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

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OPP OFFICIAL RECORD
HEALTH EFFECTS DIVISION
SCIENTIFIC DATA REVIEWS
EPA SERIES 361

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM:

1/26/99

Subject: Propiconazole: DER for a mouse carcinogenicity study (MRID# 44381401).

DP Barcode: D245245
Submission: S540845
Rereg. Case: 3125

PC Code: 122101
CAS No.: 60207-90-1
Caswell File: 323EE

From: David G Anderson, Toxicologist
Reregistration Branch-2
HED (7509C)

David G Anderson 1/26/99

To: Kathy Monk/Mark Hartman PM 52
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SRRD (7508W)

Thru: Alan Nielsen, BSS
Reregistration Branch-2
HED (7509C)

Al Nielsen 1/28/99

The registrant has submitted an oncogenicity study in males mice to support the reregistration of propiconazole. The reference ands executive summary for the study are below.

CITATION: Gerspach, R. (1997)18-months oncogenicity study in mice of CGA 64250 technical. Novartis Crop Protection AG, 4332 Stein, Switzerland. Laboratory Study No. 943126. March, 1997. MRID 44381401. Unpublished.

SPONSOR: Novartis Crop Protection AG
Human Safety Assessment
4002 Basle / Switzerland

EXECUTIVE SUMMARY: This study was conducted to satisfy a Phase-4 data call-in for propiconazole from the U.S. EPA. The phase 4 memo called for an additional mouse oncogenicity study on the basis of the Agency's determination that the high dose (2500 ppm) in the original 2-year mouse oncogenicity study was excessively toxic. A study design involving only the CD-1 males, histopathology of only the liver, and the feeding levels of 0, 100, 500, and 850 ppm was accepted by the EPA in a final communication dated 5/10/94.

In an 18-month oncogenicity study (MRID 44381401), CGA 64250 technical (Batch No.

OP.303011, Purity 92.4%) was administered to groups of 80 male CrI: CD-1^r(ICR) BR mice in the diet at concentrations of 0, 100, 500, or 850 ppm. These concentrations resulted in a nominal compound intake for each concentration level of 0, 11.0, 59.0, and 108 mg/kg/day for control, low-, mid-, and high-dose, respectively. Interim sacrifices were conducted at 9 weeks and 12 months on 10 mice/group, and 10 mice/group were designated for blood chemistry evaluation at weeks -1, 9, 14, 53, and 79, the remaining 50 mice/group were used for the main study.

No overt clinical signs were noted over the course of the study in any of the treated groups, and survival in all the treated groups was comparable to the control. Body weight and body weight gain were significantly ($p<0.01$) decreased in the 850 ppm group compared to the control group for weeks 18-50. Body weight gain was decreased by up to 10 % (n.s.) in the 500 ppm group compared to the control group. The liver was identified as a target organ based on several toxic effects to this organ. Statistically significant ($p <0.01$) concentration-effect increases in mean liver weight and mean liver to body weight ratios were observed in the high-dose group and increases in these values (n.s.) were also observed in the mid-dose group compared to the control group. At the interim, increases were 32 and 33% at the week 9 sacrifice, 11 and 29 % at the week 53 sacrifice, and 19 and 20% at the terminal sacrifices in the 850 ppm group compared to the control group liver weights and liver to body weight ratios, respectively. The high-dose group also were found to have significantly more (61%; $p<0.05$) incidences of enlarged livers compared to the control group. Increased incidences of masses and nodules (n.s.) were also observed in the 850 ppm treated animals compared to the controls. Hepatocellular hypertrophy was significantly increased by 86 and 93% ($p<0.01$), respectively in the mid- and high-exposure groups. There was a concentration-effect relationship evident for the incidence of liver necrosis at ≥ 100 ppm at the week 9 sacrifice ($p\leq 0.007$), and at the final sacrifice ($p\leq 0.003$), Cochran-Armitage test for trend, by reviewer. There was an increase in the incidences of fatty change, lymphohistiocytic infiltration, and incidence of foci of cellular change in the livers of animals in the high-dose group ($p < 0.05$) but only at the week 9 interim sacrifice.

A suggestion of altered metabolic function at ≥ 500 ppm was noted in decreased cholesterol (14% to 24%) at week 14 and at 850 ppm in increased sorbitol dehydrogenase (45%) at week 14. However, the liver enzymes, ASAT, ALAT and ALP were unaffected at the highest dose tested.

The LOAEL is 500 ppm in the diet (59.0 mg/kg/day) for males, based on hepatotoxicity and body weight gain effects observed at the interim and terminal sacrifices. The NOAEL is 100 ppm (11.0 mg/kg/day) for males.

There was a treatment related increase in hepatocellular adenoma incidences (20 %, $p<0.05$) and total hepatocellular neoplasia (adenomas and carcinomas) of 24%, $p<0.05$, in the liver of animals at the 850 ppm exposure level when compared to controls with a 2% incidence of adenomas and a 4% incidence of total neoplasia (adenomas and carcinomas). Dosing was considered adequate based on the body weight gain and hepatotoxic effects seen at 500 and 850 ppm.

