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

MEMORANDUM

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

SUBJECT: Cancer Assessment Review Committee Meeting on Action

FROM: Jess Rowland for
Executive Secretary
Cancer Assessment Review Committee
Health Effects Division (7509C)

TO: Addressees

Attached for your review is a package on Action prepared by Alan Levy.

A meeting to review the carcinogenicity classification of this chemical is scheduled for Wednesday October 7,1998 at 10:00 am in Room 817, CM2.

Addressees

K. Baetcke
L. Brennecke
L. Brunsman
W. Burnam
M. Copley
K. Dearfield
V. Dellarco
V. Dobozy
R. Hill
P. Hurley
Y. Ioannou
N. McCarroll
E. Rinde
J. Rowland
J. Stewart
L. Taylor
Y. Woo

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"A C T I O N"

(FLUTHIACET-METHYL; CGA-248757)

CHEMICAL NO.: 108803

CANCER PEER REVIEW PACKAGE

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TOXICOLOGIST: ALAN C. LEVY

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MEMORANDUM

SUBJECT: FLUTHIACET-METHYL (CGA-248757; ACTION™) - Carcinogenicity Peer Review

FROM: Alan C. Levy, Ph.D., Toxicologist
Registration Action Branch 2
Health Effects Division (7509C)

and

TO: Joanne Miller/Eugene Wilson, PM 23
Registration Division (7505C)

THROUGH:

The Health Effects Division (HED) Carcinogenicity Peer Review Committee (CPRC) met on October 7, 1998 to discuss and evaluate the weight-of-the-evidence on ACTION with particular reference to it's carcinogenic potential. The CPRC concluded

The decision to classify ACTION as a was based on

THE SUMMARY OF THE CPRC'S FINDINGS PLUS THE "INDIVIDUALS IN ATTENDANCE" PAGE WILL BE ADDED AFTER THE MEETING.

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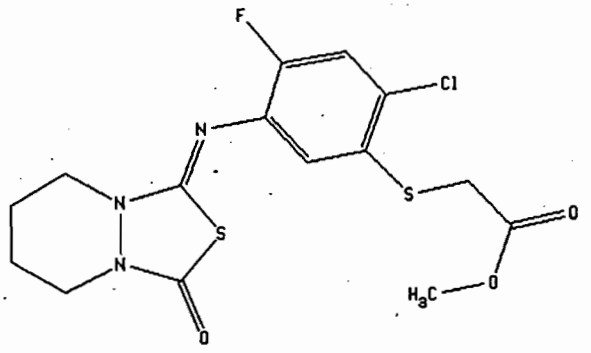
B. MATERIALS REVIEWED:

The material available for review consisted of Data Evaluation Records (DERs) and other data summaries prepared by Alan C. Levy as well as statistical analyses prepared by Lori Brunsman. The material reviewed is attached to the file copy of this memorandum. Studies were submitted by Ciba-Geigy Corporation.

C. BACKGROUND INFORMATION

Fluthiacet-Methyl (CGA-248757; ACTION™; KIH-9201) is a new photobleaching imide herbicide. The active ingredient will be formulated as a 5% (approximately) wettable powder packaged in water soluble bags (developed to minimize exposure to persons handling the chemical). The wettable powder material may be applied as a postemergence herbicide for control of velvelt leaf and other broadleaf weeds. The maximum use rate per season is 0.0089 lb ai/acre (equal to about 4.0 g/acre).

The material is as follows: [2-chloro-4-fluoro-5-[(tetrahydro-3-oxo-1H,3H-[1,3,4-thiadiazol[3,4a]pyridazin-1-ylidene)amino]phenyl]thio]acetic acid methyl ester. The PC Code is 108803. The Registrant is requesting tolerances on _____ . The structure is provided below:



CGA-248757

(2)

D. EVALUATION OF THE CARCINOGENIC POTENTIAL

1. Chronic Toxicity/Carcinogenicity Study in Rats:

Citation: Potrepka, R. and A. Richter (1995) Two-year dietary chronic toxicity/oncogenicity study with CGA-248757 Technical in rats. Ciba Crop Protection, Ciba-Geigy Corporation, Environmental Health Center, Farmington, CT. Laboratory Study No. F-00068, September 11, 1995, MRID No. 43830017. Unpublished.

a. Experimental Design

The test article (CGA-248757, 97.7% a.i.) was administered in the diet to 50 Harlan Hsd:Sprague Dawley SD rats/sex/group at concentrations of 0, 5, 50, 3,000, 5,000 (males) or 7,000 (females) ppm (mg/kg/day: males = 0, 0.2, 2.1, 130 or 219 and females = 0, 0.2, 2.5, 154 or 368) for 104 weeks. An additional 20/sex were placed in the control and high-dose groups as well as 10/sex in each of the 5, 50 and 3,000 ppm groups for a 52-week interim sacrifice.

b. Discussion of Tumor Data

There was a trend toward an increased incidence of pancreatic exocrine adenomas and pancreatic islet cell adenomas in males at 3,000 (8 and 7%, respectively) and 5,000 ppm (10 and 12%, respectively) compared with concurrent controls (1%). No evidence of carcinogenicity was reported for females.

Survival Analyses: Males showed a significant decreasing trend for mortality with increasing doses of ACTION. No significant incremental changes were noted in female rats.

Table 1

RATS: MALE MORTALITY RATES AND COX OR GENERALIZED K/W TEST RESULTS

Dose:ppm	Weeks					
	1-26	27-52	53(I)	53-78	79-106(f)	Total
0	0/70	2/70	19/68	6/48(a)	26/42	34/50(68%) **(n)
5	1/60	2/59	9/57	4/48	23/44	30/51(59%)
50	2/60	2/58	9/56	8/47	22/39	34/51(67%)
3000	1/60	1/59	10/58	5/48	13/43	20/50(40%)* (n)
5000	0/70	2/70	19.68	1/49	13/48	16/51(31%) **(n)

Mortality rates: number of animals that died during interval/number of animals alive at beginning of the interval
a:one accidental death at week 70, dose 0 ppm
I: interim sacrifice at week 53
f: final sacrifice at week 105
n: negative trend or negative change from control
NOTE: Time intervals were selected for display purposes only.
Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
Significance: * = $p< 05$; ** = $p<0.01$

Table 2

RATS: FEMALE MORTALITY RATES AND COX OR GENERALIZED K/W TEST RESULTS

Dose:ppm	Weeks					
	1-26	27-52	53(I)	53-78	79-107(f)	Total
0	1/70	1/69	20/68	2/48	16/46	20/50(40%)
5	0/60	1/60	10/59	5/49	13/44	19/50(38%)
50	0/60	2/60	9/58	7/49	16/42	25/51(49%)
3000	1/60	1/59	10/58	7/48	14/41	23/50(46%)
7000	3/70	1/67	19/66	7/47	15/40	26/51(51%)

Mortality rates: number of animals that died during interval/number of animals alive at beginning of the interval
I: interim sacrifice at week 53
f: final sacrifice at week 106
NOTES: Time intervals were selected for display purposes only.
Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
Significance: 8 = $p<0.05$; ** = $p<0.01$

Tumor Analyses

Male rats had significant increasing trends for pancreatic exocrine adenomas at $p<0.01$ and for pancreatic islet cell adenomas and adenomas and/or carcinomas combined, both at $p<0.05$. There was a significant difference in the pair-wise comparison of the 5,000 ppm dose group with the controls for pancreatic islet cell adenomas at $p<0.05$. Females had no significant test article-related tumors.

The statistical analyses of the male rats were based upon Peto's prevalence test since there were statistically significant negative trends for mortality with increasing doses of ACTION.

Table 3

RATS: MALE PANCREAS TUMOR RATES AND PETO'S PREVALENCE TEST RESULTS

TUMORS	Dose (ppm)				
	0	5	50	3000	5000
Exocrine Adenomas (%) p =	1/28 (4) 0.007**	2/36 (6) 0.349	1/31 (3) -	5/37(a) (14) 0.053	7/44 (16) 0.107
Islet Cell Adenomas (%) p =	1/43 (2) 0.017*	3/46(b) (7) 0.184	2/43 (5) 0.301	4/45 (9) 0.105	8/47 (17) 0.024*
Islet Cell Carcinomas (%) p =	1/16 (6) 0.686	0/21 (0) -	0/17 (0) -	1/30(c) (3) 0.676	0/35 (0) -
Islet Cell Tumors Combined (%) p =	2/43 (5) 0.031*	3/46 (7) 0.385	2/43 (5) -	5/45 (11) 0.202	8/47 (17) 0.081

Number of tumor bearing animals/number of animals examined, excluding those that died before observation of the first tumor.

a: First exocrine adenoma observed at week 93, dose 3000 ppm.

b: First islet cell adenoma observed at week 71, dose 5 ppm.

c: First islet cell carcinoma observed at week 106, dose 3000 ppm.

NOTE: Significance of trend denoted at control.

Significance of pair-wise comparison with control denoted at dose level.

Significance: * = $p<0.05$; ** = $p<0.01$

Historical Control Data: rat pancreatic tumors

Table 4

HISTORICAL CONTROL PANCREATIC TUMOR DATA FOR HSD:SPRAGUE DAWLEY MALE RATS - STUDIES CONDUCTED BY CIBA-GEIGY CORPORATION

In-Life End Date	4/93 a	4/94 b	10/94 c
Reference	A	B	C
Adenocarcinoma, Exocrine	1/58 (2%)	0/69 (0%)	0/58 (0%)
Adenocarcinoma, Islet Cell or Carcinoma, Islet Cell	3/58 (5%)	1/69 (1%)	2/58 (3%)
Adenoma, Exocrine	0/58 (0%)	1/69 (1%)	2/58 (3%)
Adenoma, Islet Cell	2/58 (3%)	1/69 (1%)	2/58 (3%)

a = The quality assurance review has been completed.
b = Study F-00068 (the current rat study with CGA-248757; MRID No. 43830017)
c = This report has not been issued in draft form and quality assurance has not been completed.
Data extracted from Report Table 9.35, page 423.

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c. Non-neoplastic Lesions

Histopathological changes were as noted. There were liver findings (bile duct hyperplasia, inflammation and Kupffer cell pigmentation) at 3,000 and 5,000 ppm in males as well as in females at 3,000 and 7,000 ppm (3,000<7,000). Both sexes showed signs of microcytic anemia at ≥3,000 ppm (more pronounced in males). In ≥3,000 ppm males only, effects on pancreatic acinar cells (atrophy/hyperplasia), lymph nodes, fat and inflammation were noted. Females at ≥3,000 ppm showed an increased incidence of abnormal uterine contents as well as necrohemorrhagic inflammation (7,000 ppm only).

Table 5

SELECTED NON-NEOPLASTIC MICROSCOPIC LESIONS IN A CHRONIC/CARCINOGENICITY STUDY IN RATS WITH CGA-248757

Observation ppm =	0	5	50	3000	5000/7000
Males					
LIVER No. Tissues =	70	60	60	60	70
Fatty change	3	4	2	12**	9
Focus, eosinophilic cell	2	1	1	15**	19**
Bile duct hyperplasia	36	39	43	57**	70**
Peribiliary inflammation	34	26	11**	44**	62**
Kupffer cell pigmentation	12	8	10	47**	63**
PANCREAS No. Tissues =	69	59	60	60	69
Acinar cell atrophy	21	19	30	34*	40*
Acinar cell hyperplasia	3	7	8	16**	16**
Fatty change	9	14	13	31**	37**
Inflammation	22	23	27	34*	38**
PANCREATIC LYMPH NODES No. Tissues =	70	38	41	38	70
Reactive hyperplasia	1	0	1	5*	8*
Pigmentation	1	0	1	11**	11**
Females					
LIVER No. Tissues =	70	60	60	60	70
Centrilobular fatty change	1	0	1	1	6*
Congestion	9	9	7	19**	13
Bile duct hyperplasia	40	43	34	53**	61**
Inflammation	20	35**	28	48**	38**
Leukocytosis	8	7	7	10	26**
Kupffer cell pigmentation	26	18	20	34	56**
Intracytoplasmic pigmentation	3	4	1	10	13**
UTERUS No. Tissues =	70	60	60	56	70
Hemorrhage	0	0	0	3*	1
Inflammation	2	3	3	8*	6
Necrohemorrhagic inflammation	5	3	4	7	23**

Statistical Significance: * = $p<0.05$; ** = $p<0.01$

d. Toxicological Effects

No treatment-related effects occurred in males or females at 5 or 50 ppm. Regarding survival, there was a positive trend in males (30, 42, 34, 61 and 69% at 0, 5, 50, 3,000 and 5,000 ppm) and a negative trend in females (61, 62, 51, 54 and 50% at 0, 5, 50, 3,000 and 7,000 ppm). At 3,000 and 5,000 ppm, males showed statistically significant decreases in body weights (up to 15 and 18%, respectively), food consumption during weeks 3-63 (6-12% at 3,000 and 6-14% at 5,000 ppm) and feed efficiency by as much as 48% during weeks 2-37 at 3,000 ppm as well as 66% for 5,000 ppm (weeks 2-37). There were no body weight/food consumption/feed efficiency changes for females. There was hepatocellular damage as evidenced by a statistically significant increase in one or more liver-related enzymes in males and females throughout the study (determinations at weeks 26, 52, 78 and 104) at 3,000 and 5,000/7,000 ppm (no elevation in females at 104 weeks). Statistically significant increases in the incidences of urinary bilirubin and urobilinogen were noted primarily in males (lesser extent in females) at ≥3,000 ppm. At ≥3,000 ppm, males exhibited discoloration of the kidneys, pancreatic masses and enlarged/discolored pancreatic lymph nodes.

e. Adequacy of Dosing for Assessment of Carcinogenic Potential

The highest dose tested (5,000 ppm in males and 7,000 ppm in females) produced **toxicological** (≥15% decreases in body weights/gains in males, 6-14% reduction of food consumption in males; increase in liver enzymes in both sexes; microcytic anemia in both sexes; increases in urinary bilirubin and urobilinogen primarily in males, but to a lesser extent in females; discoloration of the kidneys, pancreatic masses and enlarged/discolored pancreatic lymph nodes in males; and abnormal uterine contents in females)and **histopathological** (non-neoplastic changes as evidenced by liver bile duct hyperplasia, inflammation and Kupffer cell pigmentation; microcytic anemia; effects on pancreatic acinar cells, lymph nodes, fat and inflammation in males only; and necrohemorrhagic inflammation of the uteri). Therefore, it is concluded that the dose levels employed in this study were adequate to assess the chronic toxicity and the carcinogenicity potential of ACTION in rats. **NOEL = 50 ppm (2.1 and 2.5 mg/kg/day for males and females, respectively). LOEL = 3,000 ppm (130 and 154 mg/kg/day for males and females, respectively) based on: males = decreased body weights, liver toxicity, pancreatic toxicity and microcytic anemia; females = liver toxicity, uterine toxicity and slight microcytic anemia.**

2. Carcinogenicity Study in Mice

Citation: Chang, J.C.F. and Morrissey, R.L., (1995) CGA-248757 - Final Report - 18-Month Dietary Oncogenicity Study in Mice. Ciba-Geigy Corporation, Crop Protection Division,

Environmental Health Center, Farmington, CT. Report No. F-00069, June 26, 1995. MRID No. 43830015. Unpublished.

a. Experimental Design

The test article (CGA-248757, 97.7% a.i.) was administered in the diet to Charles River Crl:CD-1 (ICR)BR (Swiss) mice (50/sex/group) at doses of 0, 1, 10, 100 or 300 ppm (mg/kg body weight/day: males = 0, 0.1, 1.0, 10 or 32; females = 0, 0.1, 1.2, 12 or 37) for 78 weeks.

b. Discussion of Tumor Data

There was a statistically significant increase only in males for the following: carcinomas at 100 and 300 ppm in addition to adenomas/carcinomas at 300 ppm. However, regarding adenomas only, the number of males with this finding were (0, 1, 10, 100 and 300 ppm): 12, 9, 10, 19 and 22. Though not statistically significant, in females, there were greater incidences in the 100 and 300 ppm groups (compared with controls) for adenomas (2, 0, 1, 7 and 7) and adenomas/carcinomas (3, 0, 2, 9 and 8). Tumor incidences were greater than those presented in the historical control data.

