viability of the pups delivered were recorded for each litter. The number of pups surviving to lactation days 4, 7, 14, and 21 were also determined. On day 4, litters were randomly reduced to eight progeny (four males and four females when possible). Individual pups were weighed and sexed on days 0, 4, 7, 14, and 21 of lactation. Each pup was examined for gross abnormalities at birth and again at weaning (day 21).

   All pups found dead during lactation or showing gross abnormalities were necropsied for the detection of gross malformations. In addition, ten males and ten females were randomly selected from the weaned progeny of each litter interval (F_{1a}, F_{1b}, F_{2a}, F_{2b}); these animals were necropsied and their brain and gonad weights were recorded. Tissues obtained from 'b' progeny were also examined histopathologically.

4. Statistical Analyses: Body weights, food consumption, absolute organ weights, and progeny population data were evaluated using analysis of variance; significant differences were further evaluated by Tukey's or Scheffe's multiple comparison analyses. Organ weight ratios were assessed using Kruskal-Wallis analysis. Chi-square analysis and Fisher's Exact Test were used to evaluate in-life observations, histopathological findings, progeny anomalies, and reproductive indices.

B. Protocol: A study protocol was not present in the final report.

12. REPORTED RESULTS:

A. Dietary Analyses: Data from homogeneity analyses of diets prepared prior to study initiation indicate that the diets were homogeneously mixed; the concentrations of samples taken from three levels of each prepared diet were in close agreement and all assayed values were within 6% of nominal concentrations. Stability analyses showed the prepared diets to be stable at ambient and frozen temperatures through 14 days. The results of the monthly dietary analyses indicate that the concentration of the test material was generally within ±10% of the nominal concentrations.

B. Parental Effects:

1. Survival and Clinical Observations: No compound-related clinical observations were reported. In the F_0 generation, one control male was sacrificed moribund during the F_{1b} mating trials and three females died prior to terminal sacrifice; one low-dose (100 ppm) female died prior to mating, one mid-dose (500 ppm) dam died during the rest period between the F_{1a} and F_{1b} litters, and one high-dose (2500 ppm) dam died following delivery of her second litter. In the F_1 generation, a mid-dose male was sacrificed moribund after an apparent cage injury. In the high-dose group, one dam died and another was sacrificed moribund during delivery of their first litters. None of these deaths were attributed to compound administration. All other parental animals survived to terminal sacrifice.

2. Body Weights and Food Consumption: In the F_0 generation, male body weights were reduced in the mid- and high-dose groups compared to controls; however, with one exception (high-dose, week 2), these reductions were not statistically significant (Table 1). Food consumption for high-dose males was significantly reduced at weeks 1 and 7 (Table 2).

Female body weights of the F_0 generation were significantly reduced in the high-dose group at most of the body weight intervals; body weight gains were also significantly reduced compared to controls. Correspondingly, high-dose females also had significantly reduced food intake. Mid-dose food intake was significantly reduced at week 2.

Body weights of the F_1 parental males and females were reduced in the high-dose group compared to controls throughout the treatment period. Reductions were generally statistically significant for the high-dose females. Reductions in high-dose male body weights were significant at weeks 9, 10, and 11. In addition, mid-dose F_1 females had significantly reduced body weights at weeks 10 and 11 and reduced weight gain for the 12-week premating period. In the

TABLE 1. Selected Mean Parental Body Weights (g±SD) in Rats Fed CGA-64250 for Two Generations

	Dose Level (ppm)			
	0	100	500	2500
F_0 Males				
Initial	137.7± 8.9	137.6± 8.7	137.3± 9.8	138.0± 8.9
Week 2	246.8±11.3	240.4±14.0	238.6±11.0	231.3±12.3**
Week 4	339.3±16.8	326.2±25.0	327.1±20.8	320.0±21.0
Week 8	443.5±28.6	429.9±41.1	424.2±40.1	419.8±35.5
Week 12	506.7±31.1	488.4±47.6	477.2±53.0	477.7±37.8
Month 5	570.5±48.4	559.5±59.7	546.2±61.6	547.5±48.0
Final	647.6±69.6	638.6±72.1	617.1±66.7	619.2±55.1
Premating gain	369.0±32.0	350.7±45.2	339.9±58.0	339.7±35.4
Total gain	509.5±70.2	500.9±70.5	479.8±69.3	481.2±53.4
F_0 Females				
Initial	108.4±10.6	108.7± 9.4	108.4±10.2	108.4±10.2
Week 2	169.6±13.3	167.4±14.6	165.1±15.9	159.0±13.4*
Week 4	212.7±18.6	211.1±20.0	207.4±22.3	190.2±17.2**
Week 8	260.6±25.1	256.5±26.4	253.9±28.3	227.1±21.1**
Week 12	281.2±28.0	282.3±27.4	276.8±30.3	245.4±23.1**
Month 5	311.9±35.0	308.0±33.0	312.5±36.3	271.3±27.2*
Final	360.5±48.6	350.1±44.2	363.7±58.8	301.7±40.4**
Premating gain	172.7±24.5	173.1±22.7	168.4±23.1	137.0±17.6**
Total gain	252.0±47.9	240.9±41.0	254.4±53.9	193.4±34.0**

(Continued)

*Significantly different from control value ($p<0.05$).
**Significantly different from control value ($p<0.01$).

TABLE 1. Selected Mean Parental Body Weights (g±SD) in Rats Fed CGA-64250 for Two Generations (Continued)

	Dose Level (ppm)			
	0	100	500	2500
F_1 Males				
Initial	42.9± 6.4	46.0± 8.0	48.1± 6.6	36.8± 7.4
Week 2	172.6±30.0	180.2±30.8	177.6±36.1	153.1±30.8
Week 4	271.6±29.4	281.0±36.2	281.3±40.3	246.2±35.3
Week 8	403.8±33.3	406.3±45.5	409.0±37.3	367.7±38.5
Week 10	445.6±37.7	448.5±50.4	453.6±38.4	402.7±44.9*
Week 12	477.2±41.3	487.3±50.2	487.7±37.3	432.9±47.2
Month 5	550.0±52.5	559.2±67.2	564.6±46.8	508.0±61.5
Final	635.8±66.7	661.5±74.2	660.5±49.3	587.0±74.1
Premating gain	434.3±37.9	440.9±47.3	439.7±34.5	396.1±44.2
Total gain	593.0±64.3	615.5±71.4	612.3±48.1	550.2±71.4
F_1 Females				
Initial	39.7± 6.5	43.2± 6.9	43.1± 8.3	33.7± 7.5**
Week 2	129.8±22.1	134.5±19.5	131.7±27.5	114.9±19.7
Week 4	176.6±19.8	178.8±16.4	171.8±23.9	155.7±16.6**
Week 8	236.5±22.6	236.1±17.5	223.3±22.6	203.3±17.8**
Week 10	252.8±25.6	251.5±19.3	237.8±23.4*	215.9±18.9**
Week 12	265.5±27.5	263.8±21.1	249.3±25.1	224.9±21.0**
Month 5	317.7±64.7	304.9±34.8	301.7±21.7	262.3±29.3
Final	353.1±36.5	350.1±37.3	336.6±35.3	287.9±29.9**
Premating gain	225.8±23.7	220.6±19.3	206.2±22.4**	191.6±19.1**
Total gain	313.4±33.7	306.8±37.3	293.5±35.3	254.3±29.6**

(Concluded)

*Significantly different from control value ($p<0.05$).
**Significantly different from control value ($p<0.01$).

TABLE 2. Mean Parental Food Consumption (g/rat/day±SD) in Rats Fed CGA-64250 for Two Generations

	Dose Level (ppm)			
	0	100	500	2500
F_0 Males				
Week 1	19.1± 1.8	19.7± 1.7	20.1± 1.6	12.5± 2.0**
Week 4	27.7± 2.1	25.9± 2.5	26.6± 3.0	25.1± 3.1
Week 8	26.2± 5.4	26.8± 3.5	28.7± 4.0	26.4± 2.7
Week 12	29.5± 2.5	28.9± 3.0	29.3± 3.6	29.0± 2.3
F_0 Females				
Week 1	16.4± 1.7	16.9± 1.7	16.0± 1.6	10.8± 1.8**
Week 4	19.9± 2.5	19.7± 3.0	19.9± 3.1	18.1± 1.5
Week 8	21.3± 2.2	20.9± 3.1	20.4± 2.8	18.7± 2.3**
Week 12	21.8± 4.0	21.4± 2.9	20.4± 3.1	18.1± 3.3**
F_1 Males				
Week 2	23.9± 2.4	25.0± 2.9	23.7± 3.4	21.1± 2.7*
Week 6	32.1± 3.0	30.4± 3.2	30.5± 2.6	27.8± 3.1**
Week 10	29.2± 2.9	30.7± 3.6	30.5± 3.4	25.4± 3.0*
Week 12	28.1± 3.8	28.7± 4.2	29.5± 2.8	28.3± 3.7
F_1 Females				
Week 2	18.5± 1.7	18.8± 1.7	17.4± 1.9	15.8± 1.8**
Week 6	22.3± 3.0	20.7± 1.9	20.7± 3.0	18.9± 1.5**
Week 10	20.1± 2.2	20.4± 2.7	19.7± 2.3	17.3± 2.7**
Week 12	20.4± 2.4	19.3± 2.9	19.8± 2.9	19.5± 2.2

*Significantly different from control value ($p<0.05$).
**Significantly different from control value ($p<0.01$).

F_1 generation, food consumption was generally significantly reduced for high-dose females compared to controls. High-dose males had significant reductions in food intake at weeks 2, 6, and 10. Reductions were also significant for mid-dose females at week 4 and low-dose females at weeks 4 and 8.

3. Gross Pathology, Organ Weights, and Histopathology: Gross necropsies of the F_0 and F_1 parental animals that survived to termination revealed no compound-related findings.

   The brain-to-body weight ratio was significantly increased for high-dose females of both the F_0 and F_1 generations when compared to control values (Table 3). No other significant differences were noted in parental organ weight data.

   Histological examinations revealed compound-related liver changes (Table 4). The occurrence of hepatic "cellular swelling" was significantly increased in mid-dose males and high-dose males and females of the F_0 generation. In the F_1 parental animals, the change was significant for both sexes in the mid- and high-dose groups. The incidence of hepatic "clear-cell change" was significantly increased in F_0 high-dose males, F_1 mid-dose males, and F_1 high-dose males and females. The increased incidence of minimal bile duct hyperplasia reported for 6/30 high-dose F_0 females was not repeated in the F_1 generation, where it occurred in 1-2 animals per sex per group, and was not considered a compound-related effect. No compound-related histopathologic alterations were observed for the reproductive organs of F_0 or F_1 parents.

C. Reproductive Effects: Reproductive indices calculated for mating, fecundity, gestation, and male and female fertility showed no statistically significant reductions compared to controls (Table 5).

   Incidences of vaginal discharge were generally associated with litter resorption or were noted after mating and did not appear to be compound related. The incidence of litter resorption was also comparable for all groups.

   The length of gestation was comparable for all groups in each generation.