The percentage adenomas and carcinomas were within the range of the inadequate historical control data submitted with the study report. In the 850 ppm dose group, the total incidence of hepatocellular neoplasia was slightly higher (24%) than the upper limit of the historical control range of 22.4% submitted and the concurrent control (4%) was lower than of the lower range of

historical controls of 6.0% submitted. The historical controls submitted were inadequate because the collection dates were not specified and were not collected in the testing facility.

This oncogenicity study in the mouse is acceptable (guideline) and does satisfy the guideline requirement for an oncogenicity study (83-2b) in mice as agreed upon by the EPA and Novartis Crop Protection AG in a communication dated 5/10/94 (From Susan Makris to Bruce Sidwell PM 53, dated 5/10/94 & 5/16/94). This study is acceptable only when considered in conjunction with older oncogenicity study in mice (HED Doc# 004287 and 005352; MRID#073919,250784-250786 and 251237).

DATA EVALUATION REPORT

PROPICONAZOLE (CGA 64250 Technical)

STUDY TYPE: ONCOGENICITY FEEDING – MOUSE (83-2b)

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by

Chemical Hazard Evaluation Group
Toxicology and Risk Analysis Section
Life Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37831
Task Order No. 98-35

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Date: NOV 11 '98

Disclaimer

This review may have been altered subsequent to the contractor's signatures above.

Oak Ridge National Laboratory is managed by Lockheed Martin Energy Research Corporation for the U.S. Department of Energy under Contract No. DE-AC05-96OR22464

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David G Anderson Date 1/26/99.

Saujwan Diwan, Date 1/27/99

013082

DATA EVALUATION RECORD

STUDY TYPE: Oncogenicity Feeding - Mouse
OPPTS 870.4200 [§83-2b]

DP BARCODE:D245245
P.C. CODE: 122101

SUBMISSION CODE: S540845
TOX. CHEM. NO.: 323EE

TEST MATERIAL (PURITY): Propiconazole (purity, 92.4 % a.i.)

SYNONYMS: CGA 64250 technical

CITATION: Gerspach, R. (1997)18-months oncogenicity study in mice of CGA 64250 technical. Novartis Crop Protection AG, 4332 Stein, Switzerland. Laboratory Study No. 943126. March, 1997. MRID 44381401. Unpublished.

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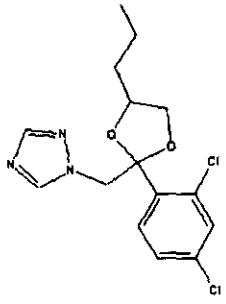
COMPLIANCE: Signed and dated GLP, Quality Assurance, Data Confidentiality, and Flagging statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. 

Test material: Propiconazole

Synonym: CGA 64250 tech. (Batch No. OP.303011)
Description: liquid
Lot/Batch #: Batch No. OP.303011
Purity: 92.4%
Stability of compound: shelf life of the test compound was not supplied. The identity of the compound was confirmed at the beginning of the study, after one year of the study, and at the end of the study.
CAS #: 60207-90-1

Structure

2. Vehicle and/or positive control

The test material was mixed with feed; a positive control was not included in this study.

3. Test animals:

Species: mouse
Strain: CrI: CD-1^r(ICR) BR
Age and weight at study initiation: age: 4 weeks, weight range: 26.59 - 39.52 g all males
Source: Charles River Deutschland Niederlassung Sulzfeld, D-97633 Sulzfeld
Housing: animals were housed individually in macrolon cages type 2 (area: 400 square centimeters), with wire mesh tops and sterilized, granulated soft wood bedding (Societe Parisienne des Sciures Pantin, 95100 Argenteuil/France).
Diet: CT1, Special Diet Services Limited, Stepfield, Witham, Essex, UK, *ad libitum*
Water: tap water, *ad libitum*
Environmental conditions:
Temperature: 22 ± 2°C
Humidity: 55 ± 10%
Air changes: 16 - 20 /hour,
Photoperiod: 12 hours light/12 hours dark
Acclimation period: 26 days

B. STUDY DESIGN

1. In life dates

Start: November 23, 1994; end: June 21, 1996

2. Animal assignment

Animals were assigned to the test groups in Table 1 by a method that ensured animals with similar weights were randomly distributed in all groups. Mice at extremes of the weight range were not included in the study.

Table 1. Study design

Dose group	Conc. in diet (ppm)	Dose (mg/kg/day) males	No. of animals			
			Interim Sacrifice*		Oncogenicity	Clinical Chem. Only
			9 wks	12 mo		
I	0	0	10	10	50	10
II	100	11.0	10	10	50	10
III	500	59.0	10	10	50	10
IV	850	107.6	10	10	50	10

Data taken from p. 26 MRID 44381401.
320 animals (total), 80 mice/dose
50 animals/group for evaluation of the carcinogenic potential of test compound.
10 animals/group used for blood chemistry investigations only, scheduled at weeks -1, 9, 14, 53, and 79.
10 animals/group for interim sacrifice at week 53.
10 animals/group for interim sacrifice at week 9.
* Macroscopic and microscopic postmortem examinations.