Tumor analyses

Male mice had significant increasing trends for liver adenomas, carcinomas, and adenomas and/or carcinomas combined, as well as significant differences in the pair-wise comparisons of the 300 ppm dose group with the controls for liver carcinomas and adenomas and/or carcinomas combined, all at $p<0.01$. There were also significant differences in the pair-wise comparisons of the 300 ppm dose group with the controls for liver adenomas, and the 100 ppm dose group with the controls for liver carcinomas and adenomas and/or carcinomas combined, all at $p<0.05$.

Female mice had significant increasing trends for liver adenomas, and adenomas and/or carcinomas combined, both at $p<0.01$. There were no significant differences in the pair-wise comparisons of the dosed groups with the controls.

The statistical analyses of the male and female mice were based upon the Exact trend test and the Fisher's Exact test for pair-wise comparisons.

Table 6

MICE: MALE LIVER TUMOR RATES AND EXACT TREND TEST AND FISHER'S EXACT TEST RESULTS (P VALUES)

Tumors	Dose (ppm)				
	0	1	10	100	300
Adenomas (%) p =	12/49 (24) 0.001**	9/49 (18) 0.312(n)	10/47 (21) 0.448(n)	19/49 (39) 0.096	22/48(a) (46) 0.023*
Carcinomas (%) p =	3/49 (6) 0.002**	5/49 (10) 0.357(n)	6/47 (13) 0.223(n)	12/49(b) (24) 0.011*	13/48 (27) 0.005**
Combined (%) p =	15/49 (31) 0.000**	13/49© (27) 0.412(n)	14/47(d) (30) 0.554(n)	26/49(e) (53) 0.020*	31/48(f) (65) 0.001**

Number of tumor bearing animals/number of animals examined, excluding those that died or were sacrificed before week 53.

a: First adenoma observed at week 60, dose 300 ppm.

b: First carcinoma observed at week 59, dose 100 ppm.

c: One animal in the 1 ppm group had both an adenoma and a carcinoma.

d:Two animals in the 10 ppm group had both an adenoma and a carcinoma.

e: Five animals in the 100 ppm group had both an adenoma and a carcinoma.

f: Four animals in the 300 ppm group had both an adenoma and a carcinoma.

n: Negative change from control.

NOTE: Significance of trend denoted at control.

Significance of pair-wise comparison with control at dose level.

Significance: * = $p<0.05$; ** = $p<0.01$

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Table 7

MICE: FEMALE LIVER TUMOR RATES AND EXACT TREND TEST AND FISHER'S EXACT TEST RESULTS (P VALUES)

TUMORS	Dose (ppm)				
	0	1	10	100	300
Adenomas (%) p =	2/48 (4) 0.003**	0/46 (0) 0.258(n)	1/49 (2) 0.492(n)	7/47 (15) 0.074	7/49(a) (14) 0.084
Carcinomas (%) p =	1/48 (2) 0.145	0/46 (0) 0.511(n)	1/49(b) (2) 0.747	2/47 (4) 0.492	2/49 (4) 0.508
Combined (%) p =	3/48 (6) 0.004**	0/46 (0) 0.129(n)	2/49 (4) 0.490(n)	9/47 (19) 0.055	8/49© (16) 0.106

Number of tumor bearing animals/number of animals examined, excluding those that died or were sacrificed before week 53.

a: First adenoma observed at week 78, dose 300 ppm.

b: First carcinoma observed at week 81, dose 10 ppm.

c: One animal in the 300 ppm dose group had both an adenoma and a carcinoma.

n: negative change from control.

NOTE: Significance of trend denoted at control.

Significance of pair-wise comparison with control at dose level.

significance: * = $p<0.05$; ** = $p<0.01$

Historical Control Data: mouse liver tumors

Table 8

HISTORICAL CONTROL LIVER TUMOR DATA FOR CHARLES RIVER CD-1 MICE - STUDIES CONDUCTED BY CIBA-GEIGY CORPORATION

STUDY	FINDING	MALE			FEMALE		
		Incidence	Combined	Percent	Incidence	Combined	Percent
A	Adenoma Carcinoma	5 1	6/55	10.9	1 0	1/60	1.7
B	Adenoma Carcinoma	5 0	5/59	8.5	2 0	2/60	3.3
C	Adenoma Carcinoma	4 1	5/57	8.8	0 0	0/60	0
D	Adenoma Carcinoma	5 3	8/50	16.0	1 0	1/51	2.0
E	Adenoma Carcinoma	14 1	15/47	31.9	1 0	1/50	2.0
F	Adenoma Carcinoma	8 5	13/59	22.0	0 0	0/60	0
G	Adenoma Carcinoma	7 0	7/60	11.7	0 0	0/60	0
H	Adenoma Carcinoma	4 2	5/60	8.3	0 0	0/60	0
I	Adenoma Carcinoma	8 0	8/50	16.0	0 0	0/50	0
Charles River	Adenoma Carcinoma	- -	- -	0-16.3 0-6.0	- -	- -	0-2.7 0-2.7

NOTE: All studies of 78 weeks duration.
Data extracted from Report Appendix 10.13.7, pages 1511 and 1512.

c. Non-neoplastic Lesions

Histopathological changes involved only the liver and were as follows: centrilobular necrosis, centrilobular cell degeneration, histiocytic pigmentation and karyomegaly ≥ 10 ppm in males and/or females in addition to bile duct hyperplasia at 300 ppm, plus focal basophilic cells ≥ 100 ppm in males and centrilobular fatty change at 300 in females.

d. Toxicological Effects

At 300 ppm, males only had a slight decrease in body weight gain during the latter portion of the study. Mean corpuscular volume and mean corpuscular hemoglobin were decreased in males ≥100 ppm. In females at 300 ppm, there were decreases in erythrocyte counts, hemoglobin and hematocrit. Absolute and relative liver weights were greater (not always statistically significant) at 100 and 300 ppm in both sexes.

e. Adequacy of Dosing for Assessment of Carcinogenic Potential

The highest dose tested (300 ppm) produced **toxicological** (slight decrease in body weight gain in males during the latter part of the study, -17% for the duration of the study; decreases in erythropoietic parameters in both sexes; absolute and relative weights were greater [not always statistically significant] in both sexes) and **histopathological** (non-neoplastic liver changes as evidenced by centrilobular necrosis, centrilobular cell degeneration, histiocytic pigmentation and karyomegaly in males and/or females; bile duct hyperplasia and focal basophilic cells in males; and centrilobular fatty change in females). Therefore, it is concluded that the dose levels employed in this study were adequate to assess the carcinogenicity in males and possibly females. **NOEL = 1 ppm (0.1 and 0.1 mg/kg/day, males and females, respectively). LOEL = 10 ppm (1.0 and 1.2 mg/kg/day, males and females, respectively) based on non-neoplastic liver findings.**

E. ADDITIONAL TOXICOLOGY DATA ON ACTION

1. Metabolism

Reference: Absorption, Distribution and Metabolism Studies in Rats with ACTION. MRID Nos. 43830018-43830020; HED Document No. 012079.

In a preliminary metabolism study (MRID No. 43830019), four groups of 2 male and 2 female rats were administered single oral doses of pyridazine 14C-CGA-248757 suspended in corn oil at nominal dose levels of either 1.0 mg/kg or 200 mg/kg. This study was designed to provide preliminary data on the elimination of orally administered pyridazine 14C-CGA-248757.

In the definitive disposition study (Appendix 2 of MRID No. 43830020), groups of 5 male and 5 female rats/dose received single oral doses of 1.0 or 200.0 mg/kg pyridazine 14C-CGA-248757 in corn oil, or repeated doses of unlabeled CGA-248757 at 1.0 mg/kg followed by a single radiolabelled dose. Excreta were collected for 7 days post-dose to determine the disposition of the test material. In a single dose biliary excretion study (MRID No. 43830018), four male and 4 female rats were surgically cannulated for bile collection, and were then administered a single oral dose of pyridazine 14C-CGA-248757 at 0.8 mg/kg. This study was designed to assess the extent of absorption and route of elimination of radioactivity after a single oral dose to bile-cannulated rats. In a metabolite identification study (MRID No. 43830020), characterization and identification of metabolites were performed on pooled (0-72 hr) samples of urine and feces containing 90% or greater of the excreted radiolabel.

Absorption at both the low and high dose was rapid for both male and female rats as inferred from excretion time course data. Repeated oral dosing had no effect on extent of absorption. Tissue levels of 14C-CGA-248757 derived radioactivity in the single and repeated low-dose groups did not exceed 0.018 ppm for any tissue. At the single high dose, female rats showed higher levels of 14C-CGA-248757 derived radioactivity in tissues than males except for muscle, brain, fat, and plasma. Excretion in males was predominantly in feces for all dose groups, with between 67-87% of administered radioactivity excreted by this route. In females, the percentage of administered radioactivity in urine across all dose groups (40-48%) was approximately equivalent to the percent excreted in feces (39-52%). The greater fecal excretion in males was based on a greater percentage excretion in bile for males (37%) vs. females (19%). Recovery of radioactivity was quantitative for all dose groups.

The major urinary metabolite observed across all dose groups was CGA-330403, identified as the free acid equivalent of the re-arranged isomer of the parent chemical. It was also observed as the major metabolite in feces, with the exception of low-dose males, where CGA-330063/330064 (identified as CGA-330403 hydroxylated in either the 6 or 7 position of the pyridazine ring) was the major product. CGA-330403 was also observed as the major metabolite in bile of female rats; whereas, CGA-330063/330064 was identified as the major product in bile of males. As CGA-330063/330064 is a hydroxylated product of CGA-330403, the data indicate a faster rate of conversion of CGA-330403 in males vs. females. A scheme for biotransformation of CGA-248757 was proposed based on the available data.

2. Mutagenicity

Reference: Mutagenicity Studies with ACTION
MRID Nos. 43348428 - 43348436

Sufficient studies are available to satisfy the data requirements for mutagenicity. Results from the nine acceptable studies indicate that CGA-248757 is not mutagenic in bacterial or cultured mammalian cells and did not cause DNA damage in bacterial or primary rat hepatocytes. There is, however, independent confirmation from in vitro cytogenetic assays performed with two different mammalian cell lines demonstrating that CGA-248757 is clastogenic both in the presence and absence of S9 activation. Although the test substance is negative for micronuclei induction in mouse bone marrow, a significant increase in micronuclei is seen in stimulated rat liver cells following in vivo exposure. Overall, these findings indicate that CGA-248757 has genotoxic potential which is expressed in vitro and confirmed in vivo. Thus, the mutagenicity results support the evidence of carcinogenic effect in the 18-month carcinogenicity study in mice (MRID No. 43830015) and a possibly carcinogenic response in the two-year chronic toxicity/carcinogenicity study in rats (MRID No. 43830017).

3. Subchronic and Chronic Toxicity

a. 28-Day Oral - Rat (MRID No. none)

This 28-day oral rat study ("4-week unaudited rangefinding study, Study No. F-00064") was referenced in a 90-day dietary admix rat study (MRID No. 43348423) and pertained to the "dose selection" for the 90-day study.

The results were as follows: MALES = at 7,000 and 20,000 ppm, body weights were 87 and 84% of control (fasted weights at necropsy); body weight gains were decreased by 25 and 30%; the NOEL was 500 ppm; FEMALES = at 7,000 and 20,000 ppm, fasted necropsy body weights were 107 and 92% of control; at week 4, body weight gains were +26 and -11%; the NOEL was 500 ppm (based on clinical chemistry, hematology and liver weight changes)

b. 90-Day Oral - Rat (MRID No. 43348423)

In a subchronic toxicity study, CGA-248757 Technical (97.1% purity) was administered by dietary admix to Sprague-Dawley rats (10/sex/group) at doses of 0, 10, 100, 3,500, 7,000 and 20,000 ppm (mg/kg/day: males = 0, 0.60, 6.19, 216, 427 and 1,224; females = 0, 0.69, 6.80, 249, 490 and 1,424) for 90 days.

Body weights and weight gains as well as food consumption were below control values in males only essentially at 3,500, 7,000 and 20,000 ppm. There were decreases in hemoglobin, hematocrit, MCV and MCH as well as decreases in total protein and albumin plus increases in alkaline phosphatase and 5'-nucleotidase primarily in males at 3,500 ppm and above. Urine color was darker and bilirubin plus urobilinogen were in greater quantity mostly in males at 3,500 and above. Absolute and relative (to-body weight) liver weights were above controls in females at 7,000 and 20,000 ppm. Histopathologically, liver centrilobular inflammation, iron pigmentation and fatty change were more severe in males and/or females at 3,500 ppm and above. **NOEL = 100 ppm (mg/kg/day = 6.19 for males and 6.80 for females). LOEL = 3500 ppm (mg/kg/day = 216 for males and 249 for females) based on decreased body weight gains as well as effects on hematology, clinical chemistry, urinalysis parameters, liver weights and microscopic pathology.**

c. <u>90-Day Subchronic Toxicity Study - Mouse</u> (MRID No. 43357804)

In a subchronic toxicity study (MRID No. 43357804), CGA-248757 (97.1% purity) was administered by dietary admix to Charles River Crl:CD-1 (ICR)Br (Swiss) mice (10/sex/group) at doses of 0, 1, 10, 500 and 5,000 ppm (mg/kg body weight/day: males = 0, 0.13, 1.3, 66 and 655; females = 0, 0.17, 1.6, 83 and 782) for 90 days.