D. Offspring Effects: The number and percent of viable pups at birth and surviving through weaning were comparable between the dose groups and controls for both the F_{1a} and F_{1b} litters. In the F_{2a} litters, however, the number of pups delivered, delivered viable and surviving to day 4 were significantly reduced in the high-dose group. The percentages of high-dose pups delivered viable and surviving to day 4 were also reduced, although differences from control did not achieve statistical

TABLE 3. Mean Organ Weight Data (±SD) for Parental Rats Fed CGA-64250 for Two Generations

Generation/ Dose Level	Final Body Weight (g)	Brain Weight (g)	Brain/Body Weight (g/100 g)	Gonad[a] Weight (g)	Gonad/Body Weight (g/100 g)
F_0 Males					
0 ppm	647.6±69.8	2.196±0.092	0.343±0.039	5.480±0.399	0.852±0.081
100 ppm	638.6±72.1	2.216±0.080	0.351±0.038	5.539±0.538	0.879±0.136
500 ppm	617.1±66.7	2.210±0.112	0.362±0.038	5.319±0.682	0.867±0.122
2500 ppm	619.2±55.1	2.227±0.099	0.362±0.032	5.998±1.053	0.971±0.160
F_0 Females					
0 ppm	360.5±48.6	2.037±0.085	0.573±0.066	0.082±0.032	0.024±0.010
100 ppm	350.1±44.2	2.019±0.103	0.584±0.070	0.088±0.027	0.026±0.009
500 ppm	363.7±58.8	2.055±0.098	0.578±0.088	0.090±0.029	0.025±0.009
2500 ppm	301.7±40.4**	2.049±0.117	0.689±0.082**	0.091±0.036	0.031±0.013
F_1 Males					
0 ppm	635.8±66.7	2.160±0.117	0.342±0.032	5.218±0.532	0.830±0.126
100 ppm	661.5±74.2	2.131±0.181	0.326±0.045	5.404±0.740	0.828±0.164
500 ppm	660.5±49.3	2.232±0.127	0.340±0.036	5.208±0.743	0.793±0.128
2500 ppm	587.0±74.1	2.134±0.078	0.368±0.041	5.582±0.660	0.961±0.134
F_1 Females					
0 ppm	353.1±36.5	1.992±0.082	0.569±0.050	0.096±0.033	0.027±0.009
100 ppm	350.1±37.3	1.996±0.145	0.576±0.066	0.085±0.020	0.025±0.006
500 ppm	336.6±35.3	1.980±0.123	0.594±0.072	0.100±0.031	0.030±0.010
2500 ppm	287.9±29.9**	1.976±0.110	0.693±0.077**	0.084±0.027	0.030±0.011

**Significantly different from control value ($p<0.01$).

[a]Testes with epididymides for males, ovaries for females.

TABLE 4. Incidence of Selected Hepatic Microscopic Findings in Parental Rats Fed CGA-64250 for Two Generations

	Dose Level (ppm)							
	0		100		500		2500	
	M	F	M	F	M	F	M	F
F_0 Parental Animals								
Number examined	15	30	15	29	15	30	15	30
Clear-cell change	0	1	2	1	3	1	14**	1
Cellular swelling	7	4	3	3	13*	6	14**	29**
Bile Duct Hyperplasia	2	2	1	1	1	1	0	6
F_1 Parental Animals								
Number examined	15	30	15	30	15	30	15	30
Clear-cell change	2	2	5	4	8*	7	11**	10*
Cellular swelling	0	0	1	2	5*	15**	15**	29**
Bile Duct Hyperplasia	0	2	2	1	0	2	0	1

*Significantly different from control value ($p<0.05$).

**Significantly different from control value ($p<0.01$).

TABLE 5. Summary of Reproductive Indices in Rats Fed CGA-64250 for Two Generations

Generation/ Dose Level	Index (%)					Mean Length of Gestation (days)
	Mating[a]	Fecundity[b]	Gestation[c]	Female Fertility[d]	Male Fertility[e]	
F_{1a}						
0 ppm	67.4	82.8	100.0	80.0	93.3	23
100 ppm	57.4	81.5	100.0	75.9	86.7	22
500 ppm	83.3	73.3	100.0	73.3	80.0	23
2500 ppm	75.0	73.3	90.9	73.3	86.7	23
F_{1b}						
0 ppm	62.9	72.7	93.8	66.7	66.7	22
100 ppm	78.6	90.9	95.0	90.9*	93.3	22
500 ppm	77.8	85.7	100.0	85.7	86.7	23
2500 ppm	81.5	81.8	100.0	81.8	80.0	22
F_{2a}						
0 ppm	61.7	86.2	96.0	83.3	93.3	22
100 ppm	83.3*	86.7	96.2	86.7	93.3	22
500 ppm	85.7*	90.0	96.3	90.0	100.0	22
2500 ppm	83.3*	73.3	100.0	73.3	93.3	22
F_{2b}						
0 ppm	67.6	87.0	95.0	80.0	93.3	22
100 ppm	100.0*	73.1	89.5	73.1	93.3	22
500 ppm	73.0	96.3	92.3	96.3	100.0	22
2500 ppm	73.1	94.7	100.0	90.0	93.3	22

*Significantly different from control value ($p < 0.05$).

[a]Mating index = $\frac{\text{No. confirmed copulations}}{\text{No. of 5-day estrous cycles}} \times 100.$

[b]Fecundity index = $\frac{\text{No. pregnancies}}{\text{No. copulations}} \times 100.$

[c]Gestation index = $\frac{\text{No. parturitions}}{\text{No. pregnancies}} \times 100.$

[d]Female fertility index = $\frac{\text{No. pregnancies}}{\text{No. females mated}} \times 100.$

[e]Male fertility indiex = $\frac{\text{No. sires}}{\text{No. males mated}} \times 100.$

significance. The F_{2b} litters of these dams had significantly reduced survival rates (both number and percent of surviving pups) at lactation days 7, 14, and 21 (Table 6).

Body weights of high-dose progeny were significantly reduced at days 14 and 21 for all four litter intervals (Table 7). Reductions were also significant on days 4 and 7 (except for F_{1b} litters) and at birth (F_{2b} litters only).

Significant changes in low- and mid-dose progeny weights included increases as well as reductions. Significant reductions occurred sporadically. In addition, reduced birth weights of low-dose F_{2b} progeny were associated with increased litter sizes.

The incidences of runts and pups with hematomas in the dose groups were comparable to controls. Except for a control F_{2a} pup with an umbilical hernia, all other gross abnormalities occurred in the progeny of the dose groups (Table 8). The overall incidence of pups with gross abnormalities (excluding runts and hematomas) was one control pup, three low-dose pups (three litters), two mid-dose pups (two litters), and four high-dose pups (three litters). Eye and/or eyelid-related findings were reported for one mid-dose F_{2b} pup and all four high-dose pups (two F_{1a} pups from same litter, and two F_{1b} pups) that had gross abnormalities. These findings included unopened eyelid, small or shrunken eye, and enlarged eye with opacity. Statistical analysis of the progeny anomaly data revealed no significant differences between the controls and dose groups.

Necropsies of the progeny selected for pathological evaluation revealed no gross findings associated with exposure to the test material.

High-dose male progeny of the F_{1a} litters that were selected for pathological evaluation had significantly reduced body weights, brain weights, and testes (with epididymides) weights and increased brain-to-body weight ratios when compared to controls (Table 9). All other organ weight data for the F_{1a} litters, and data for the F_{1b} litters were comparable to control values. In the second generation, high-dose F_{2a} males had significantly reduced body weights, testes with epididymides weights, and gonad-to-brain weight ratios, and increased brain-to-body weight ratios. Both the high-dose males and females of the F_{2b} progeny had significantly reduced body weights when compared to controls. The males also had significantly increased brain-to-body weight ratios, whereas the females had significantly reduced absolute brain weights.

TABLE 6. Population Data at Delivery and During Lactation for Progeny of Rats Fed CGA-64250 for Two Generations

Generation/ Dose Level	Mean Number Pups per Litter±SD and Percent Survival[a]											
				Viable on Lactation Day								
	Total Delivered	Delivered Viable		4		4[b]	7		14		21	
		No.	%	No.	%[c]	No.	No.	%[d]	No.	%[d]	No.	%[d]
F_{1a} Litters												
0 ppm	12.5±3.6	12.2±3.8	97	11.0±5.0	90	6.8±2.6	6.8±2.6	99	6.8±2.6	99	6.8±2.6	99
100 ppm	12.2±3.0	11.7±3.4	96	11.4±3.4	97	7.5±1.8	7.4±1.8	99	7.2±2.1	96	7.2±2.1	96
500 ppm	11.5±3.6	11.2±3.6	96	11.0±3.4	98	7.5±1.3	7.5±1.3	99	7.5±1.4	99	7.4±1.4	98
2500 ppm	12.1±2.9	11.8±2.8	98	11.4±2.2	96	8.0±0.0	7.7±1.0	96	7.4±1.8	93	7.4±1.8	93
F_{1b} Litters												
0 ppm	11.7±4.7	11.4±4.5	98	10.3±5.2	91	6.6±2.6	6.4±2.5	97	6.3±2.5	96	6.3±2.5	96
100 ppm	12.8±2.8	12.7±2.8	99	12.3±3.0	97	7.8±0.9	7.7±1.0	99	7.7±1.0	99	7.7±1.0	99
500 ppm	13.3±3.1	13.2±3.1	99	12.9±2.9	98	7.9±0.5	7.8±0.5	99	7.7±0.7	98	7.7±0.7	97
2500 ppm	12.8±2.9	12.4±2.8	97	12.1±2.7[e]	98	7.8±0.7[e]	7.4±2.0[e]	94	7.2±2.0[e]	93	7.2±2.0[e]	93
F_{2a} Litters												
0 ppm	12.8±1.7	12.5±2.1	98	12.0±2.4	97	7.9±0.6	7.8±0.8	99	7.8±0.8	99	7.8±0.8	99
100 ppm	13.0±2.8	13.0±2.8	100	11.6±4.2	89	7.2±2.0	7.2±2.3	99	7.2±2.3	99	7.1±2.3	98
500 ppm	11.0±3.5	10.8±3.3	98	10.4±3.4	96	7.5±1.4	7.2±1.8	95	7.0±2.0	93	7.0±2.1	93
2500 ppm	10.0±3.2*	9.2±3.4**	92	8.1±3.6**[e]	84	6.7±2.4[e]	6.5±2.5[e]	97	6.2±2.6[e]	93	6.2±2.6[e]	93
F_{2b} Litters												
0 ppm	12.9±3.0	12.8±3.1	99	12.5±2.9	97	7.8±0.9	7.7±0.9	99	7.7±0.9	99	7.7±0.9	99
100 ppm	13.5±2.5	13.4±2.5	100	13.2±2.3	99	8.0±0.0	8.0±0.0	100	8.0±0.0	100	8.0±0.0	100
500 ppm	12.7±3.2	12.7±3.2	100	12.5±3.1	99	7.8±0.7	7.8±0.7	100	7.8±0.7	100	7.8±0.7	100
2500 ppm	11.7±3.7	11.3±3.8	97	10.6±3.6	93	7.3±2.0	6.2±2.7*	85*	5.7±2.9*	78**	5.7±2.9**	78**

*Significantly different from control value ($p<0.05$).
**Significantly different from control value ($p<0.01$).

[a]Survival data represent progeny survival on a group basis.
[b]Litters were reduced on day 4 to eight pups.
[c]Percent of liveborn pups alive on day 4.
[d]Percent of pups surviving after culling on day 4.
[e]Omits dam(s) that died.