3. Dose selection

In a previously submitted 2-year combined oncogenicity/chronic study for CGA 64250 , doses of 0, 100, 500, or 2500 ppm were fed to CD-1 mice. At 2500 ppm the incidence of liver tumors was increased compared to controls, and the effects on survival, liver clinical pathology, histopathology, and body weight and body weight gain were sufficient to indicate that this level clearly exceeded the maximum tolerated dose (MTD). It was also apparent from this study that 500 ppm approached the MTD as evidenced by effects on body weight gain throughout the study and liver damage (noted at 6 months) as assessed through clinical pathology.

Two subchronic studies (17- and 18- weeks) were conducted in order to substantiate the perception of 500 ppm as approximating the MTD. In the 17-week study (MRID

42050501), both male and female mice were fed levels of 0, 20, 500, 850, 1450, or 2500 ppm. Liver weights and ratios were increased at ≥ 500 ppm and at ≥ 850 ppm hepatic clinical pathology changes and hepatocellular hypertrophy were observed, as well as hepatic necrosis at ≥ 1450 in male mice. Female mice seemed to tolerate the test substance better than males with an MTD at 2500 ppm compared to an MTD at 1450 ppm for males.

In the second subchronic study (MRID 42050502) male mice were fed levels of CGA 64250 at concentrations of 0, 40, 500, 850, 1450, and 2500 ppm. After 13 weeks of treatment, liver weight increases were noted in mice fed ≥ 500 ppm; after 4 to 8 weeks of treatment, absolute liver weights and liver to brain weight ratios were increased at ≥ 850 ppm and liver to body weight ratios were increased at ≥ 500 ppm. Significant reductions in body weight at ≥ 500 ppm and body weight gain at ≥ 850 ppm occurred at 4, 8, and 13 weeks. Hepatocellular toxicity was evidenced at ≥ 500 ppm as decreased serum cholesterol, increased alanine aminotransferase at ≥ 1450 ppm and increased sorbitol dehydrogenase at ≥ 850 ppm. Statistically significant increases in hepatocellular hypertrophy were noted at ≥ 500 ppm, hepatocellular necrosis at ≥ 850 ppm, hepatocellular vacuolation at ≥ 1450 ppm, and mineralization at 2500 ppm. The MTD for this study was determined to be less than or equal to 850 ppm.

Based on the results of the previous studies, the feeding levels for the current 18-month oncogenicity study in CD-1 male mice were set at 0, 100, 500, and 850 ppm;

100 ppm expected NOAEL

500 ppm expected LOAEL

850 ppm expected MTD

4. Diet preparation and analysis

Test diets were prepared in 4-week intervals (2-week intervals during treatment weeks 29-32) and stored in stainless steel containers at room temperature until used. Aliquots of CGA 64250 were weighed (without adjustment for purity), dissolved in acetone and added to a fixed amount of bolus alba and pulverized food, for each test concentration. Quantities of acetone were added to stock mixtures such that all groups had equal amounts of vehicle. After concentration premixes were mixed thoroughly and the acetone was evaporated, the stock mixtures were diluted with quantities of the diet to achieve the required concentrations of test article for each group. A 1:4 ratio of water to food was used to ensure pellet homogeneity, food pellets were air dried before use. Food for the controls was prepared in the same matter with omission of the test substance. Analysis of diets prepared for the first 4 weeks of the study was performed on samples (200 g) taken at the A) beginning, B) middle, and C) end of the pelleting process. Dietary mixtures containing 100, 500, and 850 were prepared and

stored at room temperature up to 4 weeks. Concentration analyses were performed before initiation of the study, after one year, and at the end of the study.

Results –

Homogeneity: Actual concentrations for the targeted 100 ppm group ranged from 75.9 - 93.68 ppm which corresponds to 75.9 - 93.7 % of nominal; for the 500 ppm group, actual concentrations ranged from 387.8 - 515.1 ppm corresponding to 77.6 - 103.0 % of nominal; for the 850 ppm group, actual concentrations ranged from 812.4 - 860.8 ppm corresponding to 81.2 - 85.4 % of nominal. The conclusion of these analyses was that the methods used in this study were sufficient to yield diets with a homogeneous distribution of test substance.

Stability: Analyses of these mixtures revealed that the test substance was stable for 35 days at room temperature. Stability analysis revealed actual concentration ranges of 85.76 - 94.85 ppm with corresponding values of 85.8 - 94.8 % of nominal for the targeted 100 ppm group; actual concentrations of 452.3 - 472.2 ppm which were 90.5 - 94.4 % of nominal were reported for the 500 ppm target group; 825.2 - 854.5 ppm which were 85.4-97.1 % of nominal were the ranges reported for actual concentrations of diets prepared for a target concentration of 1000 ppm. These were considered acceptable ranges for stability of the test substance after storage at room temperature for 35 days.