The following treatment-related effects were noted usually in both sexes at 500 and 5,000 ppm (slightly more severe in males than in females): erythropoietic system(decreases in hemoglobin, hematocrit, mean corpuscular volume and mean corpuscular hemoglobin; elevation of platelet counts; decreases in body marrow smear myeloid:erythroid [M:E] ratio and Erythrocyte Maturation Index; increases in granulopoiesis in bone marrow; and extramedullary hematopoiesis as well as hemosiderin pigmentation in the spleen [5,000 ppm only]); liver (increased absolute weights and relative-to-body weight as well as to-brain weight ratios; increased levels of sorbitol dehydrogenase, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase [5,000 ppm only], 5'-nucleotidase, bile acids; and microscopic fatty changes, active chronic inflammation, karyomegaly, single cell necrosis and ceroid/lipofuscin pigmentation). **NOEL = 10 ppm (mg/kg/day: males = 1.3 and females = 1.6). LOEL = 500 ppm (mg/kg/day: males = 66 and females = 83) based on effects on the erythropoietic system and the liver.**

d. <u>6-Week Subchronic Oral Toxicity Study - Dog</u> (MRID No. 43348427)

In a subchronic toxicity study (MRID No. 43348427), CGA-248757 (97.1% purity) was administered by dietary admix to beagle dogs (3/sex/group, 2/sex at 50,000 ppm) at doses of 0, 500, 2,000, 6,500, 20,000 and 50,000 ppm (mg/kg/day: males = 0, 18.1, 75.1, 236, 709 and

1,943; females = 0, 19.6, 77.7, 232, 766 and 2,126) for 6 or 8 weeks (females at 50,000 ppm, 4 weeks).

The only definitive test article effect was a decrease in body weight gain in males at ≥20,000 ppm and in females at ≥6,500 ppm. There were suggestive effects on the erythropoietic system in males and females at 50,000 ppm and on absolute as well as relative liver weights in males at ≥20,000 ppm. No effects were noted on the following parameters: mortality, clinical signs, food consumption, ophthalmology, clinical chemistry, urinalysis, fecal analysis, macroscopic pathology and microscopic pathology. **NOEL = males, 6,500 ppm (236 mg/kg/day) and females, 2,000 ppm (77.7 mg/kg/day). LOEL = males, 20,000 ppm (709 mg/kg/day) and females, 6,500 ppm (232 mg/kg/day) based on a decrease in body weight gain.**

e. <u>104-Week Feeding Study - Rat</u> (MRID No. 43830017)

Discussed in detail on pages 3-9 **.** Hsd:Sprague-Dawley rats (50/sex/group) were fed diets containing CGA-248757 (97.7% purity) at concentrations of 0, 5, 50, 3,000, 5,000 (males) or 7,000 (females) ppm (mg/kg/day: males = 0, 0.2, 2.1, 130 or 219 and females = 0, 0.2, 2.5, 154 or 368) for 104 weeks. An additional 20/sex were placed in the control and high-dose groups as well as 10/sex in each of the 5, 50 and 3,000 ppm groups for a 52-week interim sacrifice. **The systemic toxicity NOEL = 50 ppm (2.1 and 2.5 mg/kg/day for males and females, respectively). The systemic toxicity LOEL = 3,000 ppm (130 and 154 mg/kg/day for males and females, respectively) based on: males = decreased body weights, liver toxicity, pancreatic toxicity and microcytic anemia; females = liver toxicity, uterine toxicity and slight microcytic anemia. CGA-248757 in males only at 3,000 and 5,000 ppm (130 and 219 mg/kg/day, respectively), caused an increase in the trend toward pancreatic exocrine adenomas and pancreatic islet cell adenomas.**

f. <u>18-Month Carcinogenicity Study - Mouse</u> (MRID No. 43830015)

Discussed in detail on pages 9-14 **.** CGA-248757 (97.7% purity) was administered by dietary admix to Charles River Crl:CD-1 (ICR)BR (Swiss) mice (50/sex/group) at doses of 0, 1, 10, 100 or 300 ppm (mg/kg body weight/day: males = 0, 0.1, 1.0, 10 or 32; females = 0, 0.1, 1.2, 12 or 37) for 78 weeks. **The systemic toxicity NOEL = 1 ppm (0.1 and 0.1 mg/kg/day, males and females, respectively). The systemic toxicity LOEL = 10 ppm (1.0 and 1.2 mg/kg/day, males and females, respectively) based on non-neoplastic liver findings. CGA-248757 in males (and possibly females) at 100 and 300 ppm (mg/kg/day, 10 and 32 for males and 12**

and 37 for females) may cause an increase in the number of mice with hepatocellular adenomas, carcinomas and/or adenomas/carcinomas.

I. One-Year Oral Toxicity Study - Dog (MRID No. 43830014)

In a toxicity study (MRID No. 43830014), CGA-248757 (97.7% purity) was administered by dietary admix to beagle dogs (4/sex/group) at doses of 0, 10, 150, 2,000 or 20,000 ppm in males (0, 0.351, 4.19, 57.6 or 582 mg/kg/day) and 0, 10, 150, 1,000 or 5,000 ppm in females (0, 0.313, 5.00, 30.3 or 145 mg/kg/day) for 52 weeks.

The following effects were noted and only at the high dose (20,000 ppm in males and 5,000 ppm in females): suggestive decrease in male and female body weight gains; a decrease in mean corpuscular volume in both sexes as well as a decrease in mean corpuscular hemoglobin in males only; and effects on the liver (both sexes) as shown by an increase and/or severity of hepatocyte degeneration, leukocytosis, Kupffer cell pigmentation and intracytoplasmic pigmentation. **NOEL = 2,000 ppm (57.6 mg/kg/day) in males and 1,000 ppm (30.3 mg/kg/day) in females. LOEL = 20,000 ppm (582 mg/kg/day) in males and 5,000 ppm (145 mg/kg/day) in females based on effects observed in the erythropoietic system and the liver.**

F. WEIGHT-OF-EVIDENCE CONSIDERATIONS

1. Carcinogenicity

Evidence for carcinogenicity was demonstrated by the presence of pancreatic tumors in male rats and liver tumors in male and female mice. There was no evidence of carcinogenicity in female rats.

a. Male Rats

There was a significant increasing trend (p=0.007) for exocrine adenomas but there was no pair-wise significance for this tumor type at any dose level.

There was a significant trend (p=0.017) as well as pair-wise significance (p=0.024) for pancreatic islet cell adenomas at the 300 ppm (high) dose (8/47, 17%) when compared to controls (1/43, 2%).

There was a significant increasing trend (p=0.031) for the combined islet cell tumors (adenomas + carcinomas), but tyhere was no pair-wise significance for either adenomas or carcinomas at any dose level.

When compared with historical controls from two other studies, the trends and/or incidences observed in dosed rats in the current study exceeded those in the other studies (≤3% for any tumor type).

b. Male Mice

For liver tumors, there was a significant increasing trend (p=0.001) for adenomas, 0.002 for carcinomas and 0.000 for adenomas + carcinomas. Regarding pair-wise comparison, at 100 ppm for carcinomas plus combined adenomas + carcinomas there were significant increases (p=0.11 and 0.020, respectively; 12/49, 24% for carcinomas versus 3/49, 6% for controls; 26/49, 53% for adenomas + carcinomas versus 15/49, 30%) for controls). At 300 ppm, pair-wise comparison was as follows: adenomas = 0.023 with 22/48 (46%) versus controls of 12/49 (24%); carcinomas = 0.005 with 13/48 (27%) versus controls of 3/49 (6%); and adenomas + carcinomas = 0.001 with 31/48 (65%) versus controls of 15/49 (31%).

Historical controls from 9 studies indicated the following: adenomas = 7-30%; carcinomas = 0-8%; combined = 8-32%. Charles River control data: adenomas = 0-16%; carcinomas = 0-6%.

c. Female Mice

For liver tumors, there was a significant increasing trend for adenomas (p=0.003) and adenomas + carcinomas (p=0.004), but there was no pair-wise significance for either adenomas or carcinomas at any dose level.

Historical controls from 9 studies indicated the following: adenomas = 0-3%; carcinomas = 0%; combined = 0-3%. Charles River control data: adenomas = 0-3%; carcinomas = 0-3%.

2. Mutagenicity

There is evidence that ACTION is mutagenic in vitro in two different mammalian cell lines, both in the presence and absence of metabolic activation. Also, the in vitro mutagenic potential was confirmed in vivo.

3. Structure-Activity-Relationship

ACTION (Fluthiacet-Methyl; CGA-248757; KIH-9201) is a new photobleaching imide herbicide. There are no other structurally similar chemicals for comparison.

DATA EVALUATION REPORT

CGA-248757

STUDY TYPE: COMBINED CHRONIC/ONCOGENICITY FEEDING - RAT (83-5)

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. 98-12A

Primary Reviewer: William J. Spangler, Ph.D.	Signature: Date:	RAR for W. J. Spangler 4-9-98
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Disclaimer

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

Managed by Lockheed Martin Energy Research Corporation for the U.S. Department of Energy under Contract No. DE-AC05-96OR22464.

HAS BEEN SIGNED OFF AND TOX-SAC REVIEWED

CGA-248757

Chronic/Oncogenicity Oral Study (83-5)

EPA Reviewer: Alan Levy, Ph.D.
Registration Action Branch 2 (7509C)
EPA Work Assignment Manager:
Sanjivani Diwan, Ph.D.
Toxicology Branch 1 (7509C)

_____, Date _____

_____, Date _____

DATA EVALUATION RECORD

STUDY TYPE: Combined chronic/oncogenicity feeding - Rat
OPPTS 870.4300 [§83-5]

BP BAR CODE: D224320
P.C. CODE: 108803

SUBMISSION CODE: S500090
TOX. CHEM. NO.: None

TEST MATERIAL (PURITY): CGA-248757 Technical (97.7% a.i.)

SYNONYMS: Fluthiacet-methyl, KIH-9201

CITATION: Potrepka, R. and A. Richter (1995) Two-year dietary chronic toxicity/oncogenicity study with CGA-248757 Technical in rats. Ciba Crop Protection, Ciba-Geigy Corporation, Environmental Health Center, 400 Farmington Avenue, Farmington, CT. Laboratory Study No. F-00068, September 11, 1995, MRID 43830017. Unpublished.

SPONSOR: Ciba Crop Protection, Ciba-Geigy Corporation, Post Office Box 18300, Greensboro, NC

EXECUTIVE SUMMARY: In a combined chronic toxicity/oncogenicity study (MRID 43830017), CGA-248757 (97.7% a.i.) was administered in the diet to 50 Hsd:Sprague Dawley®SD™ rats/sex at concentrations of 0, 5, 50, 3,000, 5,000 (males) or 7,000 (females) ppm (0, 0.2, 2.1, 130, 219 mg/kg/day for males and 0, 0.2, 2.5, 154, 368 mg/kg/day for females) for 104 weeks. An additional 20 animals/sex in the control and high-dose groups and 10 animals/sex in each intermediate group were included for interim sacrifice at 52 weeks.

No treatment-related effects occurred in male or female rats fed the 5 or 50 ppm test diets. The positive trend in survival for males and clinically observed diarrhea in males and pallor in females at ≥ 3,000 ppm were statistically but likely not toxicologically significant. At 3,000 and 5,000 ppm, male rats had treatment-related decreases in mean body weights (up to 15 and 18%, respectively, $p \leq 0.05$ or 0.01), lower food consumption (up to 14% lower during weeks 3-63), and food efficiency was reduced by up to 48% at 3,000 ppm and 66% at 5,000 ppm during weeks 2-37. The overall body weight gains of the males up to 10% lower than of controls. There were no significant changes in these parameters for females at any dose level.

Blood enzymes indicative of hepatocellular damage were elevated at ≥3,000 ppm in both males and females ($p \leq 0.05$ or 0.01) and correlated with significantly increased microscopic observations including bile duct hyperplasia, inflammation, and Kupffer cell pigmentation. Both males and females given ≥3000 ppm CGA-248757 had altered hematological parameters suggestive of mild microcytic hemolysis; bilirubin and urobilinogen were also seen in the animals' urine. Males had significant increases pancreatic masses, acinar

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cell atrophy and hyperplasia, fatty changes, and inflammation at ≥3,000 ppm; these were not present in females. Organ weights were comparable to controls.

The LOEL for CGA-248767 is 3,000 ppm (130 and 154 mg/kg/day for males and females, respectively), based on decreased body weights, liver and pancreatic toxicity in male rats and on liver toxicity in females. The corresponding NOEL is 50 ppm (2.1 and 2.5 mg/kg/day for males and females, respectively).

The potential oncogenicity of CGA-248757 was exhibited by a trend toward increased incidence of pancreatic exocrine adenoma and pancreatic islet cell adenoma in male rats that were statistically significant at 5,000 ppm (10-12% vs. 1% in controls; $p \leq 0.05$). No evidence of oncogenic potential was seen in female rats. The dosing was considered to be adequate for both male and female rats although an additional intermediate dose to males would have been helpful.

This chronic/oncogenicity toxicity study in the rats is acceptable (83-5) and satisfies the guideline requirement for a chronic/ oncogenicity oral study (83-5) in rats.

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

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: CGA-248757 Technical

Description: beige powder
Lot/Batch #: FL-920346
Purity: 97.7% a.i.
Stability of compound: stable for the duration of the study
CAS #: none found
Structure: not given

2. Vehicle and/or positive control

The test material was administered in PMI Feeds Certified Rodent Diet® #5002; no positive control was included.

3. Test animals

Species: rat
Strain: Hsd:Sprague Dawley®SD™
Age and weight at study initiation: ≈6 weeks of age; males weighed 130.3-164.0 g; females weighed 101.7-124.7 g
Source: Harlan Sprague Dawley, Frederick, MD
Housing: individually housed in suspended polycarbonate cages (19×21×20 cm) except overnight in metabolism cages for interim urine collection; rack positions were rotated monthly
Diet: ground meal, PMI Feeds Certified Rodent Diet® #5002, *ad libitum*, except overnight before urine collection
Water: municipal water supply, *ad libitum* except during urine collection

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Environmental conditions:
Temperature: 19 to 24°C
Humidity: 40 to 60%
Air changes: ≥15 per hour
Photoperiod: 12 hours light/12 hours dark
Acclimation period: 14-15 days, males; 16-17 days, females

B. STUDY DESIGN

1. In life dates

Start: March 31-April 3, 1992; end: April 15, 1994

2. Animal assignment

Animals were assigned to the test groups listed in Table 1, by weight using a computer-generated randomization procedure.

TABLE 1: Study design

Test group	Conc. in diet (ppm)	Dose to animals (mg/kg/day)		Number of animals			
				Main study 104 Weeks		Interim sac. 52 Weeks	
		Male	Female	Male	Female	Male	Female
1 (control)	0	0	0	50	50	20	20
2	5	0.2	0.2	50	50	10	10
3	50	2.1	2.5	50	50	10	10
4	3,000	130	154	50	50	10	10
5	5,000	219	-	50	-	20	-
6	7,000	-	368	-	50	-	20

Data taken from pp. 17 and 39, MRID 43830017.