TABLE 7. Mean Pup Body Weights (g±SD) During Lactation of Rats Fed CGA-64250 for Two Generations

Generation/ Dose Level	Lactation Day 0	4	7	14	21 Male	21 Female
F_{1a} Pups						
0 ppm	6.1±0.9	8.9±1.5	14.6±2.9	28.2±4.6	48.0±8.3	46.1±6.4
100 ppm	6.0±0.8	8.8±1.4	13.0±2.7**	25.4±4.0**	44.1±6.1**	42.5±6.2**
500 ppm	6.1±0.9	9.2±2.7	15.2±2.4	26.9±3.9*	47.7±7.1	44.9±5.6
2500 ppm	6.2±0.8	8.1±1.8**	12.3±2.7**[a]	21.9±3.6**[b]	35.8±6.3**[b]	34.7±5.8**[b]
F_{1b} Pups						
0 ppm	6.1±0.8	8.8±1.7	13.4±2.8	25.7±4.8	44.3±6.6	40.1±6.3
100 ppm	6.0±0.9	9.2±1.5	14.5±2.9	27.7±4.3*	46.1±6.8	43.5±6.7
500 ppm	6.4±0.9**	9.5±1.8**	14.5±3.2*	28.0±4.9**	47.7±7.3	43.5±7.4
2500 ppm	6.1±0.8	8.6±1.4	13.3±2.2	22.9±4.4**	35.6±7.6**[a]	32.5±7.5**[b]
F_{2a} Pups						
0 ppm	5.4±0.9	8.5±1.5	13.7±2.7	25.6±4.4	43.8±8.3	41.1±7.0
100 ppm	5.3±0.9	7.9±1.5**	13.3±2.4	25.4±3.8	42.5±6.4	40.4±6.4
500 ppm	5.7±0.8*	8.3±2.1	13.0±3.2	25.2±4.3	42.5±7.5	39.8±7.2
2500 ppm	5.4±0.8	7.5±1.5**	10.8±2.2**[b]	20.0±3.0**[b]	31.5±5.4**[b]	30.2±4.9**[b]
F_{2b} Pups						
0 ppm	5.8±0.8	8.8±1.7	14.6±2.4	29.4±4.0	48.8±6.9	46.2±5.9
100 ppm	5.4±0.7**	8.6±1.4	14.7±2.1	28.7±3.2	48.6±6.5	46.6±6.7
500 ppm	5.5±0.9**	8.5±1.7	13.8±2.7	26.8±3.9**	45.1±7.7**	42.3±7.1**
2500 ppm	5.4±0.8**	7.3±1.9**[a]	10.9±2.8**[b]	21.9±3.5**[b]	36.7±4.7**[b]	33.3±5.7**[b]

NOTE: Values presented are based on individual pup weights rather than litter means.

*Significantly different from control value, $p<0.05$ (using individual pup weights).
**Significantly different from control value, $p<0.01$ (using individual pup weights).

[a]Significantly different from control value, $p<0.05$ (using mean pup weight per litter data).
[b]Significantly different from control value, $p<0.01$ (using mean pup weight per litter data).

TABLE 8. Gross Findings in Progeny of Rats Fed CGA-64250 for Two Generations

Dose Level (ppm)	Generation	Anomalies
0	F_{2a}	Umbilical hernia
100	F_{1a}	Anurous, clubbed limbs
	F_{1a}	Cleft lip, bipartite spleen
	F_{2b}	Short tail and torso, clubbed limbs
500	F_{2a}	Agnathia, cardiomegaly, possible exencephaly
	F_{2b}	Closed eyelid, shrunken eyes
2500	F_{1a}	Unopened eyelid[a]
	F_{1a}	Partially closed eyelid, small eye[a]
	F_{1b}	Domed forehead, dilated brain (small eyes were noted externally, but were not confirmed at necropsy)
	F_{1b}	Enlarged eye with opacity

[a]The two F_{1a} pups of the 2500 ppm group were from the same litter.

TABLE 9. Mean Organ Weight Data (±SD) for Progeny of Rats Fed CGA-64250 for Two Generations

Generation/ Dose Level	Final Body Weight (g)	Brain Weight (g)	Brain/Body Weight (g/100 g)	Gonad[a] Weight (g)	Gonad/Body Weight (g/100 g)	Gonad/Brain Weight (g/g)
F_{1a} Males						
0 ppm	153.0±25.4	1.766±0.098	1.181±0.145	1.732±0.381	1.127±0.155	0.970±0.183
100 ppm	143.1±29.9	1.741±0.100	1.263±0.258	1.669±0.567	1.138±0.216	0.949±0.293
500 ppm	140.7±33.7	1.766±0.116	1.331±0.362	1.620±0.533	1.123±0.180	0.906±0.270
2500 ppm	100.1±21.2**	1.580±0.117**	1.623±0.251*	1.148±0.334*	1.131±0.140	0.720±0.174
F_{1a} Females						
0 ppm	113.5±29.0	1.604±0.100	1.491±0.343	0.037±0.011	0.003±0.006	0.023±0.006
100 ppm	114.9±22.9	1.642±0.085	1.474±0.255	0.040±0.016	0.034±0.009	0.024±0.009
500 ppm	117.1±18.9	1.640±0.060	1.434±0.242	0.049±0.011	0.043±0.009	0.030±0.007
2500 ppm	97.6±27.4	1.562±0.113	1.813±0.891	0.034±0.013	0.035±0.007	0.022±0.008
F_{1b} Males						
0 ppm	130.7±21.7	1.673±0.061	1.311±0.211	1.260±0.366	0.949±0.183	0.751±0.209
100 ppm	127.9±24.7	1.645±0.078	1.346±0.356	1.343±0.399	1.028±0.198	0.813±0.234
500 ppm	140.4±25.6	1.739±0.091	1.273±0.213	1.378±0.302	0.978±0.082	0.789±0.152
2500 ppm	112.3±23.1	1.625±0.091	1.517±0.406	1.219±0.372	1.062±0.185	0.745±0.209
F_{1b} Females						
0 ppm	111.2±25.0	1.592±0.086	1.503±0.373	0.044±0.009	0.041±0.009	0.028±0.005
100 ppm	118.1±21.5	1.621±0.095	1.422±0.312	0.042±0.014	0.036±0.009	0.026±0.009
500 ppm	123.4±13.4	1.638±0.070	1.342±0.161	0.044±0.009	0.036±0.007	0.027±0.006
2500 ppm	96.0±17.1	1.533±0.069	1.646±0.326	0.034±0.009	0.036±0.008	0.022±0.006

(Continued)

*Significantly different from control value ($p<0.05$).
**Significantly different from control value ($p<0.01$).

[a]Testes with epididymides for male, ovaries for females.

TABLE 9. Mean Organ Weight Data (±SD) for Progeny of Rats Fed CGA-64250 for Two Generations (Continued)

Generation/ Dose Level	Final Body Weight (g)	Brain Weight (g)	Brain/Body Weight (g/100 g)	Gonad[a] Weight (g)	Gonad/Body Weight (g/100 g)	Gonad/Brain Weight (g/g)
F_{2a} Males						
0 ppm	142.3±14.6	1.718±0.066	1.217±0.110	1.662±0.271	1.169±0.169	0.966±0.146
100 ppm	138.0±27.2	1.731±0.124	1.306±0.302	1.558±0.418	1.106±0.159	0.891±0.219
500 ppm	139.7±12.1	1.707±0.118	1.229±0.119	1.575±0.371	1.124±0.240	0.925±0.216
2500 ppm	106.0±15.5**	1.633±0.117	1.564±0.209**	1.214±0.192*	1.147±0.107	0.743±0.059*
F_{2a} Females						
0 ppm	121.2±19.0	1.629±0.060	1.370±0.187	0.044±0.014	0.036±0.010	0.027±0.009
100 ppm	110.8±31.1	1.668±0.164	1.604±0.399	0.038±0.012	0.034±0.006	0.022±0.006
500 ppm	119.7±13.2	1.682±0.117	1.412±0.116	0.045±0.013	0.038±0.009	0.027±0.008
2500 ppm	98.7± 7.3	1.555±0.068	1.582±0.121	0.033±0.011	0.034±0.012	0.022±0.008
F_{2b} Males						
0 ppm	212.9±22.6	1.792±0.117	0.846±0.058	2.361±0.296	1.108±0.072	1.317±0.133
100 ppm	210.8±26.3	1.821±0.143	0.870±0.061	2.508±0.472	1.189±0.177	1.373±0.220
500 ppm	208.5±17.7	1.846±0.081	0.890±0.079	2.326±0.211	1.117±0.060	1.261±0.108
2500 ppm	168.2±10.4**	1.721±0.061	1.026±0.062**	2.002±0.138	1.192±0.066	1.164±0.076
F_{2b} Females						
0 ppm	150.0±16.6	1.708±0.079	1.148±0.106	0.062±0.018	0.041±0.011	0.037±0.011
100 ppm	159.4±11.6	1.735±0.099	1.091±0.059	0.059±0.016	0.037±0.008	0.034±0.008
500 ppm	151.0±13.3	1.733±0.062	1.154±0.086	0.058±0.012	0.039±0.009	0.034±0.008
2500 ppm	127.2± 9.1**	1.579±0.111*	1.244±0.086	0.050±0.013	0.039±0.010	0.031±0.006

(Concluded)

*Significantly different from control value ($p<0.05$).
**Significantly different from control value ($p<0.01$).

[a]Testes with epididymides for male, ovaries for females.

Histopathological evaluation of tissues from selected F_{1b} and F_{2b} progeny revealed significantly increased incidences of hepatic "cellular swelling" in high-dose males and females (Table 10). No compound-related histopathologic alterations were noted in the reproductive organs.

13. STUDY AUTHORS' CONCLUSIONS/QUALITY ASSURANCE MEASURES:

A. The study authors summarized the study results, but did not make any concluding statements about the LOELs or NOELs for parental toxicity, reproductive effects, or offspring toxicity.

B. A signed quality assurance statement, dated March 13, 1985, was presented in the final report.

14. REVIEWERS' DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

A. We assess that clinical observations and survival of parental animals were not adversely affected by compound administration.

These reviewers consider the decreased food consumption seen in high-dose animals and decreased body weights and increased incidence of hepatic alterations at the mid- and high-dose levels evidence of parental toxicity.

Body weights and weight gains during the premating period were decreased at the high-dose level for both generations. At the mid-dose level, F_0 males and F_1 females also showed decreases from control. Differences from control were frequently significant for the high-dose females and occasionally significant for the high-dose males and mid-dose females. Total gains for the premating period were significantly lower than control for F_0 high-dose females and F_1 mid- and high-dose females.

Significantly lower food consumption was noted for high-dose males and females of both generations. Food consumption values for the low- and mid-dose groups were generally comparable to control values; occasional significant differences were considered incidental and not compound related.

Gross necropsy findings were generally comparable between the control and dose groups for both parental generations. Absolute and relative organ weights were similar for all groups with the exception of significantly increased brain/body weight ratios for high-dose F_0 and F_1 females. Because the absolute brain weights were similar to controls, the higher ratios probably reflect the decreased terminal body weights for the high-dose females, and are not considered a direct compound effect.

TABLE 10. Incidence of Hepatic Microscopic Findings in Progeny of Rats Fed CGA-64250 for Two Generations

	Dose Level (ppm)							
	0		100		500		2500	
	M	F	M	F	M	F	M	F
F_{1b} Progeny								
Number examined	10	10	10	10	10	10	10	10
Clear-cell change	0	0	0	0	0	0	0	0
Cellular swelling	2	1	1	1	2	2	10**	8**
F_{2b} Progeny								
Number examined	10	10	10	10	10	10	10	10
Clear-cell change	0	1	0	0	0	0	1	0
Cellular swelling	0	0	0	0	2	1	10**	9**

**Significantly different from control value ($p<0.01$).

We attribute the liver alterations reported for mid- and high-dose adults of both generations to compound administration. The changes observed were increased incidences of hepatic "cellular swelling" and "clear-cell change." We concur with the study authors that the increased incidence of bile duct hyperplasia reported for high-dose F_0 females did not appear to be a compound-related effect.

There were no adverse compound-related effects indicated for the reproduction parameters, including mating, fecundity, gestation, and male and female fertility indices.

These reviewers attributed the decreased survival, decreased body weights during lactation, and increased incidence of liver alterations seen in high-dose offspring to compound administration and consider these effects as indicators of developmental toxicity.

Significantly decreased survival of offspring was reported for high-dose litters in the second generation. In the F_{2a} litters, total litter size, number of liveborn pups, and number of pups surviving to day 4 of lactation were significantly reduced at the high-dose level compared to controls. Nonsignificant reductions were also evident in the percentages of high-dose pups delivered viable and surviving to day 4. In the F_{2b} high-dose litters, significantly reduced survival (both number and percent of surviving pups) was reported at lactation days 7, 14, and 21.

In all four litter intervals, body weights of high-dose offspring were significantly lower than controls on days 14 and 21 of lactation. Significant reductions were also noted for various litter intervals on days 0, 4, or 7.

External observations of offspring were generally comparable between the control and dose groups. Findings reported at gross necropsy occurred with low frequency in all groups, including the controls. Eye and/or eyelid-related findings were reported for one F_{2b} mid-dose pup and four high-dose pups (two F_{1a} and two F_{1b}). However, the sporadic incidence and variation (small eyes versus enlarged eye) of these findings did not suggest a compound-related effect.