Concentration analysis: Content analyses revealed ranges of 80.90 to 101.4 ppm which were 80.9 - 101.4 % (mean of 90.9%) of nominal for the 100 ppm target group, 429.3 - 508.7 ppm corresponding to 85.9 - 101.7 % (mean of 96.9%) of nominal for the 500 ppm group, and 785.1 - 985.7 ppm corresponding to 92.4 - 116.0 % (mean of 103%) of nominal for the 850 ppm group. These were considered acceptable and the dose levels reported in mg/kg/day were corrected for these differences..

5. Statistics

An univariate statistical analysis was performed for each time point and parameter. Non normal data was allowed for by the incorporation of nonparametric statistical analysis. Treated groups were compared to controls by Lepage's two-sample test. Increasing or decreasing trends compared to controls were tested for using Jonckheere's test for ordered alternatives. A method introduced by Cox, a regression model (partial likelihood) was used to compare survival time of treated animals with that of control animals.

For the analysis of the tumor incidences, the investigators used a method by Peto et al. called the "context of observation" method which employs three contexts of observation: 1) incidental, 2) fatal, and 3) mortality independent. In this study two analyses were used, incidental, and fatal. Assuming that all tumors were either fatal or incidental a test for positive trend was used to analyze these parameters separately. When a trend was detected, the data were further analyzed to determine the "highest

non-significant group" using a "closed" procedure which detects the nominal significance level. Analyses were carried out for all lesions observed. Comparisons were considered significant at $p < 0.05$.

C. METHODS

1. Observations

Animals were inspected daily for signs of toxicity and mortality and were given a detailed examination once each week.

2. Body weight

Animals were weighed at weekly intervals throughout the first 3 months of the study and monthly thereafter.

3. Food consumption and compound intake

Food consumption for each animal was determined weekly throughout the first 3 months of the study and monthly thereafter and was calculated as g food/mouse/day for each cage. Food consumption ratios (g food consumed weekly / weekly body weight gain) were calculated for the first 3 months of the study and monthly thereafter. The compound intake (mg/kg/day) was calculated for each concentration from the food intake and body weight data.

4. Blood was collected from the orbital sinus using glass capillary tubes after anesthesia with ether. Food was withheld overnight and blood was collected in the morning. Heparin was utilized as an anticoagulant. Blood analysis was performed pretest, and at weeks 9, 14, 53, and 79 on 10 animals / dose. The CHECKED (X) parameters were examined.

a. Hematology

No hematological analysis was performed.

b. Clinical chemistry

X	**ELECTROLYTES**	X	**OTHER**
	Calcium		Albumin
	Chloride		Blood creatinine
	Magnesium		Blood urea nitrogen
	Phosphorus	X	Total Cholesterol
	Potassium		Globulins
	Sodium		Glucose
			Total bilirubin
	ENZYMES		Total serum protein (TP)
X	Alkaline phosphatase (ALK)		Triglycerides
	Cholinesterase (ChE)		Serum protein electrophores
	Creatine phosphokinase		
	Lactic acid dehydrogenase (LDH)		
X	Serum alanine amino-transferase (also SGPT)		
X	Serum aspartate amino-transferase (also SGOT)		
	Gamma glutamyl transferase (GGT)		
	Glutamate dehydrogenase		
X	Sorbitol dehydrogenase		

5. Urinalysis

Urinalysis tests were not conducted and are not required for oncogenicity studies based on Subdivision F guidelines.

6. Sacrifice and pathology

At scheduled sacrifices, weeks 9, 53, and 79, all surviving animals were killed by exsanguination under ether anesthesia and necropsied. Necropsies with tissue preservation were also performed on animals which died during the test period or were sacrificed in moribund condition. The CHECKED (X) tissues from all groups were collected and stored in neutral buffered 4% formalin. Two tissue samples from the livers of all animals were embedded in paraffin, sectioned, and stained with hematoxylin and eosin. Livers were the only organs examined microscopically as per the agreement for this study. The (XX) organs from all animals were weighed. No microscopic analyses were conducted on those animals designated for clinical chemistry.