3. Dose selection rationale

Dose selection was based on a 90-day feeding study (EHC Study No. F-00066) during which rats were fed 0, 10, 100, 3,500, 7,000, or 20,000 ppm CGA-248757 in the diet. Body weight gain of males was significantly decreased (≈25%) at ≥7,000 ppm. For both sexes at ≥3,500 ppm, histopathological findings in the liver included fatty changes with centrilobular necrosis. Based on this study the treatment levels for the 104-week study were set at 0, 5, 50, 3,000, 5,000 (males) and 7,000 (females) ppm.

4. Diet preparation and analysis

Diet was prepared weekly; the test substance was blended with the basal diet (PMI Feeds Certified Rodent Diet® #5002) to prepare the desired nominal concentrations. The prepared diets were stored at 4°C for no longer than 30 days. Concentration and homogeneity was tested on 2 samples taken from the bottom, left and right of each batch for each of the first 4 weekly diet preparations and monthly thereafter. Stability data was obtained during the 90-day

preliminary study. Stability was tested on 1, 500, and 20,000 ppm dietary samples after storage for 35, 35, and 28 days, respectively, in bulk at 4°C.

Results -

Homogeneity analysis: 5 ppm: the measured concentrations of the samples taken from the three positions for each of the first four weekly diet preparations and monthly thereafter varied by -12 to +19% of the nominal concentration; 50 ppm: the measured concentrations of the samples taken varied by -15 to +9% of the nominal concentration; 3,000 ppm: the measured concentrations of the samples taken varied by -7 to +6% of the nominal concentration; 5,000 ppm: the measured concentrations of the samples taken varied by -7 to +5% of the nominal concentration; 7,000 ppm: the measured concentrations of the samples taken varied by -9 to +6% of the nominal concentration. All diets analyzed were within the acceptable Relative Standard Deviation (RSD) ranges of ≤ 20% at 5 ppm, ≤ 15% at 50 ppm, and ≤ 10% at ≥ 3,000 ppm. Therefore, the diets prepared throughout the study were judged to be homogeneous (Appendix 10.2.2, pp. 459-478, MRID 43830017).

Stability analysis: 1 ppm: the measured concentration on day 35 was 97% of the nominal concentration on day 0; 500 ppm: the measured concentration on day 35 was 99% of the nominal concentration on day 0; 20,000 ppm: the measured concentration on day 28 was 87% of the nominal concentration on day 0. All the above diets were stored at 4°C during the stability testing period. In addition, the bulk stored diet at 1 ppm CGA-248757 was stable for up to 21 days at room temperature in open feed jars after storage for 26 days at 4°C. Diets were always used within 30 days of preparation. Therefore, the test substance showed adequate stability in the diet (Appendix 10.2.3, p. 479, MRID 43830017).

Concentration analysis: All measured concentrations were within ±10% of the target concentration with the following exceptions: 5 ppm, weeks 1 (+17%), 9 (-12%), 31 (+11%), 70 (+11%), 76 (+14%), 84 (+19%), 94 (+13%), and 104 (+14%); 50 ppm, week 3 (-15%). The grand mean values for all diets analyzed throughout the study ranged from 98-102% of nominal (RSD 3.76-5.18). The dietary preparations are judged to be adequate for the study (Table 9.1 and Appendix 10.2.2, pp. 60 and 459-478, MRID 43830017).

5. Statistics

Body weight, body weight change, food consumption, feed efficiency, hematology, blood chemistry, and organ weight data: Bartlett's test for homogeneity of variance was used. If heterogeneous ($p \leq 0.001$), the data were transformed and retested using Bartlett's test until homogeneity was achieved. If data could not be stabilized by transformation, the data were rank ordered and parametric methods were applied to the ranks. One-way analysis of variance (ANOVA) was performed on the untransformed/ transformed/ranked data. When the F-statistic was significant (p

≤ 0.05), the two-way Dunnett's test was used to test for significance between each treatment group and the control. Control and treatment values where N < 3, were not compared further and standard deviations were not calculated for these values.

Mortality: The Kaplan-Meier adjusted survival rates were used to compare survival between the treatment and control groups.

Clinical observations, gross pathology and neoplastic lesions: Fisher's Exact test with Bonferroni's correction. Because the statistical analysis was not presented with the data in the report, it was also performed by the reviewer using Fisher's exact test for gross pathology and neoplastic lesions.

Graded non-neoplastic data: ANOVA techniques. Lesions were graded as grade 0 (negative) through grade 5 (severe). Scores were rank ordered and one-way ANOVA performed on the ranked data. When the F-statistic was significant ($p \leq 0.05$), significance between each treatment group and the control was compared using Dunnett's test.

C. METHODS

1. Observations

Animals were inspected a minimum of twice daily for general appearance, behavior, signs of toxicity, and mortality. The animals were given a weekly detailed physical examination including palpation for tissue masses.

2 Body weight

Animals were weighed weekly for the first 13 weeks, at least every two weeks thereafter, and at termination.

3. Food consumption and compound intake

Food consumption for each animal was determined weekly for the first 13 weeks and at least once every four weeks thereafter. Mean food consumption was reported as g/day. Food efficiency ([body weight gain in kg/food consumption in kg per unit time] X 100) was calculated for each weekly interval for the first 13 weeks of treatment and every 4 weeks thereafter through week 55. Compound intake (mg/kg/day) values were based on the food consumption and body weight gain data.

4. Ophthalmoscopic examination

Eyes of all animals were examined before treatment started, of all rats scheduled for necropsy at 52 weeks and of control and high-dose animals at 104 weeks.

5. Blood was collected from the orbital plexus of 20 rats/sex/group for hematology at 3, 6, 18, and 24 months and from 30 rats/sex/group at 12 months. Those animals designated for blood

collection at interim and terminal sacrifice were bled from the abdominal aorta during necropsy. Bone marrow smears were prepared from the left femur of all rats at the 12-month sacrifice. Ten rats/sex/group were bled from the orbital plexus at 6, 12, and 18 months for clinical chemistry and from the abdominal aorta at termination. The animals were fasted overnight and anesthetized before sampling. The CHECKED (X) parameters were examined.

a. Hematology

X	Hematocrit (HCT)*	X	Leukocyte differential count*
X	Hemoglobin (HGB)*	X	Mean corpuscular HGB (MCH)
X	Leukocyte count (WBC)*	X	Mean corpusc. HGB conc.(MCHC)
X	Erythrocyte count (RBC)*	X	Mean corpusc. volume (MCV)
X	Platelet count*	X	Reticulocyte count
	Blood clotting measurements*		
	(Thromboplastin time)		
	(Thromboplastin time)		
	(Clotting time)		
X	(Prothrombin time)		

* Required for chronic/oncogenicity studies based on Subdivision F Guidelines

b. Clinical chemistry

X	ELECTROLYTES	X	OTHER
X	Calcium*	X	Albumin*
X	Chloride*	X	Blood creatinine
	Magnesium	X	Blood urea nitrogen*
X	Phosphorus*	X	Total Cholesterol*
X	Potassium*	X	Globulins
X	Sodium*	X	Albumin/globulin ratio
		X	Glucose*
	ENZYMES	X	Total bilirubin*
X	Alkaline phosphatase (ALK)	X	Total serum protein (TP)*
	Cholinesterase (ChE)	X	Triglycerides
X	Creatine phosphokinase*		Serum protein electrophoresis
	Lactic acid dehydrogenase (LDH)		
X	Serum alanine amino-transferase (also SGPT)*		
X	Serum aspartate amino-transferase (also SGOT)*		
X	Gamma glutamyl transferase (GGT)		
	Ornithine carbamyl transferase (OCT)		
	Glutamate dehydrogenase		
X	5'Nucleotidase		
X	Sorbitol dehydrogenase		

* Required for chronic/oncogenicity studies based on Subdivision F Guidelines

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6. Urinalysis

Urine was collected at 3, 6, 12, and 18 months from the first 10 rats/sex of groups designated for orbital plexus bleeding and at 24 months for the first 10 rats/sex/group designated for abdominal aorta bleeding. The animals were fasted overnight in metabolism cages prior to urine collection. The CHECKED (X) parameters were examined.

X	Appearance*	X	Glucose*
X	Volume*	X	Ketones*
X	Specific gravity*	X	Bilirubin*
X	pH	X	Blood*
X	Sediment (microscopic)*		Nitrate
X	Protein*	X	Urobilinogen
			Total reducing substance

* Required for chronic/oncogenicity studies

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7. Sacrifice and pathology

All animals that died and those sacrificed in extremis or killed on schedule by exsanguination under anaesthesia were subjected to detailed gross pathological examination and the CHECKED (X) tissues were collected for microscopic examination. All "checked " tissues from all controls and high-dose animals and from animals that died or were sacrificed in extremis were examined microscopically. The kidney, liver, lungs, mammary gland, and pancreas and all gross lesions and masses were examined in all animals. The cervix, ovaries, uterus and vagina from all females were examined. Bone marrow smears were prepared from each animal killed. The [XX] organs, in addition, were weighed.

X	DIGESTIVE SYSTEM	X	CARDIOVASC./HEMAT.	X	NEUROLOGIC
X	Tongue	X	Aorta*	XX	Brain*'
X	Salivary glands*	XX	Heart*	X	Periph. nerve*
X	Esophagus*	X	Bone marrow*	X	Spinal cord
X	Stomach*	X	Lymph nodes*		(4 levels)*
X	Duodenum*	XX	Spleen*	X	Sciatic nerve
X	Jejunum*	XX	Thymus*	X	Pituitary*
X	Ileum*			X	Eyes (optic n.)*
X	Cecum*		UROGENITAL		
X	Colon*	XX	Kidneys*'		GLANDULAR
X	Rectum*	X	Urinary bladder*	XX	Adrenal gland*
XX	Liver*'	XX	Testes*		Lacrimal gland
	Gall bladder	X	Epididymides*	X	Mammary gland'
X	Pancreas*	X	Prostate*	X	Parathyroids*
		X	Seminal vesicle*	X	Thyroids*
	RESPIRATORY	XX	Ovaries*	X	Harderian
X	Trachea*	X	Uterus* and cervix		
XX	Lung*	X	Vagina		OTHER
X	Nasal turbinates	X	Coagulating glands	X	Bone* (tibia-femur)
	Pharynx			X	Joint
	Larynx				(femorotibial)
				X	Skeletal muscle*
				X	Skin*
				X	All gross lesions and masses*

'Required for chronic/oncogenicity studies based on Subdivision F Guidelines.
'Organ weight required in chronic/oncogenicity studies.

II. RESULTS

1. Toxicity

Clinical signs of toxicity observed during the study included diarrhea in 18/70 (26%) males fed the 5,000 ppm diet compared with 5/70 controls (7%) and pallor in 18/60 (30%) and 21/70 (30%) females fed the 3,000- and 5,000-ppm diets, respectively, compared with 7/70 controls (10%). In females, a discharge from the vulva was seen in all treatment groups after 9-11 months of observation.

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There was no significant difference from controls for any treatment group. In males there was a significantly lowered incidence in swollen pelvic region at 3,000 ppm and a reduced incidence of chromorhinorrhea, dehydration, hypothermia, and pallor at 5,000 ppm. There were no treatment-related differences in the location, incidence, or distribution of palpable masses in either sex.

2. Mortality

In the interim sacrifice groups, there was one death each in the 0, 5, 50, and 5,000 ppm treatment groups for males and one death each in the 50 and 7,000 ppm group females. In the main study, 35, 30, 34, 20, and 16 males receiving 0, 5, 50, 3,000, and 5,000 ppm, respectively, of the test material died or were sacrificed in extremis before termination; 20, 19, 25, 23, and 26 females, respectively, in the 0, 5, 50, 3,000, and 7,000 ppm treatment groups died or underwent unscheduled sacrifice. There was one accidental death in the male control group. There was a positive trend in survival for males; mortality rates in treated groups of females were comparable to the control group. The Kaplan-Meier adjusted survival rates after 24 months were 30, 42, 34, 61, and 69% for the 0, 5, 50, 3,000, and 5,000 ppm males and 61, 62, 51, 54, and 50% for the 0, 5, 50, 3,000, and 7,000 ppm females.

B. BODY WEIGHT

Table 2 summarizes the effect of treatment with CGA-248757 on body weights and body weight gains in male and female rats. Treatment-related effects on body weights and body weight gains were seen only in male rats receiving 3,000 and 5,000 ppm of the test material. Males fed 3,000 and 5,000 ppm of the test material weighed significantly less than controls throughout the first 100 weeks ($p \leq 0.01$ or 0.05) of the study (up to 15% and 18%, respectively). Although not statistically significant, there was a treatment-related decrease in body weight gain in males, but not females, for the 104-week treatment period. Females fed CGA-248757 at 3,000 ppm showed only sporadic significant ($p \leq 0.05$) decreases in body weight during weeks 6-28 (4% maximum). At 7,000 ppm there was no statistically significant or treatment-related decrease in body weight for the 104-week treatment period.

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TABLE 2. Selected mean body weights and body weight gain in male and female rats fed CGA-248757 for 104 weeks

Week	Concentration (ppm)									
	0	5	50	3,000	5,000	0	5	50	3,000	7,000
	Males					Females				
	Mean body weight (g)									
0	194	193 (99)[a]	192 (99)	193 (99)	192 (99)	143	144 (101)	143 (100)	141 (99)	142 (99)
4	338	339 (100)	333 (99)	313** (93)	305** (90)	204	203 (100)	205 (101)	198 (97)	206 (101)
8	398	397 (100)	394 (99)	353** (89)	343** (86)	239	241 (101)	239 (100)	230* (96)	235 (99)
12	432	433 (100)	431 (100)	377** (87)	365** (84)	255	257 (101)	258 (101)	245* (96)	251 (98)
25	511	513 (100)	507 (99)	439** (86)	427** (84)	289	294 (102)	293 (101)	279* (97)	283 (98)
51	578	582 (101)	579 (100)	493** (85)	479** (83)	317	322 (102)	331 (104)	315 (99)	312 (98)
77	585	610 (104)	589 (101)	532** (91)	516** (88)	355	356 (100)	368 (104)	355 (100)	363 (102)
104	542	551 (102)	542 (100)	518 (96)	508 (94)	364	371 (102)	352 (97)	375 (103)	348 (96)
	Body weight gain (g)[b]									
0-4	144	146 (101)	141 (98)	121 (84)	113 (78)	61	59 (97)	62 (102)	57 (93)	64 (105)
4-8	60	58 (97)	61 (102)	40 (67)	39 (65)	35	37 (106)	34 (97)	32 (91)	30 (86)
8-12	34	36 (106)	37 (109)	24 (71)	22 (65)	16	16 (100)	19 (119)	15 (94)	16 (100)
12-51	146	149 (102)	148 (101)	116 (79)	114 (78)	62	65 (105)	73 (116)	70 (113)	61 (98)
51-104	-35	-31 (89)	-37 (106)	25 (171)	29 (183)	47	49 (104)	22 (47)	59 (126)	36 (77)
0-104	353	357 (101)	351 (99)	325 (92)	317 (90)	223	226 (101)	211 (95)	232 (104)	207 (93)

Data taken from Tables 9.9-9.12, pp. 86-107, MRID 43830017.
[a]Numbers in parentheses are percents of control calculated by the reviewer.
[b]Body weight gain calculated by the reviewer except for weeks 0-104.
*p<0.05, **p<0.01; body weight gain was not analyzed statistically except for weeks 0-104 by study authors..