No clear pattern of compound effect was evident for the organ weight data of the progeny. Significantly increased brain/body weight ratios reported for high-dose F_{1a}, F_{2a}, and F_{2b} males occurred in conjunction with significantly reduced body weights.

As in the parental animals, a significantly increased incidence of hepatic "cellular swelling" was reported for high-dose male and female offspring. We consider this a compound effect. Hepatic clear-cell change in the offspring was seen in only one control female and one high-dose male of the F_{2b} generation.

B. The reviewers assessment of the study findings differs from that of the study authors with regard to the incidence of hepatic clear-cell change in parental animals. Whereas the authors stated that an increased incidence of this finding was seen at the mid- and high-dose levels, we assess that F_1 parents had increased incidences of clear-cell change at all dose levels compared to controls.

Item 15--see footnote 1.

16. CBI APPENDIX: Appendix A, Materials and Methods, CBI pp. 10-22.

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APPENDIX 14

TABLE 4. Incidences of Hyperemic Changes in the Gastrointestinal Tract of Male Dogs Fed CGA-64250

	Dose Level (ppm)			
Tissue	0	5	50	250
No. examined	5	5	5	5
Caecum	0	0	0	1
Colon	0	0	0	1
Duodenum	0	0	1	2
Jejunum	0	0	0	1
Rectum	0	1	0	1
Stomach	0	0	1	3

13. STUDY AUTHORS' CONCLUSIONS/QUALITY ASSURANCE MEASURES:

A. The study authors concluded, "oral administration (via feed) of CGA 64250 Technical for 53 consecutive weeks to beagle dogs at the levels of 5, 50, and 250 ppm resulted in no toxicological or pathological effect which were related to the administration of the test material. Thus the no-observable-effect level for this study was 250 ppm."

B. A signed, undated quality assurance statement was presented in the report. An initialed and dated master schedule sheet indicated that quality assurance inspections were conducted throughout the study and that the final report was reviewed.

14. REVIEWERS' DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

The dietary content of CGA-64250 technical for the high-dose groups varied significantly for the four batches of feed mix prepared between 12/12/83 and 1/16/84 when compared to the diets for the same animals during the rest of the study. According to the study authors, this was due to feed mixing problems and crystallization of the test material.

The test material, CGA-64250 technical, was reported in the protocol to be stable at refrigerated temperatures; but, it was stored at room temperature during the later half of the study, heated three times to 98°C for 30 minutes, and routinely heated to 50°C for 15-20 minutes prior to each feed preparation. However, the gas chromatographic data furnished for the feed analysis throughout the course of the study provided indirect evidence that the test material was stable throughout the study.

The dose selection for this study was based on a prior 90-day subchronic study in dogs, where it was stated that 250 ppm of CGA-64250 induced changes in the pyloric stomach. In the present study, hyperemia of the stomach in males receiving 250 ppm probably indicates mild irritation of the mucosa and was related to dosing since the finding was not present after a 4-week recovery period.

CGA-64250 technical, under the conditions of this study, did not appear to elicit any other effect on the beagle dogs. The NOEL is 50 ppm and the LOEL is 250 ppm.

Item 15--see footnote 1.

16. CBI APPENDIX: Appendix A, Protocol, CBI pp. 148 to 172.

DISCLAIMER

I did not participate in any aspect of the conduct and evaluation of the study entitled "One-year subchronic oral toxicity study in beagle dogs with CGA-64250 technical" while employed at Food and Drug Research Laboratories, Inc., although my name is included in the list of emloyees.

Asit K. Lahiri, D.V.M., Ph.D.
Principal Reviewer
Dynamac Corporation

Signature: Asit K Lahiri

Date: July 15, 1986

APPENDIX A

Protocol

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APPENDIX 15

EPA: 68-02-4225
DYNAMAC No. 1-073-C1
June 23, 1986

DATA EVALUATION RECORD

BANNER (CGA 64250)

Enzyme Induction Study

STUDY IDENTIFICATION: Waechter, F., Bentley, P., and Stäubli, W. The effect of propiconazole on drug metabolizing enzymes in the livers of male rats and mice. (Unpublished report prepared by CIBA-GEIGY Ltd., Basle, Switzerland, for CIBA-GEIGY Corp., Greensboro, NC; dated July 1984.) Accession No. 073929.

APPROVED BY:

I. Cecil Felkner, Ph.D.
Department Manager
Dynamac Corporation

Signature: I. Cecil Felkner

Date: 6-25-86

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1. CHEMICAL: Banner, CGA 64250, propiconazole, 1[2-(2,4-dichlorophenyl)-4-propyl-1,3-dioxalan-2-yl-methyl]-1H-1,2,4-triazole.

2. TEST MATERIAL: Propiconazole, a clear yellow viscous liquid, had a 90.7% purity.

3. STUDY/ACTION TYPE: Enzyme induction study in rats and mice.

4. STUDY IDENTIFICATION: Waechter, F., Bentley, P., and Stäubli, W. The effect of propiconazole on drug metabolizing enzymes in the livers of male rats and mice. (Unpublished report prepared by CIBA-GEIGY Ltd., Basle, Switzerland, for CIBA-GEIGY Corp., Greensboro, NC; dated July 1984.) Accession No. 073929.

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Marcia Van Gemert, Ph.D.
Section Head
Toxicology Branch, HED
EPA

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Date: 7/29/86

7. CONCLUSIONS:

A. Daily oral administration of propiconazole to mice and rats for 14 consecutive days at concentrations of 20, 80, 160, or 320 mg/kg produced a statistically significant and dose-dependent increase in liver-to-body weight ratios in both species at all doses tested. Hepatic DNA content was also increased in both species. The protein content of the subcellular liver fractions was generally increased in the mouse and in the rat, where the effect was more pronounced in the microsomal fraction. Both species showed increases in microsomal phospholipids, but the phospholipid/protein ratio remained relatively constant. Ethoxycoumarin O-deethylase, epoxide hydrolase, γ-glutamyltranspeptidase, glutathione S-transferase, and UDP-glucuranosyltransferase activities were induced in both species especially at the two higher doses, a pattern which is consistent with a phenobarbital-like induction. However, the authors did not provide data indicating that propiconazole does not also induce cytochrome P-448-related enzymatic activities.

B. This was a special study on the hepatic induction capacity of propiconazole; the study is acceptable.

Item 8--see footnote 1.

9. BACKGROUND: In previous chronic studies with rats and mice, dietary levels of propiconazole at 500 ppm (male mice) and 2500 ppm (both species) produced increased liver weights and changes in the activity of blood plasma enzymes presumed to be indicative for liver function. In male and female mice, 2500 ppm increased the incidence of liver tumors (statistically significant only in the males).

Item 10--see footnote 1.

11. MATERIALS AND METHODS (PROTOCOLS):

A. Materials and Methods: (See Appendix A for details.)

1. Male rats of the RAI strain weighing 190 g and male mice of the Mag strain weighing 26 g were maintained at 22°C and 55±5% humidity with a 12-hour light/dark cycle. Food (Nafag No. 890, Gossau, Switzerland) and water were available ad libitum.

---

[1] Only items appropriate to this DER have been included.

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2. Groups of six rats and six mice were administered propiconazole adsorbed to 2% carboxymethylcellulose daily for 14 days by gavage. The doses used were 20, 80, 160, or 320 mg/kg body weight. Groups of eight control animals were dosed with carboxymethylcellulose only.

3. After the last dose, the rats were fasted for 24 hours and killed. Livers were removed, weighed, and then homogenized in a buffered solution. The microsomal and cytosolic fractions were prepared by differential centrifugation. In addition, a small part of each rat liver homogenate was used to prepare a '50'-g supernatant.

4. Total protein was estimated in homogenates and '50'-g supernatants of rat livers, microsomes, and cytosolic fractions. The DNA concentration was determined fluorimetrically in liver homogenates of control and high-dose animals. In the microsomal fractions, the following parameters were evaluated: the contents of phospholipid and cytochrome P-450, the activities of ethoxycoumarin O-deethylase in presence or absence of monooxygenase inhibitors, epoxide hydrolase, and UDP-glucuranosyltransferase. The activity of glutathione S-transferase with 1-chloro-2,4-dinitrobenzene as substrate was determined in the cytosolic fraction; γ-glutamyltranspeptidase activity was measured in rat liver '50'-g supernatants.

5. Sections of the livers from control (two rats and two mice) and high-dose animals (two rats and three mice) were fixed in glutaraldehyde, postfixed with buffered 1% osmium tetraoxide, dehydrated in graded acetone, and embedded in Epon. These sections were stained with uranyl acetate and lead citrate and examined by electron microscopy.

6. Differences between dosed groups and controls were evaluated statistically using Student's t test.

B. Protocol: A protocol was not provided.

12. REPORTED RESULTS:

A. Propiconazole administration produced no effects on body weight (Table 1) at any dose. However, a dose-dependent increase in relative liver weights of both species occurred and was statistically significant ($p < 0.05$ to $p < 0.001$) at all dose levels (Table 1).

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B. DNA content was increased in liver fractions from rats and mice exposed to the high dose (Tables 2 and 3). Liver homogenate protein content was not changed in the rats at any dose and was significantly ($p < 0.05$ to $p < 0.001$) increased in the mouse at all levels when compared to control values. Sporadic changes were noted in cytosolic protein content in the rats (decrease) and mice (increase) when compared to control; these changes are probably unrelated to the test compound. Microsomal protein and phospholipid content was higher ($p < 0.001$) in rats receiving 80, 160, and 360 mg/kg and in mice receiving 160 and 360 mg/kg when compared to control values.

However, the phospholipid/protein ratio of microsomal fractions remained essentially unchanged over the dose range investigated.

C. Cytochrome P-450 specific content and ethoxycoumarin O-deethylase, UDP-glucuranosyltransferase, glutathione S-transferase, γ-glutamyltranspeptidase, and epoxide hydrolase activities were significantly ($p < 0.001$) higher in rats receiving the three highest doses when compared to controls (Table 4). In the mouse, the same pattern was exhibited at the two highest doses, but the effects were less pronounced in the 80-mg/kg animals (Table 5). UDP-glucuranosyltransferase activity in mice receiving the lowest dose was significantly lower than control values.

D. The inhibition of rat microsomal ethoxycoumarin O-deethylase activity in the presence of known mixed-function oxidase inhibitors was studied in propiconazole-dosed rats as well as untreated rats. The activity was increased with tetrahydrofuran, decreased with metyrapone, and unchanged with 7,8-benzoflavone (Table 6).

E. Electron microscopic evaluation of the hepatocytes of rats and mice showed proliferation of smooth endoplasmic reticulum at the highest dose. The number of lysosomes increased in the dosed rats. A few hepatocytes of treated rats showed rearrangement of smooth endoplasmic reticulum to "fingerprints." Treated mice showed increased lipid droplets. Actual photomicrographs were not provided.

13. STUDY AUTHORS' CONCLUSIONS/QUALITY ASSURANCE MEASURES:

A. The results of the present study demonstrate a marked and dose-dependent effect on the livers of male rats and mice dosed with propiconazole daily for 14 days. Increases in relative liver weights were observed in both species, even at the lowest dose (20 mg/kg). These were accompanied by proliferation of the smooth-surfaced endoplasmic reticulum and pronounced induction of several enzymes that are known to be involved in xenobiotic metabolism.

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A NOEL for liver enlargement was not demonstrated in either rats or mice since the marginal increases in liver weight observed at 20 mg/kg (about 10%) were statistically significant in both species (Table 1). However, the measured enzyme activities were not increased following treatment with 20 mg/kg propiconazole. Consequently, for these parameters, this dose may indicate a "NOEL" in rats and mice (Tables 4 and 5).