X	DIGESTIVE SYSTEM	X	CARDIOVASC./HEMAT.	X	NEUROLOGIC
X	Tongue	X	Aorta*	XX	Brain*
	Oral tissue	XX	*Heart**	X	*Periph. nerve**
X	Salivary glands*	X	Bone marrow*	X	Spinal cord (3 levels)*
X	Esophagus*	X	Lymph nodes*	X	Pituitary*
X	Stomach*	X	Spleen*	X	Eyes* with optic nerve
X	Duodenum*	X	Thymus*		
X	Jejunum*				**GLANDULAR**
X	Ileum*		**UROGENITAL**	X	Adrenal gland*
X	Cecum*	XX	Kidneys*+	X	Lacrimal/Harderian glands
X	Colon*	X	Urinary bladder*	X	Mammary gland*
X	Rectum*	XX	Testes*+	X	Parathyroids*
XX	*Liver*+*	X	*Epididymides*	X	*Thyroids**
X	Gall bladder*	X	Prostate	X	orbital gland, both
X	Pancreas*	X	Seminal vesicle	X	Auditory sebaceous gland
			Coagulating gland		(Zymbal's gland)
	RESPIRATORY		Preputial gland		
X	Trachea*		Ovaries*†		**OTHER**
X	Lung*		Uterus*†	X	Bone*
X	Nose		Cervix †		Femur with joint
	Pharynx		Oviduct †		Sternum with marrow
	Larynx		Vagina †	X	Skeletal muscle*
				X	*Skin**
					Mediastinal tissue
					Mesenteric tissue
				X	All gross lesions and masses*

* Required for oncogenicity studies based on Subdivision F Guidelines.
+ Organ weight required in oncogenicity studies.
† No females in the current study

II. RESULTS

A. OBSERVATIONS

1. Toxicity

There were no increased incidences in clinical signs of toxicity in the treated groups compared to controls. There were however, numerous incidences of hunched posture and piloerection seen in control and all treated groups that were associated with early death in most cases.

2. Mortality

The percent survival at selected times during the study is given in Table 2. There were no significant trends or differences in survival of treated males compared to the control group.

Table 2. Summary of animal fate.				
Number of mice/Interval	Dietary concentration (ppm)			
	0	100	500	850
Males				
Study initiation	80	80	80	80
Found dead	40	40	36	36
Moribund Sacrifice	0	1	1	4
Accidental death	3	3	0	0
9-weeks sacrifice	10	9	10	10
53-weeks sacrifice	10	9	10	10
18-month sacrifice	17	18	23	20
Adjusted survival at 18 months *	17/77	18/77	23/80	20/80
Survival at 18 months **	16/50	17/50	20/50	18/50
Percent survival	32	34	40	36

* all animals on test, excluding accidental deaths
** carcinogenicity subgroup only (used for survival analysis)
Data taken from p. 76 MRID 44381401

B. BODY WEIGHT AND BODY WEIGHT GAIN

During the first three months of the study, the treated groups exhibited mean body weights that were similar to controls. Statistically significant lower mean body weights were measured for the mice receiving 850 ppm of test substance for weeks 18-50. This group seemed to equilibrate with controls after week 50 and remained comparable to controls throughout the remainder of the study. Mean body weights for animals in the 500 and 100 ppm groups were similar to controls throughout the study. Animals in the treated and control groups gained weight steadily for the first 3 weeks of the study. By week 14, a decreasing trend in body weight gain was discernible in the mid- and high-dose groups. During weeks 18-46 body weight gain was significantly lower than controls in the 850 ppm group (11 to 19 % ; $p < 0.01$) and in the 500 ppm group up to 10 % (n.s.) lower than controls. After week 46, weight gains in these groups stabilized and was comparable to controls throughout the remainder of the study. Body weight gain values in the 100 ppm exposed mice were comparable to the control group throughout the study. Body weights and weight gains for selected weeks are presented in Table 3.

Table 3. Group mean body weights (g) and cumulative body weight gains (g) in male mice fed CGA 64250.

Weeks of study	Dietary concentration (ppm)			
	0	100	500	850
Body Weight				
1	34.70±2.23	35.33±2.43	35.78±2.22	35.0±2.21
11	40.55±3.26	40.93±3.10	40.59±2.95	40.11±3.06
18	43.13±4.14	43.42±3.78	42.96±3.72	41.75±3.58*
22	43.22±4.41	44.06±4.08	43.22±3.80	41.70±3.85*
30	44.56±4.56	45.10±4.34	44.36±4.22	42.53±4.30*
46	46.22±5.11	46.75±4.58	45.47±4.41	43.60±4.48*
59	45.55±5.34	45.99±4.28	45.48±4.47	44.07±5.49
71	45.60±5.86	45.18±5.21	46.15±4.95	44.38±4.76
Cumulative Body Weight Gain				
2	2.31±0.83	2.14±1.02	2.29±0.90	2.42±0.95
6	4.90±1.24	4.78±1.28	4.66±1.45	5.07±1.35
10	6.70±2.34	6.90±1.80	6.09±1.85	6.29±1.93
14	7.96±2.90	7.96±2.09	7.56±2.26	7.61±2.22
18	9.46±3.46	9.20±2.35	8.78±2.60	8.38±2.47*
22	9.55±3.74	9.81±2.77	9.04±2.71	8.33±2.72*
26	10.55±3.51	10.47±3.14	9.69±2.95	9.17±2.95*
42	12.06±4.10	12.00±3.54	10.94±3.40	10.09±3.33*
59	11.88±4.34	11.90±3.34	11.44±3.83	10.52±4.20
75	12.18±4.57	12.37±3.97	13.05±4.97	12.24±4.63

Data taken from pp. 79-95, MRID 44381401
*$p < 0.01$, Significantly different from control.