C. FOOD CONSUMPTION AND COMPOUND INTAKE

1. Food consumption

Food consumption in male rats receiving test material at 3,000 and 5,000 ppm was statistically significantly increased ($p \leq 0.01$) compared to controls during week 1 (3%) and significantly decreased ($p \leq 0.01$) from weeks 3 through 63 (6-12% at 3,000 ppm

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and 6-14% at 5,000 ppm). Food consumption in females at all dose levels of the test material was similar to that of controls. A single statistically significant increase ($p \leq 0.05$) in food consumption that occurred during week 45 in 50 ppm females was not treatment-related.

2. Compound consumption

Compound intake is summarized in Table 1.

3. Food efficiency

There was a treatment-related decrease in food efficiency in 3,000 and 5,000 ppm males during weeks 2-37. During this period, food efficiency at 3,000 ppm was reduced by as much as 48% (week 3) and at 5,000 ppm by as much as 66% (week 3). No treatment-related changes in food efficiency were observed for females in any treatment group.

D. OPHTHALMOSCOPIC EXAMINATION

No treatment-related effects on the eyes were observed during the 12- and 24-month ophthalmoscopic examinations. All ocular changes observed in all groups at 12 months and in the high-dose and control groups at 24 months were random and sporadic.

E. BLOOD WORK

1. Hematology

Statistically significant changes ($p \leq 0.05$ or 0.01) were observed at various intervals throughout the study in males and/or females given ≥ 3,000 ppm CGA-248757. Alterations included a 2-12% decrease in the hematocrit, 5-6% decrease in hemoglobin (males only), 4-14% increase in RBC count, 3-16% decrease in the MCV, 2-14% decrease in the MCH, 2-6% increase or decrease in the MCHC, and 15-25% increase in the platelet count. These changes were small but were generally dose-related and possibly caused by compound treatment. The slightly decreased hematocrit, hemoglobin, and MCV were compensated for physiologically by the increase in RBC production (i.e. increased RBC count), and the overall toxicological significance of the hematological changes was minor. Both sexes given ≥ 3000 ppm CGA-248757 also had small, statistically but not biologically significant changes in the RBC osmotic fragility (i.e., the EC-50 and slope) and in the bone marrow myeloid:erythroid ratio and erythrocyte maturation indices. These changes are all consistent with compensated hemolytic microcytosis.

2. Clinical chemistry

Selected clinical chemistry values are summarized in Table 3. Alkaline phosphatase activity was significantly increased in male rats fed 3,000 or 5,000 ppm of the test material from week 26 to 78 or 104 (123-225% of controls, $p \leq 0.01$). In female rats,

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alkaline phosphatase activities were not significantly affected at any dose or time point except for a 208% increase at 7,000 ppm during week 78, which was likely an overlooked data entry error. In male rats given ≥ 3000 ppm CGA-248757, aspartate and alanine aminotransferase activities were significantly increased only during week 26 (140-173% of controls; $p \leq 0.01$). In females given 3000 and/or 5000 ppm compound, aspartate aminotransferase was increased for weeks 52 and 78, and alanine aminotransferase was increased for weeks 26-78 (138-202% of controls; $p \leq 0.01$). The activity of γ-glutamyl transferase was significantly increased in males receiving ≥ 3,000 ppm of test material throughout the treatment period (147-194% of controls; $p \leq 0.01$) and in females at ≥3,000 ppm (181-197% of controls) only at week 52. Sorbitol dehydrogenase activity was significantly increased at weeks 26, 78, and 104 in males and at weeks 26-78 in females (155-281% of control; $p \leq 0.05$ or 0.01). The 5'-nucleotidase activity in males was significantly increased ($p \leq 0.01$) in males at ≥3,000 ppm throughout the treatment period (182-364% of control), in females at ≥3,000 ppm during weeks 26 and 52 (152-203% of control), and in females at 7,000 ppm during week 78 (200% of control). Statistically significant changes occurred in a number of other parameters that were not considered by the study author to be biologically significant or treatment-related. These included decreased serum albumin in males and females, increased serum sodium, chloride, and total bilirubin and decreased calcium, glucose, total protein, triglycerides, and globulin in males, and decreased A/G ratio in females.

TABLE 3. Selected blood chemistry values in male and female rats fed CGA-248757 for 104 weeks

Conc. (ppm)	ALP (U/L)	AST (U/L)	ALT (U/L)	GLT (U/L)	SD (U/L)	5' Nucl. (U/L	ALP (U/L)	AST (U/L)	ALT (U/L)	GLT (U/L)	SD (U/L)	5' Nucl. (U/L)
	Males						Females					
26 Weeks												
0	45.6	81.2	31.8	3.10	6.9	15.1	33.5	92.7	26.8	3.70	5.4	14.7
5	50.0	88.6	34.4	2.70	6.8	16.8	35.3	97.0	30.0	3.90	6.9	14.5
50	52.0	85.7	32.2	2.80	7.3	14.8	31.2	79.9	28.9	4.70	5.7	13.8
3,000	102.8**	123.8**	55.0**	5.70**	12.9**	46.7**	38.4	102.1	37.6**	4.10	9.8**	26.7**
5,000	93.1**	113.3**	51.3**	5.20**	11.1**	36.9**	-	-	-	-	-	-
7,000	-	-	-	-	-	-	38.0	108.4	39.4**	4.30	12.3**	29.8**
52 Weeks												
0	55.3	124.9	79.4	3.80	13.5	24.0	29.1	58.2	23.3	3.20	3.8	13.0
5	44.2	66.4	30.8	3.30	6.0	15.3	29.3	70.5	23.9	3.10	4.9	13.4
50	49.0	73.5	31.4	3.80	6.7	14.9	27.5	60.9	23.5	4.10	4.4	12.1
3,000	103.2**	89.7	39.5	6.20**	9.2	58.0**	34.3	74.3	32.2**	6.30**	5.9*	19.7**
5,000	94.6**	85.4	38.9	5.60**	9.0	46.2**	-	-	-	-	-	-
7,000	-	-	-	-	-	-	36.0	86.2**	33.5**	5.80**	7.8**	25.3**
78 Weeks												
0	45.9	77.3	30.1	4.70	5.8	14.3	33.3	65.9	25.0	3.80	4.7	12.3
5	42.8	65.4	28.6	4.70	5.2	14.0	33.1	76.1	28.0	3.10	5.5	12.3
50	48.9	70.7	31.0	5.00	6.5	13.4	33.8	67.4	23.7	3.30	5.0	12.5
3,000	79.5**	102.3	36.6	9.10**	16.3*	45.5**	32.5	77.1	29.7	3.70	6.0	15.3
5,000	94.1**	73.7	34.9	8.40**	8.4	52.0**	-	-	-	-	-	-
7,000	-	-	-	-	-	-	102.4*	132.8**	43.1**	4.30	7.5**	24.6**
104 Weeks												
0	37.9	67.6	24.7	0.68	7.8	9.6	51.1	128.4	36.9	1.05	12.2	12.7
5	43.1	78.4	31.1	1.42	8.6	14.3	30.5	79.0	26.5	0.44	8.7	10.4
50	38.4	79.7	29.9	0.96	11.8	11.8	27.8	91.1	23.2	1.18	9.2	9.7
3,000	47.0	86.9	26.2	2.10**	13.6*	17.5**	44.2	92.1	22.1	0.70	9.7	11.0
5,000	77.2**	96.3	39.2	2.65**	15.3*	26.5**	-	-	-	-	-	-
7,000	-	-	-	-	-	-	50.3	107.0	32.9	0.50	13.7	17.8

Data taken from Tables 9.21 and 9.22, pp. 146-160, MRID 43830017.
ALP = alkaline phosphatase, AST = aspartate aminotransferase, ALT = alanine aminotransferase, GLT = γ-glutamyl transferase, SD = sorbitol dehydrogenase, 5' Nucl. = 5' nucleotidase.
*Possible data entry error.
*p<0.05, **p<0.01, compared with controls

F. URINALYSIS

Urinalysis results are summarized in Table 4. The pH values were significantly decreased ($p<0.05$ or 0.01) after 26, 52, and 78 weeks in males fed 3,000 and/or 5000 ppm of the test material. The decrease in urine pH in females ($p \leq 0.05$) receiving 5 ppm for 26 and 52 weeks was not treatment-related. In males, bilirubin and urobilinogen were significantly increased throughout the study at ≥3,000 ppm ($p \leq 0.05$ or 0.01). In females, bilirubin was significantly increased ($p \leq 0.05$ or 0.01) at 7,000 ppm during week 13, at ≥3,000 ppm during week 26 and at 7,000 ppm during weeks 13-52. Urobilinogen was also significantly increased ($p \leq 0.05$ or 0.01) in females at ≥3,000 ppm during week 13 and at 7,000 ppm during week 26. Ketones were significantly increased throughout the study in males fed ≥3,000 ppm of CGA-248757, but in females the increase was only sporadic and not treatment-related. A treatment related change in urine color from yellow to amber occurred at ≥3,000 ppm in both sexes. Statistically significantly decreased bacterial content (≤ 3,000 ppm at 52 weeks) and white blood cells (5,000 ppm at 104 weeks) observed in the urine sediments of males were not considered treatment-related due to lack of a time-response relationship.

TABLE 4. Urinalysis values or incidences in male and female rats fed CGA-248757 for 104 weeks

Parameters	Concentration (ppm)									
	0	5	50	3,000	5,000	0	5	50	3,000	7,000
	Males					Females				
13 Weeks										
pH	7.35	7.45	7.15	7.35	7.25	7.00	6.95	6.95	6.90	7.10
Bili.	0/10	0/10	0/10	10/10**	10/10**	0/10	0/10	0/10	3/10	6/10**
Urobil.	0/10	1/10	2/10	10/10**	10/10**	0/10	0/10	0/10	7/10**	7/10**
26 Weeks										
pH	7.15	7.00	7.10	6.60**	6.50**	6.75	6.20*	6.40	6.40	6.45
Bili.	0/10	0/10	0/10	10/10**	10/10**	0/10	1/10	0/10	4/10*	8/10**
Urobil.	0/10	1/10	0/10	10/10**	6/10**	0/10	1/10	1/10	4/10	5/10*
52 Weeks										
pH	7.10	6.95	7.05	6.35**	6.30**	6.70	6.15*	6.75	6.45	6.55
Bili.	0/10	1/10	1/10	10/10**	9/10**	0/10	0/10	0/10	1/10	3/10*
Urobil.	0/10	1/10	1/10	7/10**	8/10**	0/10	0/10	0/10	0/10	2/10
78 Weeks										
pH	7.00	7.00	7.20	6.65	6.25*	6.90	6.35	6.75	6.75	6.80
Bili.	0/10	0/10	0/10	7/10**	10/10**	0/10	0/10	0/10	0/10	2/10
Urobil.	0/10	0/10	0/10	8/10	7/10	0/10	0/10	0/10	0/10	1/10
104 Weeks										
pH	7.00	6.90	7.20	6.95	7.30	6.80	6.55	6.60	6.35	6.65
Bili.	0/10	1/10	0/10	4/10*	8/10**	0/10	0/10	0/10	0/10	0/10
Urobil.	0/10	1/10	0/10	4/10*	8/10**	0/10	0/10	0/10	0/10	1/10

Data taken from Tables 9.23 and 9.24, pp. 162-171, MRID 43830017.
'Bili. = bilirubin, Urobil. = urobilinogen.
*p<0.05, **p<0.01, compared with controls.

G. SACRIFICE AND PATHOLOGY

1. Organ weight

At only the interim sacrifice (week 52), there were significant differences from controls in the absolute and/or several weights of several organs; these are summarized in Table 5. After 52

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weeks, there was a significant decrease in absolute weights for the heart at ≥3,000 ppm (10-16%, $p \leq 0.5$ or 0.01), kidneys at ≥3,000 ppm (11%, $p \leq 0.05$ or 0.01), liver at ≥3,000 ppm (16-24%, $p \leq 0.05$ or 0.01), lungs at 5,000 ppm (8%, $p \leq 0.05$) and spleen at ≥3,000 ppm (20%, $p \leq 0.01$). Because of the significant drop in body weight at 52 weeks in males given 3,000 and 5,000 ppm CGA-248757 (16-18%; $p \leq 0.01$), organ weights relative to body weight were increased significantly ($p \leq 0.05$ or 0.01) for heart (10% at 5,000 ppm), kidneys (9% at 5,000 ppm), lungs (11% at 3,000 and 5,000 ppm), testes (19% at 3,000 ppm and 22% at 5,000 ppm), and brain (16% at 3,000 ppm, 20% at 5,000 ppm). The relative organ to brain weights were significantly decreased ($p \leq 0.05$ or 0.01) at 52 weeks for heart (15% at 3,000 ppm, 9% at 5,000 ppm), kidneys (10% at 5,000 ppm), liver (14% at 3,000 ppm and 21% at 5,000 ppm), and spleen (18% at 3,000 ppm and 19% at 5,000). After 104 weeks, only the absolute adrenal weights were decreased at 5,000 ppm (21%, $p \leq 0.05$) in males. The relative (to body) weights for liver and kidneys were statistically significantly increased at 50 ppm after 12 months (10% and 6%, respectively, $p \leq 0.05$), but the changes were not considered treatment-related due to lack of a dose-response relationship. Also, the statistically significant increase in absolute and relative (to brain) weights for lungs in females (11%, $p \leq 0.05$) at 3,000 ppm after 104 weeks were not considered to be treatment-related due to lack of a dose-response relationship.