The combination of increased cytochrome P-450 content without a shift in the position of the absorption maximum of the reduced cytochrome P-450/carbon monoxide complex (toward 448 nm); inhibition of propiconazole-induced ethoxycoumarin O-deethylase activity by metyrapone; and propiconazole-induced epoxide hydrolase, UDP-glucuranosyltransferase, soluble glutathione S-transferase, and γ-glutamyltranspeptidase activities all indicate that propiconazole is a phenobarbital-like inducer.

B. A quality assurance statement was not presented in the report.

14. REVIEWERS' DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

A. The conclusions presented by the authors are in agreement with the data provided. However, in their discussion, the authors used these data along with some information from the published reports of others to propose a possible rationale for the carcinogenicity of propiconazole in mice. This rationale leads the reader to believe that the carcinogenicity of propiconazole may simply be an enhancement of the spontaneous rate of hepatic tumor formation common to mice. Although this rationale, by itself, could be used as a definition of a carcinogen, the authors state that propiconazole is a phenobarbital (PB)-like inducer, not a polycyclic aromatic hydrocarbon (PAH)-type inducer; the scientific literature indicates that PB-like induction is a beneficial detoxification mechanism, whereas PAH-like induction is often associated with the metabolic conversion of procarcinogens to ultimate carcinogens. The implication is that propiconazole is not a direct-acting carcinogen nor is it metabolized to an ultimate carcinogen. In addition, it is stated that the DNA/total liver protein ratios indicate that the induction observed in mice is due to the "strong hyperplastic effect of propiconazole" while the induction seen in the rats is due to a combination of hypertrophy and hyperplasia. They also argue that the morphological studies present findings consistent with an "adaptive response to an enhanced functional load."

Our reviewers consider this rationale to be weak and flawed because it is not supported by the data presented. The authors provide convincing evidence that propiconazole acts as a PB-like inducer. However, the data presented do not show that the compound is not also a PAH-like inducer. Polychlorinated and polybrominated biphenyls are two well-known examples of mixed

inducers. The authors should have performed assays to measure enzyme activities more specific for cytochrome P-450 induction as well as cytochrome P-448 induction (such as the conversion of the 7,8-diol of benzo(a)pyrene to DNA-binding adducts or 7-ethoxyresorufin O-deethylase activities). The activity that was measured, ethoxycoumarin O-deethylase, is nonspecific and even the 80 percent inhibition of this activity by metyrapone in hepatic microsomes from propiconazole-induced rats (not mice) is not convincing evidence that cytochrome P-448 induction has not occurred. Moreover, the authors did not produce a tracing of the reduced cytochrome P-450/carbon monoxide spectra to show that induction by propiconazole does not produce a shift in the Soret maximum.

The assertion that the induction seen in mice is due to hyperplasia based solely on the ratio of mg DNA/g liver protein (3.69 mg/kg for controls and 3.78 for induced animals, an increase of 2 percent) is unacceptable. Convincing evidence should include measurements of increased incorporation of [^{3}H]thymidine, increased numbers of hepatocytes per gram of liver, and/or increased numbers of nuclei per surface of thin sections of liver.

Finally, although there is no reason to doubt the reported morphological findings, no photomicrographs were provided.

Item 15--see footnote 1.

16. CBI APPENDIX: Appendix A, Materials and Methods, CBI pp. 4-5; Appendix B, References, CBI pp. 13-15.

APPENDIX A

Materials and Methods

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APPENDIX B

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APPENDIX 16

EPA: 68-02-4225
DYNAMAC No. 1-073-C2
July 17, 1986

DATA EVALUATION RECORD

CGA-64250

Promotion Study in Rats

STUDY IDENTIFICATION: Froehlich, E., Bentley, Ph., Staeubli, W., and Waechter, F. Promotion study with CGA 64250. (Unpublished study No. 834015 prepared by CIBA GEIGY Ltd., Toxicology GU 2, Basle, Switzerland; dated October 1, 1984.) Accession No. 073929.

APPROVED BY:

I. Cecil Felkner, Ph.D.
Department Manager
Dynamac Corporation

Signature: I. Cecil Felkner

Date: 7-18-86

1. CHEMICAL: CGA-64250; propiconazole; Banner; Tilt; 1-[2-(2,4-dichlorophenyl) 4-propyl-1,3-dioxolan-2-YL methyl]-1H-1,2,4-trizole.

2. TEST MATERIAL: CGA-64250, batch No. Op.301064, technical grade with a stated purity of 89.7%.

3. STUDY/ACTION TYPE: Promotion study in rats.

4. STUDY IDENTIFICATION: Froehlich, E., Bentley, Ph., Staeubli, W., and Waechter, F. Promotion study with CGA 64250. (Unpublished study No. 834015 prepared by CIBA GEIGY Ltd., Toxicology GU 2, Basle, Switzerland; dated October 1, 1984.) Accession No. 073929.

5. REVIEWED BY:

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Marcia Van Gemert, Ph.D.
Section Head
Toxicology Branch, HED

Signature: Marcia Van Gemert
Date: 7.29-86

7. CONCLUSIONS:

A. Under the conditions of the study, CGA-64250 was found to be a promoter of nonneoplastic and neoplastic proliferative rat liver changes when fed to weaned rats for 2, 4, and 8 weeks at 2000 ppm [with or without pretreatment using the initiator N-nitroso-diethylamine (DENA)]; effects were comparable to those using 500 ppm phenobarbital (reference or positive control for promotion). Although the study demonstrated the promotion properties of CGA-64250, it was not designed to identify initiator activity that could also be present.

B. The study is inconclusive, but acceptable; it provides useful information for establishing the mechanism of action for CGA-64250.

Item 8--see footnote 1.

9. BACKGROUND:

The experimental procedure ("baby rat model") utilized in this study was originally proposed by C. Peraino and coworkers in 1981.[2] The reference (positive control) promoter, phenobarbital, and the initiator (DENA) used by Periano et al. were included in this study as well. This test system offered three major advantages: (1) increased sensitivity (newborn animals are more susceptible to chemical induction), (2) lack of confounding variables (subhepatotoxic dose of the initiating carcinogen minimizes toxic changes in the liver), and (3) rapidity (early detection of preneoplastic changes--11 weeks).

Gamma-glutamyl-transpeptidase (GGT) was selected as the histochemical marker for altered hepatocytes since the traditional stain for histological examination (hematoxylin and eosin (HE)) was not considered to be sufficiently sensitive to contribute sufficient information on the number of focal proliferative changes in liver cells. Focal changes were easily detected, counted, and measured using GGT. This permitted the quantitative analysis of the effects of CGA-64250. Since the GGT procedure acts to histochemically

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[1] Only items appropriate to this DER have been included.

[2] C. Peraino et al. Early appearance of histochemically altered hepatic foci and liver tumors in female rats treated with carcinogens one day after birth. Carcinogenesis 5(1981): 463-465.

localize aminopeptidase activity, this markerwould also provide information on the association of enzymatic or biochemical changes within specific cells.[3]

The ability of phenobarbital to promote proliferative changes in the liver has been reported in the literature.[4,5] It was therefore used as the reference promoter.

The basis of the dose selection for this study was not given in this report. However, previously conducted acute oral and feeding studies with CGA-64250 might be used as a basis for the dietary level of 2000 ppm used in this study. An acute oral LD_{50} study conducted in rats in 1978 (EPA Accession No. 244272) reported that the LD_{50} was 1517 mg/kg (958-2271 mg/kg) when doses of 500, 1000, 3000, and 4000 mg/kg CGA-64250 were tested. An acute oral LD_{50} study conducted in a different strain of rats in 1980 (EPA Accession No. 244272) reported that the LD_{50} was 1510 mg/kg (1270-1800 mg/kg) in males, 1100 mg/kg (798-1520 mg/kg) in females, and 1310 mg/kg (1130-1520 mg/kg) when the sexes were combined and when doses of 904, 1150, 1390, 1670, 2000, and 5020 mg/kg CGA-64250 were tested. A 1979 90-day rat feeding study (EPA Accession No. 244272) reported that the LOEL was 1200 ppm when doses of 0, 240, 1200, and 6000 ppm CGA-64250 were tested. Twelve hundred ppm was found to reduce the body weight gain in female rats of this study. When rats were fed doses of 0, 400, 2000, and 5000 ppm CGA-64250 in a two-generation reproduction study (EPA Accession No. 072206), 5000 ppm caused the death of all F_0 females in that treatment group. Dose-related reductions in body weight gain and increased relative liver weights were seen in groups treated at all concentrations of CGA-64250. Two thousand ppm CGA-64250 was also found to cause hypertrophy of centrilobular hepatocytes in F_1 adults. The reviewers assess that the 2000-ppm dietary level appears to be adequate for a promotion study using preneoplastic focal proliferative changes in the liver as the principal index since it caused hypertrophy of hepatocytes, increased relative liver weight, and reduced the body weight gain in F_1 adults of the two-generation reproduction study; higher doses (5000 ppm) caused deaths in all F_0 females.

Item 10--see footnote 1.

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[3] A. Rutenburg et al. Histochemical and ultrastructural demonstration of γ-glutamyl transpeptidase activity. J. Histochem. Cytochem. 17(1969): 517-526.

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II. MATERIALS AND METHODS (PROTOCOLS):

A. Materials and Methods: (See Appendix A for complete details.)

1. The test compound CGA-64250 was of technical grade (batch No. OP.301064) with a chemical purity of 89.7%. The chemical was received July 20, 1983. Lot number and storage conditions for the test compound were not reported.

2. Pregnant rats of the Tif:RAIf strain (CIBA-GEIGY breeding station) were individually housed and acclimated to the test facility for 1 week prior to parturition. They were fed standard laboratory diet Nafag No. 890 (Nafag AG, 9202 Gossau, Switzerland).

3. Twenty-four hours after birth, the newborn rats were randomly distributed into two experimental groups. The test group was injected intraperitoneally (ip) one time with DENA (15 mg/kg body weight (BW)) dissolved in 0.9% NaCl (0.05 mL/7 g BW) as the vehicle (140 µmol/kg BW DENA). The control animals were injected ip one time with the vehicle alone.

4. At 3 weeks old, the pups were weaned and each group was randomly assigned to three separate experimental subgroups of 15 rats/sex (Table 1). Each of these subgroups was further assigned to three separate subgroups of five rats/sex (Table 2). Individual diets were prepared by combining either a precalculated concentration of 5-ethyl-5-phenyl-barbituric acid (phenobarbital; reference promoter) or CGA-64250 with casein-enriched diet Nafag No. 900. The 3-week-old rats were fed their group diets containing diet alone, 500 ppm phenobarbital, or 2000 ppm CGA-64250 for a period of up to 8 weeks.

5. The frequency of diet preparation was not indicated. The two food batches supplied for use during the study were analyzed for content one time during weeks 1-4 (batch 1) and once again during weeks 5-8 (batch 2).

6. Animals were observed daily for toxic symptoms, morbidity, behavior, and mortality. Body weights of the pups were determined on the day of weaning (day 22 of the study), every 2-4 days for 2 weeks, and every 6-8 days thereafter for 42 days. Food consumption for each group was determined weekly.

   Five pups/sex/group were sacrificed at 2 weeks (first sacrifice: male I and female IV subgroups), 4 weeks (second sacrifice: male II and female V subgroups), and 8 weeks (third sacrifice: male III and female VI subgroups) after weaning. Individual final body weights and liver weights were determined. Paraffin blocks were prepared for liver samples of all animals.