C. FOOD CONSUMPTION AND COMPOUND INTAKE

1. Food consumption

Food consumption was comparable in all groups, treated and controls. There were some higher values in the high-dose group during the first month of the study, that were considered to have resulted from excessive food spillage by individual animals and were not considered to be related to toxicity.

2. Compound consumption

The calculated average intakes of CGA 64250 were 12.1, 60.9, and 104.9 mg/kg/day for groups 2 (100 ppm), 3 (500 ppm), and 4 (850 ppm), respectively. When corrected for the actual amount of test substance as determined by chemical analysis, the calculated average intakes were 11.0, 59.0, and 107.6 mg/kg/day for the 100, 500, and 850 ppm groups, respectively.

3. Food consumption ratios

The food consumption ratios were similar for treated and control groups throughout the study, with the exception of higher values for group 4 (850 ppm) during weeks 1-4 which corresponds with confounding excessive food spillage as well.

D. BLOOD WORK

1. Hematology

Hematological analysis was not performed.

2. Clinical Chemistry

For mice treated at ≥500 ppm, there were treatment-related decreases in plasma cholesterol levels (11% to 25%) throughout the study which were significantly decreased (25%) in the 850 ppm group at weeks 9 and 14. Increased sorbitol dehydrogenase activities (45%) were seen in mice treated at 850 ppm for weeks 9 and 14 (Table 4). Other parameters remained comparable to controls.

Table 4: Clinical Chemistry values (from Table 8.13 of the submitted report)

Paramenter	0 ppm	100 ppm	500 ppm	850 ppm
Cholesterol				
Wk 9	2.901	3.029	2.571(-11%)	2.190*-(-25%)
14	2.787	2.623	2.396-(-14%)	2.127*-(-24%)
53	3.118	3.353	2.642	-2.053-
79	4.746	5.803	3.595	3.332
Sorbitol dehydrogenase				
Wk 9	26.49	27.55	24.97	41.04(55%)
14	24.78	30.54	25.42	35.84*+(45%)
53	33.86	24.60	25.28	34.35
79	39.58	38.00	27.76	38.19

* = Lepage, $p<0.01$; +- = Jonckheere, $p<0.01$

E. SACRIFICE AND PATHOLOGY

1. Organ weight

In treatment groups 3 and 4 (500 and 850 ppm) the mean liver weights and the mean liver to body-weight ratios were slightly to notably increased compared to controls at the interim as well as the final sacrifices. These values along with p-values are presented in Table 5. Other organ weights were comparable to controls. Mean carcass weight of group 4 was lower compared to controls at the 53 weeks sacrifice.

Table 5. Mean liver weight percent increase compared to the control values.

Time of Sacrifice		100 ppm	500 ppm	850 ppm
week 9	absolute liver weight relative liver weight	-6% 0.17%	11 % 10 %	32 %* 33 %*
week 53	absolute liver weight relative liver weight	-1.13% 1.62%	14 % 13 %*	11 %* 29 %*
week 79	absolute liver weight relative liver weight	0.23% 1.54%	14 % 10 %	19 %* 20 %*

Data taken from p. 47 MRID 44381401
* $p < 0.01$

2. Gross pathology

Selected macroscopic necropsy findings are summarized in Table 6. There were no dose-related increases of any macroscopic findings at either the week 9 or week 53 sacrifices. At the terminal sacrifice, there was a significantly higher incidence of enlarged livers observed in the 850 ppm group than in the other groups. There were also increased incidences (n.s.) of liver masses in the 100, 500, and 850 ppm groups as well as one animal with several nodules in the liver in the high-dose group. Other macroscopic findings were comparable in all groups and were similar to spontaneous findings characteristic of this strain of mice.

Table 6. Macroscopic necropsy findings in male mice fed CGA 64250 for up to 78 weeks.

Liver (terminal sacrifice)	Dietary concentration (ppm)			
	0	100	500	850
Enlarged	12/60	9/60	12/60	21/60*
Mass	1/60	3/60	3/60	4/60
Nodule	0/60	0/60	0/60	1/60
Total masses and nodules	1/60	3/60	3/60	5/60

Data taken from Table 1, pp. 48, MRID 44381401.
*$p < 0.05$, Significantly different from controls; Fisher's exact test by reviewer

3. Microscopic pathology

a. Non-neoplastic

At the week 9 scheduled sacrifice, there were treatment-related increases in mice with hepatocellular hypertrophy in the mid- and high-dose groups. The severity was generally more pronounced in the high-dose group. Enlargement of the centrilobular hepatocytes coincident with pale eosinophilic cytoplasm was associated with this effect. In the high-dose group, centrilobular hepatocytes also were observed to have increased incidences of small-sized fatty vacuoles in the cytoplasm, and there was a higher incidence of necrosis observed , 8/10 in the 850 ppm versus 2/10 in the control group. There was a concentration-effect relationship evident for the incidence of liver necrosis at ≥100 ppm at the week 9 sacrifice ($p \leq 0.007$), Cochran-Armitage test for trend, by reviewer. The high-dose group also had slightly increased numbers of mice with lymphohistiocytic infiltration of the liver.