TABLE 5. Selected absolute organ weights (g) and relative organ weights (%) in male and female rats fed CGA-248757 for 52 weeks

Organ	Concentration (ppm)				
	0	5	50	3,000	5,000/7,000[b]
Males					
Body wt.	547.42 ± 42.83	550.00 ± 37.27	555.89 ± 55.82	461.40 ± 40.49**	446.95 ± 40.18**
Heart	1.966 ± 0.205[a] 0.361 ± 0.045 94.60 ± 11.26	1.847 ± 0.133 0.336 ± 0.020 86.69 ± 7.86	1.999 ± 0.209 0.361 ± 0.036 95.29 ± 7.56	1.645 ± 0.200** 0.357 ± 0.033 80.56 ± 7.96**	1.768 ± 0.234* 0.395 ± 0.037* 86.05 ± 9.36*
Kidneys	3.411 ± 0.353 0.623 ± 0.043 163.7 ± 14.5	3.483 ± 0.274 0.634 ± 0.031 163.5 ± 15.5	3.759 ± 0.568 0.676 ± 0.074* 179.0 ± 24.8	3.046 ± 0.262* 0.661 ± 0.024 149.3 ± 11.0	3.034 ± 0.296** 0.679 ± 0.044** 148.0 ± 13.1*
Liver	15.007 ± 1.777 2.738 ± 0.202 720.4 ± 80.3	15.187 ± 1.783 2.756 ± 0.217 714.1 ± 99.6	16.786 ± 3.311 3.003 ± 0.406* 799.4 ± 150.9	12.621 ± 1.765* 2.727 ± 0.222 618.2 ± 80.4*	11.735 ± 1.607** 2.618 ± 0.186 572.1 ± 72.3**
Lungs	2.038 ± 0.154 0.374 ± 0.035 97.894 ± 7.385	2.055 ± 0.158 0.374 ± 0.025 96.461 ± 8.954	2.065 ± 0.184 0.373 ± 0.025 98.473 ± 7.449	1.919 ± 0.211 0.417 ± 0.040** 94.078 ± 8.646	1.877 ± 0.176* 0.421 ± 0.032** 91.739 ± 10.096
Spleen	0.987 ± 0.157 0.180 ± 0.023 47.25 ± 6.17	1.019 ± 0.104 0.186 ± 0.017 47.82 ± 5.35	1.097 ± 0.189 0.197 ± 0.023 52.19 ± 8.18	0.789 ± 0.127** 0.171 ± 0.019 38.60 ±5.35**	0.789 ± 0.144** 0.176 ± 0.026 38.38 ± 6.28**
Testes	4.174 ± 0.301 0.765 ± 0.062 200.4 ± 12.0	4.246 ± 0.501 0.775 ± 0.100 199.2 ± 25.1	4.139 ± 0.488 0.752 ± 0.122 197.3 ± 20.0	4.151 ± 0.257 0.907 ± 0.102** 203.9 ± 16.1	4.130 ± 0.277 0.930 ± 0.091** 201.5 ± 12.2
Females					
Body wt.	292.00 ± 35.56	316.40 ± 40.14	329.33 ± 42.15	305.30 ± 85.83	289.16 ± 25.31
Heart	1.132 ± 0.142 0.390 ± 0.045 61.17 ± 6.72	1.223 ± 0.109 0.390 ± 0.039 65.52 ± 6.09	1.246 ± 0.112 0.380 ± 0.029 66.80 ± 5.04	1.153 ± 0.153 0.396 ± 0.084 62.58 ± 10.28	1.108 ± 0.103 0.385 ± 0.039 59.63 ± 5.79
Kidneys	1.798 ± 0.140 0.622 ± 0.070 97.2 ± 6.4	1.910 ± 0.087 0.611 ± 0.073 102.3 ± 6.2	1.899 ± 0.133 0.583 ± 0.067 101.9 ± 6.8	1.836 ± 0.162 0.624 ± 0.098 99.4 ± 9.9	1.866 ± 0.140 0.647 ± 0.041 100.5 ± 8.2
Liver	6.958 ± 1.087 2.387 ± 0.262 375.6 ± 51.3	7.320 ± 0.620 2.332 ± 0.209 391.9 ± 32.7	7.725 ± 0.884 2.358 ± 0.236 414.1 ± 41.5	8.332 ± 4.654 2.610 ± 0.508 453.6 ± 264.2	7.180 ± 0.829 2.487 ± 0.233 387.0 ± 51.1
Lungs	1.434 ± 0.106 0.497 ± 0.060 77.564 ± 4.848	1.529 ± 0.097 0.489 ± 0.058 81.776 ± 3.309	1.506 ± 0.071 0.463 ± 0.055 80.850 ± 4.252	1.528 ± 0.130 0.519 ± 0.093 82.878 ± 8.421	1.506 ± 0.143 0.524 ± 0.062 81.088 ± 8.055
Spleen	0.610 ± 0.126 0.211 ± 0.049 33.00 ± 6.60	0.610 ± 0.044 0.195 ± 0.026 32.64 ± 2.05	0.615 ± 0.048 0.188 ± 0.020 33.00 ± 2.36	0.695 ± 0.222 0.229 ± 0.051 37.82 ± 13.05	0.594 ± 0.140 0.208 ± 0.060 32.01 ± 7.86

Data taken from Tables 9.27 and 9.28, pp. 182-195, MRID 43830017.
[a]Absolute weight in the first row, organ/body weight × 100 in second row, and organ/brain weight × 100 in third row.
[b]High doses: males = 5,000 ppm, females = 7,000 ppm.
*p<0.05, **p<0.01, compared with controls.

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2. Gross pathology

Notable gross lesions are summarized in Table 6. Lesions were seen in both sexes in the liver, and also in males in the kidneys, pancreas, and pancreatic lymph nodes, and in the females in the uterus. Male rats given 3000 or 5000 ppm CGA-248757 had statistically significantly increased incidences ($p \leq 0.01$) of liver focus (I) discoloration (43-44% vs 14% of controls), general discoloration (33-43% vs 0% for controls), and rough pitted surface (13-17% vs 1% for controls). The kidneys of males given ≥ 3000 ppm had an increased incidence of general discoloration (23-27% vs 4% in controls). Masses in the pancreas were statistically significantly increased in males at 3,000 ppm (17% vs 3% in controls) and 5,000 ppm (23% vs 3% in controls). The pancreatic lymph nodes were enlarged in males at 3,000 ppm (12% vs 1% in controls) and 5,000 ppm (10% vs 1%) and showed general discoloration at 3,000 ppm (22 vs 1% in controls) and at 5,000 ppm (16% vs 1% in controls). In the female rat, statistically significant increased incidences were also seen in liver focus (I) discoloration at 3,000 ppm (33% vs 16% in controls, $p \leq 0.05$) and general liver discoloration at 7,000 ppm (17% vs 0% in controls, $p \leq 0.01$). The uteri contained abnormal contents at 3,000 ppm (15% vs 6% in controls, N.S.) and at 7,000 ppm (31% vs 6% in controls, $p \leq 0.01$).

TABLE 6. Gross necropsy observations in male and female rats fed CGA-248757 for 104 weeks[1]

Lesions	Concentration (ppm)				
	0	5	50	3,000	5000/ 7,000[2]
Males					
No. Animals per Group	70	60	60	60	70
Liver					
Focus (I) discoloration	10	9	9	26**	31**
General discoloration	0	2	0	20**	30**
Rough pitted surface	1	0	0	10**	9**
Kidney					
General discoloration	3	3	1	14	19
Pancreas					
Masses	2	2	3	10**	16**
Pancreatic lymph nodes					
Enlarged	1	2	3	7*	7*
General discoloration	1	1	4	13**	11**
Females					
No. of animals	70	60	60	60	70
Liver					
Focus (I) discoloration	11	11	9	20*	14
General discoloration	0	1	0	2	12**
Uterus - Abnormal contents	4	3	5	9	22**

Data taken from Tables 9.29 and 9.30, pp. 196-250, MRID 43830017.
[1]Statistically significantly different from controls: *$p \leq 0.05$, **$p \leq 0.01$, calculated by the reviewer using Fisher's exact test.
[2]High-doses: males = 5,000 ppm, females = 7,000 ppm.

3. Microscopic pathology

a) Nonneoplastic - The incidences of notable nonneoplastic lesions are presented in Table 7. Statistically significant increases ($p \leq 0.01$) in the incidence of liver lesions in males were observed microscopically as fatty change at 3,000 ppm (20 vs 4% of controls), eosinophilic cell focus at 3,000 and 5,000 ppm (25 and 27%, respectively, vs 3% of controls), bile duct hyperplasia at 3,000 and 5,000 ppm (95 and 100% vs 51% of controls), peribiliary inflammation at 3,000 and 5,000 ppm (73 and 89% vs 49% of controls), and Kupffer cell pigmentation at 3,000 and 5,000 ppm (78 and 90% vs 17% of controls). In the pancreas of males, there were significant increases ($p \leq 0.05$) in acinar cell atrophy at 3000 and 5,000 ppm (57 and 58%, respectively, vs 30% of controls), acinar cell hyperplasia at 3,000 and 5,000 ppm (27 and 23% vs 4% of controls), fatty change at 3,000 and 5,000 ppm (52 and 54% vs 13% in controls), and inflammation at 3,000 and 5,000 ppm (57 and 55% vs 32% of controls). In the pancreatic lymph nodes, males had statistically significantly increased ($p \leq 0.05$ or 0.01) incidences of

reactive hyperplasia at 3,000 and 5,000 ppm (13 and 11%, respectively, vs 1% of controls) and pigmentation at 3,000 and 5,000 ppm (29 and 16% vs 1% of controls). Statistically significant increases ($p \leq 0.05$ or 0.01) in the incidence of liver lesions in females were observed as centrilobular fatty change at 7,000 ppm (9 vs 1% of controls), congestion at 3,000 ppm (32 vs 13% of controls), bile duct hyperplasia at 3,000 and 7,000 ppm (88 and 87% vs 57% of controls), inflammation at 5, 3,000 and 7,000 ppm (58, 80, and 54%, respectively, vs 29% of controls), leukocytosis at 7,000 ppm (37 vs 11% of controls), Kupffer cell pigmentation at 7,000 ppm (80 vs 37% of controls), and intracytoplasmic pigmentation at 7,000 ppm (19 vs 4% of controls). Also in females, there were significantly increased incidences ($p \leq 0.05$) of uterine hemorrhage at 3,000 ppm (5% vs 0% of controls), inflammation at 3,000 ppm (14% vs 3%), and necrohemorrhagic inflammation at 7,000 ppm (33 vs 7% of controls).

TABLE 7. Selected nonneoplastic microscopic lesions in male and female rats fed CGA-248757 for 104 weeks

Lesions	Concentration (ppm)				
	0	5	50	3,000	5,000/ 7,000[a]
Males					
Liver					
Fatty change	3/70	4/60	2/60	12/60**	9/70
Focus, eosinophilic cell	2/70	1/60	1/60	15/60**	19/70**
Bile duct hyperplasia	36/70	39/60	43/60	57/60**	70/70**
Peribiliary-inflammation	34/70	26/60	11/60**	44/60**	62/70**
Kupffer cell pigmentation	12/70	8/60	10/60	47/60**	63/70**
Pancreas					
Acinar cell atrophy	21/69	19/59	30/60	34/60*	40/69*
Acinar cell hyperplasia	3/69	7/59	8/60	16/60**	16/69**
Fatty change	9/69	14/59	13/60	31/60**	37/69**
Inflammation	22/69	23/59	27/60	34/60*	38/69**
Pancreatic lymph nodes					
Reactive hyperplasia	1/70	0/38	1/41	5/38*	8/70*
Pigmentation	1/70	0/38	1/41	11/38**	11/70**
Females					
Liver					
Centrilobular fatty change	1/70	0/60	1/60	1/60	6/70*
Congestion	9/70	9/60	7/60	19/60**	13/70
Bile duct hyperplasia	40/70	43/60	34/60	53/60**	61/70**
Inflammation	20/70	35/60**	28/60	48/60**	38/70**
Leukocytosis	8/70	7/60	7/60	10/60	26/70**
Kupffer cell pigmentation	26/70	18/60	20/60	34/60	56/70**
Intracytoplasmic pigmentation	3/70	4/60	1/60	10/60	13/70**
Uterus					
Hemorrhage	0/70	0/60	0/60	3/56*	1/70
Inflammation	2/70	3/60	3/60	8/56*	6/70
Necrohemorrhagic inflamm.	5/70	3/60	4/60	7/56	23/70**

Data taken from Tables 9.33 and 9.34, pp. 418-422, MRID 43830017.
[a]High-doses: males = 5,000 ppm, females = 7,000 ppm.
*p<0.05, **p<0.01, compared to controls.

b) Neoplastic - The incidences of selected neoplastic lesions are presented in Table 8. In males, there was a positive trend toward hepatocellular adenomas; the incidence at 3,000 and 5,000 ppm was 3 and 4%, respectively, vs 0% of controls (N.S.). There was no significant trend when hepatocellular adenomas and carcinomas were combined, however. In males given ≥ 3000 ppm CGA-248757, there was a positive trend for pancreatic exocrine adenoma and total islet cell adenoma; the tumor incidences were statistically significantly increased at 5,000 ppm (10-12% vs 1% of controls). Although the incidence of islet cell adenocarcinoma was not different from controls, the total incidence of islet cell tumors had

a definite positive trend and was significant at 5,000 ppm (12% vs 3% of controls). In females, there were increased incidences of uterine adenocarcinoma at only 3,000 ppm (4 vs 0% of controls), uterine squamous cell papilloma at 7,000 ppm (4 vs 0% in controls), and uterine hemangiosarcoma at 7,000 ppm (3 vs 0% of controls). However, the incidences for these tumors do not appear to significantly different from controls and are not suggestive of a relationship to treatment. The incidence of uterine polyps was equally distributed throughout the treatment groups.

TABLE 8. Selected neoplastic microscopic lesions in male and female rats fed CGA-248757 for 104 weeks[1]

Lesions	Concentration (ppm)				
	0	5	50	3,000	5,000/ 7,000[2]
Males					
Liver					
Hepatocellular adenoma	0/70 (0)[3]	0/60 (0)	0/60 (0)	2/60 (3)	3/70 (4)
Hepatocellular carcinoma	1/70 (1)	1/60 (2)	0/60 (0)	1/60 (2)	0/70 (0)
Total hepatocellular tumors	1/70 (1)	1/60 (2)	0/60 (0)	3/60 (5)	3/70 (4)
Pancreas					
Exocrine adenoma	1/69 (1)	2/59 (3)	1/60 (2)	5/60 (8)	7/69* (10)
Islet cell adenocarcinoma	1/69 (1)	0/59 (0)	0/60 (0)	1/60 (2)	0/69 (0)
Islet cell adenoma	1/69 (1)	3/59 (5)	2/60 (3)	4/60 (7)	8/69* (12)
Total islet cell tumors	2/69 (3)	3/59 (5)	2/60 (3)	5/60 (8)	8/69* (12)
Females					
Uterus					
Adenocarcinoma	0/70 (0)	0/60 (0)	0/60 (0)	2/56 (4)	0/70 (0)
Papillary-cyst adenoma	1/70 (1)	0/60 (0)	0/60 (0)	0/56 (0)	0/70 (0)
Squamous cell papilloma	0/70 (0)	0/60 (0)	0/60 (0)	0/56 (0)	3/70 (4)
Polyp	3/70 (4)	5/60 (8)	1/60 (2)	4/56 (7)	3/70 (4)
Hemangiosarcoma	0/70 (0)	0/60 (0)	0/60 (0)	0/56 (0)	2/70 (3)
Stromal cell sarcoma	0/70 (0)	0/60 (0)	1/60 (2)	0/56 (0)	0/70 (0)

Data taken from Table 9.35, pp. 423 and Appendix 10.16, pp. 2225-2232, MRID 43830017.
[1]Statistically significantly different from controls: *p ≤ 0.05, **p ≤ 0.01, calculated by the reviewer using Fisher's exact test.
[2]High-doses: males = 5,000 ppm, females = 7,000 ppm.
[3]Values in parenthesis are percent of group with neoplastic lesions, calculated by the reviewer.