TABLE 1. Summary of Experimental Groups

Group Number	Compound Injected Intraperitoneally	Feed Preparation	No. of Animals/ Sex
Group 1	0.9% NaCl vehicle (0.05 mL/7 g BW)	Control	15/M 15/F
Group 2	0.9% NaCl vehicle (0.05 mL/7 g BW)	Phenobarbital (500 ppm)	15/M 15/F
Group 3	0.9% NaCl vehicle (0.05 mL/7 g BW)	CGA-64250 (2000 ppm)	15/M 15/F
Group 4	DENA dissolved in NaCl vehicle (15 mg/kg BW)	Control	15/M 15/F
Group 5	DENA dissolved in NaCl vehicle (15 mg/kg BW)	Phenobarbital (500 ppm)	15/M 15/F
Group 6	DENA dissolved in NaCl vehicle (15 mg/kg BW)	CGA-64250 (2000 ppm)	15/M 15/F

TABLE 2. Summary of Experimental Subgroups

Group/Subgroup[a]	Male			Female		
	I	II	III	IV	V	VI
Group 1 NaCl vehicle, control feed	*	*	*	*	*	*
Group 2 NaCl vehicle, phenobarbital feed	*	*	*	*	*	*
Group 3 NaCl vehicle, CGA-64250 feed	*	*	*	*	*	*
Group 4 DENA, control feed	*	*	*	*	*	*
Group 5 DENA, phenobarbital feed	*	*	*	*	*	*
Group 6 DENA, CGA-64250 feed	*	*	*	*	*	*

* Five animals per subgroup

[a]Subgroups I and IV were sacrificed at 2 weeks, subgroups II and V at 4 weeks, and subgroups III and VI at 8 weeks.

A series of four liver sections, three samples of which had been preserved in acetone and one sample that had been preserved in formalin, were prepared for histological examination for each animal, using three cytological staining procedures. The formalin-fixed sample and one of the acetone-fixed samples were stained with HE. One section was stained with the periodic acid-Schiff (PAS) method and one with the method of Rutenburg[6] to indicate GGT activity. A fifth section was retained if needed. All of the liver tissue sections from each animal were examined microscopically and screened for the presence of nodular lesions.

The sections stained for GGT were used to determine the presence and histological nature, both qualitatively and quantitatively, of focal or diffuse GGT-positive changes. The following criteria were used to identify GGT-positive changes: (1) clusters of at least three clearly delineated GGT-positive cells in the liver, possibly revealing morphological deviation from normal structure (recorded as foci or islands), and (2) diffuse perilobular enzymatic GGT activity (see Section 9, Background) identified by a specific enzyme-activity grading system (Table 3). Focal changes that presented histological evidence of neoplastic growth were identified. The number of focal GGT-positive changes and their cross-sectional area were determined.

Means were analyzed and graphed for body weight and food consumption. Box and whisker plots were used to show the median, interquartile range, extremes, and the symmetry of the following parameters: liver sample areas, number of foci per group, number of foci per group per cm^2, liver sample size vs. age at sacrifice, and number of foci per group per cm^2 vs. age at sacrifice. Each pair of box plots provided a test of significance for the difference of the medians of the two corresponding distributions. Reported large variations in the number of foci between individual animals in a group and between the four sample sections taken on each animal precluded the use of conventional statistical methods to compare the effects attributed to the different treatments.

B. Protocol: A protocol was not provided.

12. REPORTED RESULTS:

Dietary Analysis: The mean concentration of the test material in the diet (test weeks 1-4 and test weeks 5-8) was 2065 ppm for a nominal level of 2000 ppm. The homogeneity and stability of the test material in the diet were not reported.

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[6] A. Rutenburg et al.

TABLE 3. Enzyme-Activity Grading of GGT-Positive Foci

Grading	Microscopic Finding
----	No enzyme activity observed upon microscopic examination
0-+	Occasional hepatocytes displaying GGT activity
+	Several periportal hepatocytes of the limiting plate displaying GGT activity
+-++	Periportal zone displaying GGT activity (layer of 1-3 hepatocytes deep, starting from limiting plate)
++	Periportal zone displaying GGT activity (layer of >3 hepatocytes deep, starting from limiting plate)
+++	Entire perilobular zone displaying GGT activity (producing a netlike pattern)

Clinical Observations and Mortality: There were no clinical signs of toxicity or mortality during the 8-week feeding period from the time of weaning to the study termination.

Body Weights and Food Consumption: Table 4 presents mean body weight data at selected intervals during the study. These data were statistically evaluated by the reviewers using analysis of variance (ANOVA) and Dunnett's t test. Table 5 presents the differences (as percent) in body weight gain in NaCl- and DENA-injected mice receiving 500 ppm phenobarbital or 2000 ppm CGA-64250 when compared to their respective vehicle control groups. These data were calculated by the reviewers from Table 4. With the exception of the increase in body weight gain at 8 weeks in NaCl-injected males receiving 2000 ppm CGA-64250, the mean body weight gain of NaCl- and DENA-injected males receiving CGA-64250 was less than the NaCl- and DENA-injected males receiving control feed or 500 ppm phenobarbital at 2, 4, and 8 weeks. A similar pattern is found in DENA-injected females receiving CGA-64250 at 2 and 4 weeks.

These differences in body weight gain between experimental groups were not statistically significant; the changes were not considered toxicologically important by the study authors since no clinical symptoms or signs of toxicity were observed during the feeding period. The authors reported that between days 22 and 49, the food consumption in all groups dosed with DENA and/or exposed to phenobarbital or CGA-64250 was similar to that of the untreated controls. Food consumption values between days 50 and 77 were not interpreted by the authors; interpretation was not possible because of an error which was introduced into the data. The nature of this error was not described by the study authors.

Clinical Laboratory Measurements: There were no hematologic or clinical chemistry values reported.

Organ Weights: The liver was the only organ considered in the necropsy of the animals in this study. The authors presented tabulation of relative change in absolute liver weight and liver-to-body weight ratios in the text of the report, comparing weights at each sacrifice interval with the respective uninitiated control. No statistical notations were provided. Tables in Appendix A9 of the CBI report, titled "Descriptive Statistics," gave means, standard deviations, coefficient of variation, and maximum and minimum values but did not give levels of significance or denote significant values. Tables 6a and 6b summarize relative changes in group mean liver weight and liver-to-body weight ratios, comparing weights at each sacrifice level with the respective uninitiated control. Statistical notations have been added to these data. The liver-to-body weight ratio is found to increase after dosing with phenobarbital or CGA-64250 (with or without pretreatment with DENA) when compared to vehicle control groups of either sex.

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Using individual data, group mean liver weight and liver-to-body weight ratios were statistically evaluated by the reviewers using ANOVA, Dunnett's t test, and Duncan's multiple range test where appropriate (Table 7).

When compared to initiated and uninitiated controls, sporadic significant increases were found in mean liver weights and liver-to-body weight ratios in males and females receiving CGA-64250 and/or phenobarbital at the 2, 4, and 8 weeks sacrifice (Table 7).

In the female groups initiated with DENA injection, liver-to-body weight ratios were significantly increased compared to controls in animals receiving CGA-64250 and phenobarbital at all sacrifices. Absolute liver weights were increased in these groups when compared to controls, but only rats receiving phenobarbital at the 2-week sacrifice and CGA-64250 at the 8-week sacrifice were significantly different from controls.

When groups initiated with DENA were compared to the uninitiated controls, all absolute and relative liver weights were nonsignificantly increased in males and females receiving CGA-64250 and phenobarbital at all sacrifice times. The absolute and relative liver weights were significantly increased in males initiated with DENA and dosed with CGA-64250 or phenobarbital at the 8-week sacrifice. In the female groups initiated with DENA, the relative liver weights were significantly increased, compared to controls, in animals receiving CGA-64250 or phenobarbital at all sacrifice times. In addition, the absolute liver weights were significantly increased in females initiated with DENA and dosed with CGA-64250 at the 4- and 8-week sacrifice.

In all sacrifice periods, except at the 2-week sacrifice in males, the absolute liver weights and liver-to-body weight ratios of NaCl-injected males and females receiving the test compound were nonsignificantly increased more than that of the rats receiving phenobarbital. Absolute and relative liver weights in males receiving CGA-64250 (without DENA pretreatment) sacrificed at 8 weeks were found to be significantly increased ($p < 0.05$, Duncan's multiple range test, test of least significant differences (LSD)) when compared to phenobarbital-treated males of the same age. Liver-to-body weight ratios in females receiving CGA-64250 (without DENA pretreatment) sacrificed at 4 weeks were found to be significantly increased ($p < 0.05$, Duncan's multiple range test, LSD test) when compared to phenobarbital-treated females of the same age. In the 2-week sacrifice for males and the 4- and 8-week sacrifices for females, the absolute liver weights and liver-to-body weight ratios of mice injected with initiator (DENA) followed by CGA-64250 were also nonsignificantly increased more than that of the mice receiving phenobarbital.

Gross Pathology: The authors reported that there were no compound-related gross effects observed in animals sacrificed at 2, 4, or 8 weeks. Specific data were not presented.

TABLE 7. Mean Liver Weights and Liver-to-Body Weight Ratios[a] (±SD) in Rats[b]

Diet	Pretreatment: NaCl — Week of Sacrifice 2	NaCl — 4	NaCl — 8	Pretreatment: DENA — Week of Sacrifice 2	DENA — 4	DENA — 8
Males						
Control	4.56 ± 1.35 (4.20 ± 0.82)	8.32 ± 0.91 (3.90 ± 0.43)	9.94 ± 1.12 (2.86 ± 0.13)	3.96 ± 0.48 (3.68 ± 0.23)	7.76 ± 1.34 (3.75 ± 0.40)	11.84 ± 1.68 (3.13 ± 0.40)
Phenobarbital	4.46 ± 0.49 (4.54 ± 0.51)	9.94 ± 1.80[c] (4.65 ± 0.93)	12.38 ± 1.04[d] (3.43 ± 0.34)[d]	4.80 ± 0.70 (4.60 ± 0.31)[e]	9.37 ± 1.32 (4.67 ± 0.50)[e]	12.77 ± 1.54[d] (3.54 ± 0.18)[d]
CBA-64250	4.47 ± 0.76 (4.51 ± 0.36)	10.16 ± 1.24 (5.13 ± 0.24)[d]	14.05 ± 1.00[d,f] (3.85 ± 0.19)[d,f]	4.86 ± 0.72 (4.96 ± 0.79)[e]	9.15 ± 0.87 (4.53 ± 0.24)[e]	12.39 ± 0.95[d] (3.60 ± 0.19)[d,e]
Females						
Control	3.58 ± 0.44 (3.53 ± 0.20)	5.56 ± 0.44 (3.57 ± 0.16)	6.49 ± 0.77 (2.72 ± 0.26)	3.47 ± 0.38 (3.52 ± 0.19)	5.82 ± 0.38 (3.57 ± 0.10)	6.32 ± 0.71 (2.80 ± 0.16)
Phenobarbital	4.21 ± 0.56 (4.33 ± 0.54)	6.20 ± 0.85 (3.88 ± 0.22)	6.25 ± 1.13 (2.93 ± 0.35)	4.53 ± 0.60[e] (4.48 ± 0.42)[e]	6.31 ± 0.76 (4.10 ± 0.24)[d,e]	7.35 ± 0.60 (3.36 ± 0.22)[d,e]
CGA-64250	4.33 ± 0.26 (4.46 ± 0.49)	6.59 ± 0.33[d] 4.30 ± 0.19[d,f]	7.19 ± 0.52 (3.36 ± 0.23)[d]	3.81 ± 0.44 (4.32 ± 0.14)[e]	6.67 ± 0.56[d] (4.49 ± 0.49)[d,e]	8.38 ± 0.83[d,e] (3.67 ± 0.34)[d,e]

[a]Listed in parenthesis beneath specific mean liver weight for that treatment group.

[b]Statistically evaluated by reviewers using ANOVA, Dunnett's t test with exception of groups compared to reference control (NaCl, ip/phenobarbital diet) where Duncan's multiple range test, LSD test used in evaluation.

[c]The individual liver weight of animal No. 23 of the NaCl, ip/phenobarbital diet group should be 12.47 g instead of 12.27 g. Individual body weight percent and mean and liver body weight percent calculations will have been recorded accurately if this change in liver weight is recorded.

[d]Significantly different ($p < 0.05$) from concurrently sacrificed uninitiated negative control (NaCl, ip/control diet) value.

[e]Significantly different ($p < 0.05$) from concurrently sacrificed initiated control (DENA, ip/control diet) value.

[f]Significantly different ($p < 0.05$) from concurrently sacrificed reference control (NaCl, ip/500 ppm phenobarbital in feed) value.