At the 53 week sacrifice, an increase in the incidences of hepatocellular hypertrophy were also observed in the mid- and high-dose groups. Eosinophilic cytoplasm and large nuclei with multiple prominent nucleoli were found coincident with the enlarged hepatocytes and centrilobular distribution observed in these groups.

Selected incidences of microscopic findings at terminal sacrifice are presented in Table 7. The aforementioned effects (hepatocellular hypertrophy and associated observations) in addition to pigmentation of Kupffer cell, inflammatory cell infiltration, and change of cellular foci were seen in the mid- and high-dose animals with a dose-related increase in severity and occurrence (Cochran-Armitage test for trend $p < 0.05$, by reviewer). Hepatocellular hypertrophy was increased by 86 and 93 %, respectively, in the mid- and high-dose groups, $p < 0.01$. Other findings were considered incidental and not treatment-related.

Table 7. Microscopical findings in male mice fed CGA 64250 for up to 78 weeks.

Lesion or Abnormality in Liver at Terminal Sacrifice	Dietary concentration (ppm)			
	0	100	500	850
Hepatocellular Hypertrophy	15/50	18/50	28/50**	29/50**
Kupffer cell pigmentation	3/50	5/50	3/50	11/50*
Focus of cellular change	0/50	0/50	1/50	6/50*

Data taken from Table 2, p. 51, MRID 44381401
*p < 0.05, **p < 0.01, Significantly different from controls; Fisher's exact test by the reviewer.

b. Neoplastic

A summary of the neoplasms seen in this study is given in Table 8. There were significantly increased incidences of hepatocellular adenomas observed in the high-dose group compared to the control. Hepatocellular carcinomas, however showed no treatment-related increase. Other neoplastic observations, including hemangioma of the liver and infiltration of the liver by systemic malignant lymphoma and myeloid leukemia were not observed at incidences significantly greater than in controls.

Table 8. Neoplastic findings in male mice fed CGA 64250 for up to 78 weeks

Type of Tumor	Dietary concentration (ppm)			
	0	100	500	850
50 Males Examined				
Hepatocellular adenoma	1	0	3	10*
Hepatocellular carcinoma	1	3	2	2
Total hepatocellular tumors	2	3	5	12*

Data taken from Table 2, p. 51, MRID 44381401
* $p < 0.05$, significantly different from the control, Fisher's exact test by the reviewer.

c. Historical control Data: Adequate historical control data was not submitted. The data that was submitted was data from 4 studies from Charles River, France (1993) that indicated that hepatocellular neoplasia in 50 animals/group was 22.0%, 22.4%, 14.0% and 6.0%. Hepatocellular adenoma was 14.0%, 18.4%, 6.0% and 6.0%, respectively. Hepatocellular carcinoma was 12.0%, 6.1%, 12.0% and 0.0%, respectively. The current study shows 2.0% adenoma and 2.0% carcinoma with 4.0% combined, which is low by comparison to the 1993 data. However, the current study was conducted between 1994 and 1996 and the historical data submitted was gathered for an unspecified time period before 1993. In addition to the time period being inadequately specified, the historical control data was not gathered on animals subjected to the environment in testing facility.

Other historical control data from Charles River, USA 1995 showed 10.8% (range 0% to 19.2% hepatocellular adenomas and 4.9% (range 1.3% to 11.55) hepatocellular carcinomas in 12 studies with 770 CD-1 mice. These data are also inadequate for comparison.

III. DISCUSSION

A. INVESTIGATOR'S CONCLUSION

The investigators concluded that the administration of CGA 64250 to male mice for 18 months at concentrations of 0, 100, 500, or 850 ppm resulted in several detrimental effects. These effects included: decreased body weight gains at ≥ 500 ppm, lowered plasma cholesterol levels at ≥ 500 ppm, increased sorbitol dehydrogenase activities at 850 ppm, increased liver weights at ≥ 500 ppm, as well as enlarged livers and increased incidences of masses and nodules at 850 ppm (terminal sacrifice). The liver was identified as the target organ where toxic effects were manifested as hepatocellular hypertrophy (≥ 500 ppm), fatty change at 850 ppm (week 9 only), liver necrosis at 850 ppm (week 9 only), lymphohistiocytic infiltration of the liver at 850 ppm (week 9 only), foci of cellular change at 850 ppm, Kupffer cell pigmentation at 850 ppm (terminal sacrifice), and a higher number of hepatocellular adenomas at 850 ppm (terminal sacrifice).