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III. DISCUSSION

A. DISCUSSION

In a combined chronic toxicity/oncogenicity study (MRID 43830017), CGA-248757 (97.7% a.i.) was administered in the diet to 50 Hsd:Sprague Dawley®SD™ rats/sex at concentrations of 0, 5, 50, 3,000, 5,000 (males) or 7,000 (females) ppm (0, 0.2, 2.1, 130, 219 mg/kg/day for males and 0, 0.2, 2.5, 154, 368 mg/kg/day for females) for 104 weeks. An additional 20 animals/sex in the control and high-dose groups and 10 animals/sex in each intermediate group were included for interim sacrifice at 52 weeks.

There was a significant positive trend in survival for males at ≥3,000 ppm; mortality of females was comparable to controls. Clinical signs of toxicity included diarrhea in males and pallor in females; it is unclear whether these effects were treatment-induced. Males fed 3,000 and 5,000 ppm of the test material weighed significantly less than controls throughout the first 100 weeks (up to 15 and 18%). Although not statistically significant there was a treatment-related decrease in body weight gain in males throughout the study. In females at 7,000 ppm there was no statistically significant or treatment-related decrease in body weight throughout the treatment period. The weekly food consumption in ≥3,000 ppm males was significantly decreased during weeks 3-63 (6-12% at 3,000 ppm and 6-14% at 5,000 ppm). Food efficiency in males was reduced as much as 48% (week 3) during weeks 2-37 at 3,000 ppm and by as much as 66% during the same period at 5,000 ppm. Food efficiency in females was not significantly affected by treatment throughout the study. Both the reduced food consumption and decreased food efficiency indicate that body weight loss in males was due to both reduced appetite (palatability) and toxicity from CGA-248757.

There were minor, statistically significant changes ($p \leq 0.05$ or 0.01) in hematological parameters that suggested treatment was causing hemolytic microcytosis in males and females given ≥ 3,000 ppm CGA-248757. The slight decreases in the hematocrit (2-12%) and hemoglobin (5-6%, males only), MCV (3-16%), and MCH (2-14%) were likely compensated for by the 4-14% increase in the RBC count, and the overall toxicological significance of the hematological changes was minor. The small alterations in the RBC osmotic fragility and in the bone marrow myeloid:erythroid ratio and erythrocyte maturation indices were also consistent with compensated hemolytic microcytosis.

Changes in blood clinical chemistry values appear to be related to liver toxicity. Significantly increased alkaline phosphatase, aspartate and alanine aminotransferase, γ-glutamyl transferase, and sorbitol dehydrogenase in males at ≥3,000 ppm were indicative of hepatic damage. Alkaline phosphatase was not significantly increased in females, however the other enzymatic indicators of hepatotoxicity were increased at 3,000 ppm and significantly increased at 7,000 ppm. Microscopic correlates included hepatic bile duct hyperplasia, inflammation and Kupffer cell pigmentation which were significantly increased ≥3,000 ppm in males and females. In addition, eosinophilic foci and fatty change were observed in the

livers of male rats fed diets containing ≥3,000 ppm of the test material and may be indicative of aging.

A statistically significant increase in nodular acinar hyperplasia of the pancreas was observed microscopically in males at 3,000 and 5,000 ppm. The study authors suspected that the statistically significant increase in pancreatic acinar atrophy, inflammation, and fatty change was indicative of oxidative toxic insult with the nodular hyperplasia resulting from subsequent regenerative repair.

In females, significant increase in uterine discoloration was observed at necropsy of 3,000 ppm rats and necrohemorrhagic inflammation, inflammation, and hemorrhage was observed microscopically in 7,000 ppm females. Gross observations at 7,000 ppm revealed significant increase in dilated, fluid-, and debris-filled uteri. The authors state that this is common in the Harlin strain of Sprague Dawley rat. In this stock of rat there appears to be an unusual prolongation of the estrus phase of the reproductive cycle with excessive squamous metaplasia of uterine glands and intraluminal hyperkeratosis which can obstruct drainage through the cervix. However, in this case it is dose-responsive and may be a toxic manifestation.

In conclusion, the lowest observed effect level (LOEL) is 3,000 ppm based on decreased body weight, liver toxicity, pancreatic cell atrophy and acinar cell hyperplasia in male rats and liver toxicity in female rats. The corresponding no observed effect level (NOEL) is 50 ppm for both sexes.

Oncogenic effects of CGA-248575 were manifested by a dose-related increase in the incidences of pancreatic exocrine adenomas and pancreatic islet cell adenomas in male rats; statistical significance for both was achieved at 5,000 ppm (10-12% vs 1% of controls, $p \leq 0.05$). The incidence of exocrine adenomas in historical controls ranged from 0 to 3% (185 animals) and the incidence of islet cell adenomas ranged from 1 to 3% [historical controls reported in this DER consisted of data obtained from studies initiated within the 5-year period prior to the start of this study]. Therefore, the incidence in males receiving 3,000 and 5,000 ppm of the test material is outside the range of historical controls for both types of adenomas. The incidence of hepatocellular adenomas in males receiving the 3,000 and 5,000 ppm diet was higher than of controls (3 and 4%, respectively, vs 0% of controls), but not significantly so, and these neoplasms were not considered to be treatment-related (historical control data were not included for hepatocellular adenomas). In females low incidences of several uterine tumor types were observed such as adenocarcinoma, papillary-cyst adenoma, squamous cell papilloma, polyps, hemangiosarcoma, and stromal cell sarcoma, however, there was no dose-response relationship and CGA-248757 was not considered to be oncogenic to female rats under the conditions of this study.

The study authors presented arguments against the oncogenicity of CGA-248757, first on the basis that the incidence of pancreatic adenomas in historical controls of some rat strains are higher than those used in this study. This is not a valid argument because (45)

tumor incidences for historical controls must be obtained using the exact same strain due to interstrain differences in tumor susceptibility. They also argue that the pancreatic tumors observed commonly occur with age (or after long term exposure) and, indeed, most did occur late in the study. The same could be said for many human tumors and this is part of the criteria for using chronic studies to determine oncogenic potential. Another argument is that the MTD was greatly exceeded at the high dose (5,000 ppm), although it is questionable whether this is true since the overall weight gain at 5000 ppm was comparable to that of 3000 ppm dose males and was within 10% of controls. Use of another lower intermediate dose (perhaps 500 or 1,000 ppm) for males would possibly aid in clarification of this discrepancy by further validating the dose-response relationship. Finally, it is argued that the pancreatic adenomas observed were secondary to reparative and regenerative response to a repeated insult. True, but even if secondary it can still be argued that the tumors occurred in a dose-responsive manner as an effect of the chronic insult.

In conclusion, weak oncogenic potential of CGA-248757 is exhibited by the increased incidence of pancreatic exocrine and islet cell adenoma, but not carcinomas, in male rats. The dosing of male rats was adequate, although additional doses to males would have been helpful; the reviewer considers these results to be acceptable. There were no treatment-related increases in the incidences of neoplasms in female rats.

B. STUDY DEFICIENCIES

There were no deficiencies in this study that would classify it as unacceptable; all elements of test protocol were included and the data were adequately summarized. The greatest shortcoming of the study is the poor choice of doses i.e., the top two doses were only about 2-fold different, whereas the low dose and next higher dose (50 and 3000 ppm) were 30-fold different. The study would likely have established a more "accurate" NOEL and LOEL if the mid-doses were spaced differently.

(46)

EPA Reviewer: Alan C. Levy, Ph.D. Alan C. Levy Date: 3-17-98
Registration Action Branch II (7509C)
EPA Secondary Reviewer: Pamela M. Hurley, Ph.D. Pamela M Hurley Date: 3/17/98

Registration Action Branch II (7509C)

DATA EVALUATION RECORD

STUDY TYPE: 18-Month Carcinogenicity Study - Mouse
OPPTS NUMBER: 870.3200 OPP GUIDELINE NUMBER: 83-2

DP BARCODE: D224320 SUBMISSION CODE: S500090 MRID NO.: 43830015
PC CODE: 108803 TOX. CHEM. NO.: none CASE NO.: 287134

TEST MATERIAL (PURITY): CGA-248757 technical (97.7 %)

SYNONYM: KIH-9201; [2-chloro-4-fluoro-5-[(tetrahydro-3-oxo-1H,3H-[1,3,4-thiadiazol[3,4a]pyridazin-1-ylidene)amino]phenyl]thio]acetic acid methyl ester

CITATION: Chang, J.C.F. and Morrissey, R.L., (1995) CGA-248757 - Final Report - 18-Month Dietary Oncogenicity Study in Mice. Ciba-Geigy Corporation, Crop Protection Division, Environmental Health Center, Farmington, CT. Report No. F-00069, June 26, 1995. MRID 43830015. Unpublished.

SPONSOR: Ciba-Geigy Corporation, Crop Protection Division, Greensboro, NC

EXECUTIVE SUMMARY:

In a carcinogenicity study (MRID No. 43830015), CGA-248757 (97.7% purity) was administered by dietary admix to Charles River Crl:CD-1 (ICR)BR (Swiss) mice (50/sex/group) at doses of 0, 1, 10, 100 and 300 ppm (mg/kg body weight/day: males = 0, 0.1, 1.0, 10 and 32; females = 0, 0.1, 1.2, 12 and 37) for 78 weeks.

The following effects were noted: at 300 ppm, males only had a slight decrease in body weight gain during the latter portion of the study; mean corpuscular volume and mean corpuscular hemoglobin were decreased in males ≥100 ppm; at 300 ppm in females, there were decreases in erythrocyte counts, hemoglobin and hematocrit; absolute and relative liver weights were greater (not always statistically significant) at 100 and 300 ppm, both sexes; liver changes (centrilobular necrosis, centrilobular cell degeneration, histiocytic pigmentation and karyomegaly ≥10 ppm in males and/or females in addition to bile duct hyperplasia at 300 ppm, plus focal basophilic cells ≥100 ppm in males and centrilobular fatty change at 300 ppm in females; and

increased incidences of adenomas and/or carcinomas and/or adenomas/carcinomas in males at 100 and 300 ppm.

The systemic toxicity NOEL = 1 ppm (0.1 and 0.1 mg/kg/day, males and females, respectively)

The systemic toxicity LOEL = 10 ppm (1.0 and 1.2 mg/kg/day, males and females, respectively) based on non-neoplastic liver findings

CGA-248757 in males (and possibly females) at 100 and 300 ppm (mg/kg/day 10 and 32 for males and 12 and 37 for females) may cause an increase in the number of mice with hepatocellular adenomas, carcinomas and/or adenomas/carcinomas.

This study is **acceptable** and satisfies the data requirement for OPPTS 870.3200 (Guideline 83-2) for a carcinogenicity study in mice.

COMPLIANCE:

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

The Flagging statement was as follows: "I have applied the criteria of 40 CFR 158.34 for flagging studies for potential adverse effects to the results of the attached study. This study meets or exceeds criteria 1 & 2." [No. 1 = an incidence of neoplasms in male or female animals which increases with dose; No. 2 = a statistically significant ($p \leq 0.05$) incidence of any type of neoplasm in any test group (male or female animals at any dose level) compared to concurrent control animals of the same sex]

I. MATERIALS AND METHODS

A. MATERIALS

1. TEST MATERIAL: CGA-248757 Technical
   Description: beige solid, demonstrated stable stored at 4°C protected from light
   Lot No: FL-920346; ECH Code No.: 0003-45
   Purity: 97.7%
   CAS No.: 117337-19-6

2. Vehicle: none

3. Test animals:
   Species: mouse
   Strain: Crl:CD-1 (ICR) BR (Swiss)
   Age: about 6 weeks old at start of dosing
   Body Weights: males =27.7 ± 1.2 g and females = 21.6 ± 1.2 g at time of computer assignment to study groups
   Source: Charles River Laboratories, Raleigh, NC
   Housing: 2/cage during acclimation; this Reviewer "assumes" 1/cage during treatment (study Report states on page 17 that individual food consumption was measured); polycarbonate cages.
   Diet: basal diet was Purina Certified Rodent Chow # 5002 ground meal
   Water: automatic system, ad libitum
   Environmental conditions: Temperature: 19-24°C
   Humidity: 40-60%
   Light/Dark Cycle: 12 hours each
   Airflow: 15 changes/hour
   Acclimation period: males = 14 days; females = 16 days

B. PROCEDURES AND STUDY DESIGN

1. Experimental Design

   Start of dosing: males = June 30, 1992; females = July 2, 1992
   Terminal sacrifice: completed on January 21, 1994
   Animal assignment: by computer

Table 1

STUDY DESIGN FOR AN 18-MONTH DIETARY ADMIX CARCINOGENICITY STUDY IN MICE WITH CGA-248757

Dose (ppm)	Number of Mice/Group	
	Males	Females
0	50	50
1	50	50
10	50	50
100	50	50
300	50	50

Data extracted from the table on Report page 16.

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2. Dose Selection (Report page 14)

Doses were chosen on the basis of the results from a 90-day dietary admix study (F-00067) with doses of 0, 1, 10, 500 and 5,000 ppm (mg/kg/day for males = 0, 0.13, 1.3, 66 and 655 and for females = 0, 0.17, 1.6, 83 and 782). There were no effects on mortality, body weight or food consumption/feed efficiency in males or females. The following effects were seen at ≥500 ppm (both sexes): elevated serum cholesterol and bile acid concentrations; elevated sorbitol dehydrogenase, alanine aminotransferase, 5'nucleotidase, aspartate aminotransferase and alkaline phosphatase; decreases in hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin as well as increased platelet counts; increased liver weights; and microscopically, liver necrosis, karyomegaly, fatty changes, subacute inflammation and pigment deposition.

As the liver and hematology effects were moderate at 500 ppm, a top dose of 300 was chosen for the 18-month study. One and 10 ppm were chosen for a possible NOEL.

3. Dietary Admix Preparation

Appropriate amounts of the test article were mixed (Patterson-Kelly twin shell blender) with basal diet to obtain the desired concentrations. The prepared diets were

stored at 4°C. Fresh diets were prepared every two weeks. Jars of fresh feed were provided every 7 ± 1 days.