Histopathology: Focal proliferative changes that stained for GGT activity were classified as foci of altered hepatocytes. The authors reported that equal size sample areas were used for foci evaluation.

Microscopic examination revealed no changes in the liver parenchyma that could mask the occurrence or observation of focal proliferation (i.e., necrosis, inflammation).

Slides that were not stained specifically for focal proliferative changes (HE or PAS stains) revealed few foci, indicating little morphological deviation from normal hepatocyte appearance. The HE stain found the foci to resemble clear cell foci or mixed cell foci.

The authors reported that GGT-positive foci were primarily found in the periportal region of the liver lobule directly adjacent to the portal field (periphery of lobule). This was interpreted to mean that the focal changes originated in the limiting plate of the liver lobule.

An attempt was made to distinguish GGT-positive focal changes (identified as foci or islands) from diffuse GGT activity. Diffuse perilobular GGT activity and GGT-positive focal changes were observed in groups exposed to phenobarbital, the reference promoter, or to CGA-64250 (with or without prior initiator treatment), whereas the NaCl control and DENA control animals showed only slight GGT activity. The foci identified after application with CGA-64250 alone or following pretreatment with the initiator, DENA, did not differ qualitatively from those found after treatment with DENA alone or with DENA followed by phenobarbital. The morphological appearance of GGT-positive foci was similar in males and females.

In males and females in all age groups, the highest number of foci was found in animals treated with DENA followed by either phenobarbital or CGA-64250 (Table 8). Following DENA initiation, CGA-64250 treatment resulted in almost twice as many foci as seen with phenobarbital in females sacrificed at 4 weeks and males and females sacrificed at 8 weeks (Table 8). The total number of foci more than doubled between the sacrifice at 4 weeks and that at 8 weeks in all initiated groups of females and initiated and uninitiated groups of CGA-64250-treated males (Table 8).

NaCl + control groups of females produced a larger number of foci than their male counterparts. With the exception of males sacrificed at 2 weeks, the exposure of 2000 ppm CGA-64250 was more effective than 500 ppm phenobarbital in both sexes and in all age groups.

The foci density (number of foci per cm^2 liver sample) was found to be proportional to the number of foci per treatment group. Using this parameter, exposure to 2000 ppm CGA-64250 (with or without pretreatment with DENA) was reported to be equal to or more effective than treatment with phenobarbital (with or without pretreatment with DENA).

TABLE 8. Estimated Total Number of GGT-Positive Foci At Each Week of Sacrifice Per Treatment Group

Treatment		Total Number of Foci at Week of Sacrifice		
Pretreatment	Diet	2	4	8
Males				
NaCl[a]	control	0	1	0
NaCl	phenobarbital[b]	0	53	11
NaCl	CGA-64250[c]	0	142	422
DENA[d]	control	55	103	121
DENA	phenobarbital[e]	493	367	613
DENA	CGA-64250[f]	552	382	1178
Females				
NaCl	control	14	15	38
NaCl	phenobarbital	0	69	34
NaCl	CGA-64250	143	165	244
DENA	control	179	71	198
DENA	phenobarbital	612	284	660
DENA	CGA-64250	488	516	1189

[a]0.9% NaCl, 7.14 mL/kg BW, ip.

[b]Dietary concentration = 500 ppm.

[c]Dietary concentration = 2000 ppm.

[d]140 µmol DENA/kg BW, ip.

[e]Dietary concentration = 500 ppm.

[f]Dietary concentration = 2000 ppm.

Source: CBI page 44; standard deviations not provided.

The transsectional areas of the foci of vehicle control animals were found to be smaller than 0.01 mm^2, with the range in size between 0.0001-0.01 mm^2. A treatment group was reported to have a bimodal distribution when at least one focus was larger than 0.01 mm^2 and at least one focus was smaller than 0.001 mm^2. In bimodal distributions, the first peak of distribution was located at a focus size of 0.001 mm^2, the second frequency peak at 0.015 mm^2, peak defined as that focus size which occurred with greatest frequency. If all foci were found to be smaller than 0.01 mm^2 unimodality was reported. Foci larger than 0.01 mm^2 were not found to occur alone. Bimodal distribution was found in all initiated groups and the uninitiated CGA-64250-treated females sacrificed at 2 weeks.

The size of the individual GGT-positive foci in all uninitiated groups were found to be smaller than 0.01 mm^2; however, CGA-64250 or phenobarbital were found to quantitatively enhance formation of these 0.01 mm^2 foci in males. A similar, but less pronounced, result was obtained in females. Following pretreatment with the initiator DENA, the size of the GGT-positive foci ranged from 0.0001-0.2 mm^2. When CGA-64250 or phenobarbital were given after pretreatment with DENA, larger foci were found in greater quantity. When compared to 500 ppm phenobarbital, 2000 ppm CGA-64250 produced more foci that were smaller than 0.01 mm^2 and similar numbers that were larger than 0.01 mm^2 after 8 weeks of exposure. The first peak was found to occur at a frequency that was approximately twice as high in CGA-64250 animals pretreated with DENA as in their phenobarbital-treated counterparts. Neoplastic nodules, designated as GGT-negative nodules, were reported in two male rats pretreated with DENA followed by 2000 ppm CGA-64250 and sacrificed at 8 weeks.

13. STUDY AUTHORS' CONCLUSIONS/QUALITY ASSURANCE MEASURES:

A. Under the conditions of the study, CGA-64250 was found to be a promoter of nonneoplastic and neoplastic proliferative rat liver changes. The effect found with CGA-64250 was comparable to that found with phenobarbital, the reference promoter. The small differences in body weight gain between experimental groups were not considered toxicologically important. There were no effects of dosing on signs of toxicity or on mortality. Liver-to-body weight ratios increased in CGA-64250- or phenobarbital-treated animals (with or without pretreatment with DENA) when compared to vehicle control groups of either sex.

GGT-positive foci (few in number) were found in the livers of uninitiated controls; their size was less than 0.01 mm^2. The foci of rats receiving DENA alone ranged from 0.0001-0.2 mm^2; this GGT activity was also slight. CGA-64250 and phenobarbital alone were found to quantitatively enhance the formation of foci smaller than 0.01 mm^2. With the exception of males sacrificed at 2 weeks, the exposure to 2000 ppm CGA-64250 was more effective in males and females at 2, 4, and 8 weeks in inducing focal proliferative changes than 500 ppm phenobarbital.

When CGA-64250 or phenobarbital were given after pretreatment with DENA, larger foci were found in greater quantity. When compared to 500 ppm phenobarbital, 2000 ppm CGA-64250 produced more foci that were smaller than 0.01 mm^2 and similar numbers that were larger than 0.01 mm^2 after 8 weeks of exposure.

A neoplastic nodule that was GGT negative was found at the 8-week sacrifice in two of five males exposed to CGA-64250 after pretreatment with DENA.

B. Quality assurance measures were not indicated for this study.

14. REVIEWERS' DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

The design and conduct of this study were acceptable. However, the following deficiencies were noted:

1. There was no indication that study animals (pregnant or newborn rats) were examined for their general health status prior to testing.

2. The frequency of diet preparation and results of homogeneity and stability analyses were not reported.

3. The authors reported that between days 22 and 49, the food consumption in all groups dosed with DENA and/or exposed to phenobarbital or CGA-64250 was similar to that of the uninitiated controls. However, the food consumption of DENA-injected males receiving CGA-64250 was found to be 14% less from weeks 0-2 and 8.4% less from weeks 0-4 than that of uninitiated controls. The food consumption in all other groups was found to be similar to that of the control animals. Even though an unexplained error was reported to have been introduced into the food consumption data for days 50-77, tabulated mean values were reported, and were analyzed by the reviewers.

4. Group means were reported for food consumption and group means and standard deviations were reported for body weights, and absolute and relative liver weights. There were no other statistical calculations performed on any parameter of the study. For this reason, body weights, liver weights, and liver-to-body weight ratios were statistically evaluated using ANOVA and Dunnett's t test (Tables 4 and 7). Body weight change was found to be nonsignificant. The absolute and relative liver weights of males and females treated with phenobarbital and CGA-64250 (with or without pretreatment with DENA) were found to be significantly increased when compared to vehicle control and DENA control animals. The liver weights and liver-to-body weight ratios of rats treated with phenobarbital and CGA-64250 were statistically compared by the reviewers using Duncan's multiple range test and the LSD test. (See Section 12, Reported Results, Organ Weights, and Table 5 for specific statistical results.)

5. There was no descriptive information given for the differentiation between "diffuse GGT activity" and "GGT-positive activity." The authors reported some technical difficulty in discriminating between diffuse and GGT-positive activity, which could have resulted in an under- or overestimation of the actual number of foci. The intra-group and intra-animal variation reported might have been due in part to this problem.

6. Acetone was the primary preservative used for this study. This deviates from the protocol of Rutenburg[7] who preserved liver in 3% glutaraldehyde, 10% aqueous formalin, or a formol-calcium solution for a comparative preservation of tissue for use in the histochemical demonstration of GGT activity. Rutenburg found that morphology was well preserved in each fixative used; however, glutaraldehyde fixation for 4 hours was found to result in the least inhibition of the GGT reaction for light microscopy.[8] Acetone has been found to dehydrate tissue and extract lipids.[9]

   We assess that it would be a poor preservative for an extended fixation process. There is no indication for the basis of this change. From the data presented, we assess that the HE stain (general cell structure) and the PAS stain (insoluble carbohydrates and mucopolysaccharides) were used to compare degenerative changes in the liver ultrastructure. The authors reported that microscopic examination revealed no change in the liver parenchyma of the samples of this study that would mask the observation of focal proliferation.

   The hypothesis that CGA-64250 may act as a promoter of liver cell tumors was prompted by results from prior studies. A chronic feeding study in mice showed an increase in the incidence of liver cell tumors (CBG 196 81827; reference 1 in this study). Specific data from the mouse study were not included with this report.

   The liver weights and liver-to-body weight ratios of rats treated with phenobarbital and CGA-64250 were statistically compared by the reviewers using Duncan's multiple range test and the LSD test. In all sacrifice periods, except at the 2-week sacrifice in males, the absolute liver weights and liver-to-body weight ratios of NaCl-injected males and females receiving the test

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[7] A. Rutenburg et al.

[8] A. Rutenburg et al.

[9] W. Bloom and D. Fawcett. A Textbook of Histology. W. B. Saunders Co., 1975.

compound were nonsignificantly increased more than that of rats receiving phenobarbital. Absolute and relative liver weights in males receiving CGA-64250 (without DENA pretreatment) sacrificed at 8 weeks were found to be significantly increased ($p < 0.05$) when compared to phenobarbital-treated males of the same age. Likewise, liver-to-body weight ratios in females receiving CGA-64250 (without DENA pretreatment) sacrificed at 4 weeks were found to be significantly increased ($p < 0.05$) when compared to phenobarbital-treated females of the same age. At the 2-week sacrifice interval for males and the 4- and 8-week sacrifice for females, the absolute liver weights and liver-to-body weight ratios of rats injected with DENA followed by CGA-64250 were also nonsignificantly increased more than that of the rats receiving phenobarbital. DENA application followed by CGA-64250 dosing was found to produce twice the number of foci in females sacrificed at 4 and 8 weeks and males sacrificed at 8 weeks as DENA application followed by phenobarbital (Table 7). Uninitiated CGA-64250-treated animals produced at least twice the number of foci as phenobarbital, with the exception of males sacrificed at 2 weeks (Table 8). The reported density of foci (number of foci per cm^2 liver sample) followed the same pattern. This might suggest the initiation as well as promotion potential of CGA-64250. In addition, after the 8-week exposure period to 2000 ppm CGA, more foci that were smaller than 0.01 mm^2 were found than after the same exposure period to 500 ppm phenobarbital.