B. REVIEWER'S DISCUSSION

CGA 64250 was administered in the diet to 320 male albino mice at concentrations of 0, 100, 500, or 850 ppm for up to 18 months. These concentrations resulted in a nominal compound intake for each concentration level of 12.1, 60.9, and 104.9 mg/kg/day for the 100, 500, and 850 ppm groups, respectively. When these values were corrected for the actual amount of test article as determined by chemical analysis, the calculated average intakes were 11.0, 59.0, and 108.0 mg/kg/day for males in the 100, 500, and 850 ppm groups, respectively.

There were no signs of overt toxicity resulting from compound administration and the survival of the treated groups did not vary significantly compared to the controls. Body weight was depressed in the mid- and high-dose groups over a major portion of the study (weeks 18-50). These body weight decreases corresponded with body weight gain decrements in these groups as well during the same period of time. The reviewer agrees with the concept, as expressed by the author, that liver weight increases may have masked this effect during the first three months of the study. Body weight gains were depressed up to 10 % in the 500 ppm group and up to 19 % in the 850 ppm group for weeks 18-50. After week 50, the treated groups seemed to recover and remained comparable to controls in body weight and weight gain throughout the remainder of the study. Food consumption and food consumption ratios were unaffected by the administration of the test substance as they both remained comparable to the control group throughout the study.

Neither hematological analysis nor ophthalmoscopic examinations were performed in this study. As this study is ancillary to that original study the reviewer does not question the absence of data for the current study, if indeed it was presented in the original study and/or there was an agreement made with the EPA based on the previous studies (MRID 44381401 text p. 18).

There were significant clinical chemistries variations reported in this study including: treatment-related decreases in plasma cholesterol levels ($\geq$500 ppm), and increased sorbitol dehydrogenase levels in mice treated at 850 ppm at the weeks 9 and 14 analysis. These effects concur with the concept that the liver is the target organ for toxicity.

The liver was identified as the target organ of the test substance based on macroscopical and microscopic observations as well as increased liver weights. The mean liver weights and mean liver to body weight ratios of mice treated with 500 and 850 ($p<0.01$) ppm groups were increased at the interim and terminal sacrifices. Macroscopic observations revealed enlarged livers and an increase in the incidences of masses and nodules found in mice treated with 850 ppm CGA 64250 at the terminal sacrifice. Microscopic observations revealed several treatment-related effects in the livers of mice fed 500 and/or 850 ppm CGA 64250. These effects were detected and identified as fatty change, hepatocellular hypertrophy ($\geq$500 ppm), necrosis of the liver, and increased incidence of lymphohistiocytic infiltration of the liver compared to controls at the week 9 sacrifice in the 850 ppm group. At the 53 weeks sacrifice, mice in the 500 and 850 ppm groups had

increased incidences of hepatocellular hypertrophy compared to the controls. The animals at the terminal sacrifice presented with increased incidence of hepatocellular hypertrophy (500 and 850 ppm), deposition of pigment in the Kupffer cells (850 ppm), increased incidence of foci of cellular change and hepatocellular adenomas (850 ppm) compared to controls.

The percentage adenomas and carcinomas were within the range of the inadequate historical control data submitted with the study report. In the 850 ppm dose group, the total incidence of hepatocellular neoplasia was slightly higher (24%) than the upper limit of the historical control range of 22.4% submitted and the concurrent control (4%) was lower than of the lower range of historical controls of 6.0% submitted. The historical controls submitted were inadequate because the collection dates were not specified and were not collected in the testing facility.

The lowest-observed-adverse-effect-level (LOAEL) in this study was 500 ppm (59.0 mg/kg/day for males) based on body weight effects and liver toxicity. A no-observed-adverse-effect-level (NOAEL) of 100 ppm (11 mg/kg/day for males) was determined.

Treatment of male CrI: CD-1^{r}(ICR) BR mice for up to 79 weeks resulted in a significant increase in the incidence of hepatocellular adenomas in males at 850 ppm compared to the control group ($p < 0.01$ Cochran-Armitage test; $p < 0.05$, Fisher's exact test). The incidence of other tumors was comparable to controls.

C. STUDY DEFICIENCIES

The lack of hematological data and ophthalmoscopic examinations should have been justified by citation of the lack of an experimental effect on these parameters from the original 2-year study and/or an explicit agreement with the EPA that analysis of these parameters was not required. This is not sufficient to invalidate the study provided that this data does exist and is negative in the original 2-year combined study in mice. The homogeneity analysis revealed actual concentrations that were 75-81% of nominal, this is lower than the normally accepted 15 % window, but is not sufficient to invalidate the study based on the dose separation. There was a serious problem with the presentation of data in the results section of the document, results were not presented clearly with summary statistics, associated p-values, and statistical tests. This made interpretation of the data a difficult task.