The increase in absolute and relative liver weight in rats receiving CGA-64250, as well as the increased pattern of focal proliferation, could be interpreted as evidence for initiator activity, or may have been caused by the induction of enzymes known to be involved in xenobiotic metabolism (study No. 1-073-C1). The study authors repeatedly equated the focal proliferation of CGA-64250 with that of phenobarbital. Since phenobarbital has been described in the literature as a promoter and the test material was found to behave similarly and to be at least as effective, CGA-64250 was assessed to be a promoter of proliferative changes in the rat liver. The initiation or complete carcinogenic potential of CGA-64250 could not be assessed from the data in this study and would require protocol modifications before it could be adequately investigated.

Item 15--see footnote 1.

16. CBI APPENDIX: Appendix A, Materials and Methods, CBI pp. 31-38.

APPENDIX A

Materials and Methods

TILT CGA-64250 Reviews

The next 8 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.

EPA: 68-02-4225
DYNAMAC No. 1-073-B
July 11, 1986

DATA EVALUATION RECORD

CGA-64250

Chronic Feeding Study in Dogs

STUDY IDENTIFICATION: Johnson, W. D., Thompson, S. W., and Becci, P. J. One-year subchronic oral toxicity study in beagle dogs with CGA-64250 technical. (Unpublished study No. 7737 prepared by Food and Drug Research Laboratories, Inc., Waverly, NY, for CIBA-GEIGY Corporation, Agricultural Division, Greensboro, NC; dated May 28, 1985.) Accession No. 073928.

APPROVED BY:

I. Cecil Felkner, Ph.D.
Department Manager
Dynamac Corporation

Signature: I. Cecil Felkner

Date: 7-11-86

2-94

1. CHEMICAL: Banner; CGA-64250.

2. TEST MATERIAL: CGA-64250 technical, batch No. FL-831527, had a purity of 90.2 percent.

3. STUDY/ACTION TYPE: Chronic feeding study in dogs.

4. STUDY IDENTIFICATION: Johnson, W. D., Thompson, S. W., and Becci, P. J. One-year subchronic oral toxicity study in beagle dogs with CGA-64250 technical. (Unpublished study No. 7737 prepared by Food and Drug Research Laboratories, Inc., Waverly, NY, for CIBA-GEIGY Corporation, Agricultural Division, Greensboro, NC; dated May 28, 1985.) Accession No. 073928.

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7. CONCLUSIONS:

A. Under the conditions of the study, no apparent toxicologic effects resulted when CGA-64250 was fed to beagle dogs for 53 weeks at dietary levels of 5 or 50 ppm. Administration of CGA-64250 did not cause any dose-related effects on mortality, organ or body weights, food consumption, and clinical laboratory parameters. Necropsy and histopathologic examinations revealed evidence of mild irritation of the stomach in males given the highest dietary concentration of CGA-64250 (250 ppm). The NOEL was 50 ppm and the LOEL was 250 ppm.

B. Core Classification: The study is considered Core Minimum since the test compound was not homogeneously distributed in the diet during weeks 14-21.

Items 8 and 10--see footnote 1.

9. BACKGROUND: Feeding levels in the chronic study were based on a 3-month study in dogs where 50, 250, and 1,250 ppm were tested. In the 3-month study, a LOEL of 250 ppm was set based on pyloric changes in the stomach of some dogs at 250 ppm.

11. MATERIALS AND METHODS (PROTOCOLS):

A. Materials and Methods:

1. The test material, CGA-64250 technical, batch No. FL831527, was 90.2 percent pure; it was stored refrigerated during the initial 24 weeks of the study and at room temperature thereafter.

2. Four- to 6-month-old purebred beagle dogs (Buckshire Corp., Perkasie, PA) were acclimated in the laboratory for 6 weeks. The dogs were distributed into different dose groups by randomized assignments from a body weight stratification list. Seven animals/dose/sex constituted the control and high-dose groups, whereas five animals/dose/sex made up the mid- and low-dose groups. The dogs were housed individually in pens with hardwood chip bedding in environmentally controlled rooms. The dose levels used in this study were 0, 5, 50, and 250 ppm of the test material mixed with Purina certified canine diet No. 5007. Four hundred grams of the feed, containing appropriate levels of the test material, were offered for 2 hours daily to all animals of both sexes for the first 18 weeks. After 18 weeks, all male animals were offered 500 g of food containing the prescribed levels of the test material. Tapwater was available ad libitum.

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[1] Only items appropriate to this DER have been included.

3. Test diets were offered to appropriate dogs until the end of week 52 when euthanasia and necropsy were performed on all dogs except for two dogs/sex for the control and the high-dose groups; following week 52 these eight dogs were fed diets free of CGA-64250 technical for an additional 28 days. They were then sacrificed and necropsied.

4. Test diets were prepared approximately every 10 days. Three samples of diets for each dose level taken from three different locations of the mixing chamber were analyzed to determine homogeneity of the test material and diet content.

5. Animals were observed twice a day for survival and daily for toxic signs. Palpation for masses and clinical examinations were performed weekly. Individual body weight and food consumptions were measured weekly for the first 13 weeks and monthly thereafter.

   Ocular examinations were performed on all dogs prior to study initiation and at the end of week 52 and on two dogs/sex for the high-dose and control groups at the conclusion of the 4-week recovery period.

6. Evaluation of hematologic and clinical chemistry parameters of blood samples and urinalysis were performed on all dogs once prior to study initiation and three times during the course of study (at 3, 6, and 12 months) and on two dogs/sex in the control and high-dose groups at the end of the 4-week recovery period.

7. All the animals in mid- and low-dose groups and five dogs/dose/sex for the control and high-dose groups were sacrificed for necropsy at the end of treatment week 52. The remaining two dogs/sex for the control and the high-dose groups were sacrificed and necropsied after a period of 4 weeks of recovery following a year of treatment.

   Approximately 40 representative specimens of different tissues and organs were collected and fixed in 10 percent formalin (eyes in Zenker's fixative) for microscopic examination at necropsy from each dog. Weights of approximately 10 different organs from each dog were recorded prior to fixation.

   One way analysis of variance, least significant difference, Kruskal-Wallis and Mann-Whitney U-test, Mantel Haenszel chi-square test, and Fisher's exact test were used where appropriate to analyze the data. Significance for all statistical analysis was judged at $p \leq 0.05$.

B. Protocol: See Appendix A.

12. REPORTED RESULTS:

Dietary Analysis: Dietary analyses indicated that CGA-64250 technical was stable in the feed for 21 days at room temperature.

The distribution of the test compound in the mid- and high-dose groups was not homogeneous during weeks 14-21 (Table 1).

It was observed that CGA-64250 crystallized during storage in the refrigerators. This was considered to be the reason for its uneven distribution in the feed. Therefore, the test material was heated to 98°C for 30 minutes on three occasions prior to diet preparation, kept at room temperature from day 172 of the study, and routinely heated to 50°C in water bath prior to diet preparation.

Time-weighted average dietary concentrations of CGA-64250 were 4.92 ± 0.8 ppm for the low dose, 47.6 ± 5.0 ppm for the mid dose, and 252 ± 68 ppm for the high dose.

Clinical Observations and Mortality: No death occurred during the course of the study. Alopecia, skin sores, thinning of hair, and thick skin were observed both in males and females of all groups and were attributed to demodectic mange infestation in the colony. Seven dogs were treated by rubbing 2-3 mL of a solution of Canolene● on the infested area (once); one dog received three applications. Skin conditions did not affect the health of the animals except for one mid-dose female whose body weight declined from 11.9 kg at week 13 to 9.0 kg at week 53. Ophthalmoscopic examinations of all dogs did not reveal any toxic effects.

Body Weights: Except for one incidence of a sporadic decrease in mean body weight gain of the mid-dose females at week 33, no statistically significant change occurred in body weight or body weight gain in any of the dose groups when compared to controls. The decrease in body weight of one female animal in the mid-dose group from weeks 13 to 53 was considered to be caused by demodectic mange.

Food and Test Substance Consumption: Mean food consumption for all dosed groups were comparable to control values. The average consumption of CGA-64250 for the 5-, 50-, and 250-ppm male dogs were 1.2 ± 0.2, 13.0 ± 2.0 and 59.0 ± 8.0 mg/kg, respectively. The average consumption for the females was 1.3 ± 0.2, 13 ± 2.0, and 62 ±10 mg/kg for the low-, mid-, and high-dose groups, respectively.

Hematologic Parameters: None of the group mean values of the hematologic parameters examined at 3, 6, and 12 months of the study for the dosed animals of either sex were significantly different from the respective control groups.

Clinical Chemistry Parameters: The group mean values of all the individual parameters in clinical chemistry examined at 3, 6, and 12 months of the study for the dosed animals of both sexes were comparable to the respective control groups except for a sporadic change in calcium value.

TABLE 1. Selected Results from Analysis of CGA-64250 in the Diet

Dietary Concentration (ppm)	Replicate	CGA-64250 Found (ppm) on Mix Date (No. of Days Fed)[a] 12/12/83 (10)	12/19/83 (10)	12/28/83 (10)	01/06/84 (10)	01/16/84 (10)	01/26/84 (10)	02/02/84 (10)
50	1	48.2	43.8	55.0	46.6	23.7	50.8	45.2
	2	44.9	32.2	30.6	48.3	57.8	25.7	44.1
	3	-	-	-	-	-	-	42.8
	Mean	46.6	38.0	42.8	47.4	40.8	38.2	44.0
250	1	210.2	209.1	242.5	189.2	185.6	282.4	246.5
	2	288.0	182.5	794.6	197.5	136.5	283.4	241.6
	3	-	295.0	-	-	-	-	242.3
	Mean	249.1	228.9	518.6*	193.4*	161.0*	282.9	243.5

[a]Mix dates encompass weeks 14 to 21 of the study.

*Statistically significant ($p \leq 0.05$) when compared to the mean content of CGA-64250 from the rest of the food mix at this level.

Urinalysis: The specific gravity of the urine was significantly ($p \leq 0.05$) increased in the mid- and high-dose males at the 3-month interval (Table 2). However, the values were found to be comparable to control values both at the 6- and 12-month intervals. Urine specific gravity for all female dose groups was comparable to that of the controls at all intervals during the study. The increase in the urine specific gravity for males at the 3-month interval was considered inconsequential to the administration of CGA-64250.

Organ Weights: The following statistically significant changes in organ weights were noted when compared to the respective controls:

a. Decrease in brain weights of mid-dose females;

b. Increase in the organ-to-body weight ratio of the adrenals in the 50- and 250-ppm females;

c. Increase in the organ-to-brain weight ratio of the adrenals in the 50-ppm females;

d. Decrease in the organ-to-body weight ratio of the pituitary glands in the 250-ppm male dogs; and

e. Increase in the organ-to-brain weight ratio in the pituitary glands in the 50-ppm female dogs (Table 3).

The study authors did not attribute the above changes to the administration of CGA-64250 because there was a lack of histopathological correlation. In addition, in support of their conclusion, they pointed to the lack of a dose-response relationship and inconsistencies in the occurrences of the above changes.

Necropsy: At necropsy, no gross finding indicative of a compound-related effect was seen in any tissue or organ of the dosed animals. Color alterations were noted in the large and small intestines and the stomach of 3/5 males receiving 250 ppm CGA-64250. This was attributed to a focal variation in the amount of blood in the vasculature at time of sacrifice and was not considered of pathological significance by the report authors. Skin changes were the most frequently encountered gross lesions in this study.

Histopathologic Examination: No neoplasms were found in any of the animals in this study. A wart-like growth noted in life on the left ear of a female receiving 50 ppm (days 350-370) was not noted at necropsy or on histologic examination. Three of five males receiving 250 ppm had hyperemia of the mucosa of the stomach (Table 4) but no comparable findings were seen in two control males or two males that had received 250 ppm following a 4-week recovery period. Functional hypertrophy of the mammary gland was found in 1/5 control females, 2/5 receiving 50 ppm, and 3/5 receiving 250 ppm. Microscopic examination did not reveal any effects that were attributable to the test compound. The results of both necropsy and histopathological examination of the animals in the recovery groups were considered unremarkable by the study authors.

TILT CGA-64250 Reviews

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