4. Stability, Concentration and Homogeneity

A. Test Article Stability (Report page 17)

Stability was determined from the 90-day study (Lot No. FL-911622), where the bulk mixture of 1 ppm was stored at 4°C for up to 35 days or in open feed jars at room temperature for 9 days. From this same study, the 500 ppm diet was stable at 4°C for up to 35 days or in open feed jars at room temperature for 13 days. The stability in this 18-month study (Lot No. FL-920346) was examined at 1 ppm. Based on the 1 ppm data (26 days at 4°C followed by 21 days at room temperature), "... test diets may be stored at room temperature for up to 21 days and was usable within 47 days from the blend date." [Report appendix 10.2.3, page 412]

B. Concentration and Homogeneity (Report Table 9.1, pages 40-43; Report Appendix 10.2.1, pages 405-409)

Table 2

TEST ARTICLE DIETARY ADMIX CONCENTRATION AND HOMOGENEITY IN AN 18-MONTH DIETARY ADMIX CARCINOGENICITY STUDY IN MICE WITH CGA-248757

Date Prepared	1 ppm		10 ppm		100 ppm		300 ppm	
	ppm	% diff	ppm	% diff	ppm	% diff	ppm	% diff
6/22/92	0.99	-1	9.35	-7	88.4	-12	285.1	-5
10/8/92	0.88	-12	9.51	-5	95.3	-5	283.5	-6
4/21/93	0.97	-3	9.34	-7	94.7	-5	288.8	-4
10/19/93	1.20	+20	9.79	-2	98.8	-1	289.8	-3

ppm = ppm found
NOTE: Data from 21 dates were analyzed. This Reviewer chose the above dates randomly.
Each value is a mean of up to 6 samples.
Data extracted from Report Table 9.1, pages 40-43.

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Analytical data for stability, concentration and homogeneity were considered to have been within acceptable limits.

Animals received fresh diet every 7 ± 1 days.

5. Mortality and Clinical Signs

All mice were observed A.M. and P.M. each day (appearance, behavior, signs of toxicity and mortality). General physical exams (including tissue mass palpation) were performed each week on all animals.

6. Ophthalmology (results in Appendix 10.5, pages 534-542)

These were performed on all animals prior to the start of dosing as well as on all control and high-dose (300 ppm) mice at 12 and 18 months. **[There was no mention of the type of equipment used.]**

7. Body Weights

Each mouse was weighed every 7 ± 1 days for the first 13 weeks and at least once every 4 weeks thereafter. Terminal body weights were taken.

8. Food Consumption

This was measured for each animal for 7 ± 1 day periods for the first 13 weeks and at least once every 4 weeks thereafter.

9. Clinical Laboratory Tests (only hematology)

Blood smears for differential leukocyte counts were prepared from tail vein blood of all survivors at 12 and 18 months. In addition, smears were made from abdominal aorta blood of moribund sacrificed mice.

At 18 months, under sodium pentobarbital anesthesia, blood samples were obtained from the abdominal aorta of 10/sex/group.

The following parameters were measured:

Erythrocyte count	Reticulocyte count (when HCT≤34%)
Hematocrit	Differential leukocyte count (0 and 300 ppm only)
Hemoglobin	Mean corpuscular volume
Total leukocyte count	Mean corpuscular hemoglobin
Platelet count	Mean corpuscular hemoglobin concentration

10. Sacrifice and Necropsy

Mice were sacrificed by exsanguination under intraperitoneally administered sodium pentobarbital anesthesia. The following organs were weighed and their weights expressed as absolute, relative-to-body weight and relative-to-brain weight:

liver, kidneys, brain, heart, thymus, testes (without epididymides), ovaries, adrenals and spleen

The following tissues were preserved (x) and the organs weighed (xx):

DIGESTIVE	RESPIRATORY	UROGENITAL
-Tongue	xTrachea*	xxKidneys*
xSalivary glands*	xLungs*	xUrinary bladder*
xEsophagus*	xNasal passages	xxTestes*
xStomach*		xEpididymides*
xDuodenum*	CARDIOVASC/HEMAT	xProstate*
xJejunum*	xAorta*	xSeminal vesicles*c
xIleum*	xxHeart*	xxOvaries
xCecum*	xBone marrow*	xUterus*
xColon*	xLymph nodes*	xCervix
xRectum*	xxSpleen*	-Oviducts
xxLiver*	xxThymus*	xVagina
xPancreas*		
xGallbladder*		

NEUROLOGIC	GLANDULAR	OTHER
xxBrain*	xxAdrenals*	xBone*
xPeripheral nerve*	xLacrimal gland	xSkeletal muscle*
xSpinal cord (3 levels)a	xMammary gland*	xSkin*
xPituitary*	xParathyroids*	xAll gross lesions and masses*
xEyes (optic nerve)*b	xThyroids*	xHarderian glands; tibial-femoral joint

* = EPA Guideline requirement - = not examined
a = 4 levels b = no optic nerve indicated c = coagulating glands

The following tissues were examined microscopically by the study pathologist:

a. Tissues from all control and high-dose (300 ppm) mice
b. All mice that died or were sacrificed moribund
c. All gross lesions and masses in all mice
d. From all animals in all groups: sternal bone, sternal bone marrow, bone from tibia and femur, femortibial joint, lungs, liver, gallbladder, thymus, spleen, mesenteric lymph node, pancreas, kidneys and mandibular lymph node
e. Adrenal glands from all males

Histology preparation and evaluation are described on Report pages 21-23.

The study pathologist examined tissues from all mice knowing to which dose group the tissue belonged. Re-evaluations were made of selected lesions which seemed to be involved in determining a NOEL and/or were subjective in nature. These re-evaluations were performed by mixing the slides of tissues from all mice of a sex, evaluating the tissue for the lesion (without knowing which dose group) and putting the slide in an appropriate group based on the grade of severity or type of tumor (benign or malignant). Re-evaluation was performed for the following:

Male Livers - hepatocellular tumors, centrilobular hypertrophy, karyomegaly and pigmentation of histiocytes
Female Livers - centrilobular hypertrophy and chronic active inflammation
Female Thymuses and Mesenteric Lymph Nodes - lymphoma and lymphoid hyperplasia
Female Spleens - erythropoiesis
Male and Female (0 and 300 ppm) Adrenals - ceroid/lipofuscin degenereation
Male (0 and 300 ppm) Testes - ceroid/lipofuscin degeneration
Females (0 and 300 ppm) Glandular Stomach - crypt dilatation, hyperplasia and necrosis

A limited pathology review was performed by Dr. M. Stedham of Pathology Associates, Inc. and consisted of: 10% of all tissues from the 0 and 300 ppm mice, all neoplasms from 0 and 300 ppm groups, as well as all potential target tissues (livers in both sexes and adrenals from males) from all dose groups. The 0 and 300 ppm animals

chosen for review were selected by computer randomization and included the following mice:

males 0 ppm = 103, 124, 128, 140 and 150
males 300 ppm = 503, 508, 531, 533 and 539
females 0 ppm = 1016, 1022, 1025, 1026 and 1035
females 300 ppm = 4911, 4931, 4934, 4942 and 4949

Neoplastic and non-neoplastic liver lesions were reviewed. For male adrenals, only ceroid/lipofuscin degeneration was evaluated. When the study pathologist diagnosed malignant lymphoma, only tissues considered sufficient to establish diagnosis were reviewed.

Diagnoses from the study pathologist and reviewing pathologist were compared. Differences and resolutions were kept in the study file. "The results shown in this report represents the concurred diagnoses from the study and reviewing pathologists."

11. Data Analyses (Report page 24)

Body Weight, Food Consumption and Feed Efficiency: Bartlett's Test for homogeneity of variance was performed at each interval. If not homogenous, appropriate transformations were made to the data. Transformed data were retested (Bartlett's Test) until there was homogeneity. If variances could not be stabilized, data were rank ordered and the usual parametric methods applied. ANOVA was performed on the untransformed/transformed/ranked data. Dunnett's Test was used if the F-statistic was $p \leq 0.05$, between treatment and control groups.

Hematology and Organ Weights: These were analyzed by ANOVA followed by two-way Dunnett's Test.

Non-Neoplastic Findings: Graded findings were analyzed by ANOVA. Severity of lesions was given scores from 0-5. Scores from all animals were rank ordered and one-way analysis was performed. If the overall F-statistic from the analysis was $p \leq 0.05$, Dunnett's Test was used to test for significant effects between treatment and control groups. The Fisher's Exact Test was used to analyze incidences.

Survival and Time-to-Tumor: Analyses were described in Report Appendix 10.14, pages 1513-1530.

II. RESULTS

A. OBSERVATIONS

1. Toxicity (Report Tables 9.3 and 9.4, pages 45-57)

There were no clinical signs which appeared to be related to test article administration.

2. Mortality

Table 3

ANIMAL FATE IN AN 18-MONTH DIETARY ADMIX CARCINOGENICITY STUDY IN MICE WITH CGA-248757

	Males (ppm)					Females (ppm)				
	0	1	10	100	300	0	1	10	100	300
Study Start	50	50	50	50	50	50	50	50	50	50
Found Dead.....	6	7	5	4	5	6	5	5	5	5
Unscheduled Sacrifice	9	7	10	11	5	11	8	8	4	5
Accidental Death	0	0	0	0	0	1	0	0	0	0
Overall Survival %	70	72	70	70	80	65a	74	74	82	80

a = 32/49 (one accidental death)
Data extracted from Report Table 9.2, page 44.

The only effect exhibited by the test article was an increase in the number of male and female survivors compared with the number of survivors in the respective control groups.

B. Body Weight

Table 4

GROUP MEAN BODY WEIGHTS AND WEIGHT GAINS IN AN 18-MONTH DIETARY ADMIX CARCINOGENICITY STUDY IN MICE WITH CGA-248757

Week	Males (ppm)					Females (ppm)				
	0	1	10	100	300	0	1	10	100	300
Body Wt - g										
0	31	31	30	30	30	24	24	24	23	23
24	47	47	46	46	46	35	35	37	37	36
52	48	48	47	49	48	38	38	40	40	39
78	49	48	46	48	45**	40	39	39	41	39
B.W. gain										
0-24 g	17	16	16	16	16	12	12	13	14*	13
%	-	-6	-6	-6	-6	-	0	+8	+17	+8
0-52 g	18	18	17	18	18	14	14	17	17	16
%	-	0	-6	0	0	-	0	+21	+21	+14
0-78 g	18	18	16	17	15*	16	15	15	17	16
%	-	0	-11	-6	-17	-	-6	-6	+6	0

Statistical significance: * = $p<0.05$; ** = $p<0.01$
NOTE: All values were "rounded off" from 2 decimal places to the whole number of grams.
Data extracted from Report Tables 9.7, 9.8, 9.9 and 9.10, pages 60-71.

Group mean body weights ($p<0.01$) and weight gains ($p<0.05$) for 300 ppm males were statistically lower than the control values only at the week 78 determination. However, it appeared that for the weighing intervals of weeks 56-78, the group means (300 ppm) were less than the controls (body weights and weight gains). The group mean body weight gains as percent of controls for males for weeks 0-78 were (1, 10, 100 and 300 ppm): 0%, -11%, -6% and -17%.

For females, treated group mean body weights or weight gains were ≥ controls except at 1 and 10 ppm where the decrease was 6% (one gram).

C. Food Consumption and Compound Intake

1. Food Consumption (Report Tables 9.11 and 9.12, pages 72-77)

There was a slight, though statistically significant, decrease in group mean food consumption (g/day) in treated males during study week one only (ppm): 0 = 5.16, 1 = 4.98 ($p<0.05$), 10 = 4.95 ($p<0.05$), 100 = 4.99 (not significant) and 300 = 4.93 ($p<0.01$). This week one decrease was not noted in treated females. Other than the week one decrease in males, there did not appear to be a pattern of differences between treated and control males; whereas, primarily in 300 ppm females, there were some intervals where the group mean was greater than the control ($p<0.05$ or 0.01).

2. Food Efficiency (Report Tables 9.13 and 9.14, pages 78-81)

Values were calculated by dividing the % body weight change/day by the daily food intake. Calculations were made only for the intervals through study week 40 as body weights appeared to have plateaued during weeks 44-78.

There was no indication in either sex that test article administration had a consistent effect on feed efficiency.

3. Compound Intake (Report Tables 9.15 and 9.16, pages 82-85)

The time-weighted average compound consumption in mg/kg/day was as follows (1, 10, 100 and 300 ppm): males = 0.1, 1.0, 10 and 32; females = 0.1, 1.2, 12 and 37.

D. Ophthalmology (Report Appendix 10.5, pages 534-542)

No 12- and 18-month findings in the 0 and 300 ppm groups were considered to have been the result of test article administration. Therefore, no examinations were performed on the 1, 10 or 100 ppm animals.

E. Hematology

The only statistically significant differences between treated and control groups

were mean corpuscular volume and mean corpuscular hemoglobin in males; and, in females, erythrocytes, hemoglobin and hematocrit.

Table 5

SELECTED GROUP MEAN 18-MONTH HEMATOLOGY VALUES IN AN 18-MONTH DIETARY ADMIX CARCINOGENICITY STUDY IN MICE WITH CGA-248757

Parameter	Males (ppm)					Females (ppm)				
	0	1	10	100	300	0	1	10	100	300
Erythrocyte	7.8	7.5	8.2	8.4	8.6	8.6	8.3	7.5	7.8	6.1**
Hemoglobin	11.8	11.3	11.9	11.7	12.1	12.3	12.5	11.4	11.4	9.0**
Hematocrit	35.8	33.9	36.0	35.7	37.0	35.1	34.7	31.8	32.5	26.1**
MCV	46	45	44	43**	43**	41	42	43	42	44
MCH	15.0	15.0	14.5	14.0**	14.0**	14.2	15.0	15.2	14.6	15.0

Erythrocyte: mil/μL Hemoglobin: G/dl Hematocrit: %
MCV = Mean Corpuscular Volume: cu. microns
MCH = Mean Corpuscular Hemoglobin: PG
Statistical significance: ** = $p<0.01$
Data extracted from report Tables 9.17 and 9.18, pages 86-89.

F. Sacrifice and Pathology

1. Organ Weights (Report Tables 9.19 and 9.20, pages 90 and 91)

There was an apparent treatment related statistically significant increase in absolute and relative (to-body weight and to-brain weight) liver weights of males and females. In males, there was an increase ($p<0.05$) in the relative-to-body weight value in 100 ppm mice as well as increases ($p<0.05$ or 0.01) in absolute and relative-to-body weight values at 300 ppm. In females at dose levels of 100 and 300 ppm, there were significant ($p<0.05$ or 0.01) increases in absolute and relative (to-body weight and to-brain weight) liver weights.

Significantly lower ($p<0.05$) absolute and/or relative thymus weights were noted in males only. This was due to the elevated weight of the thymus in one control mouse (#149) 0.168 g with the range for the other mice being 0.005-0.063 g and the group mean being 0.025 g with a Standard Deviation of 0.028 (Report Appendix 10.11.1, page 726).

Table 6

GROUP MEAN ABSOLUTE AND RELATIVE LIVER WEIGHTS IN AN 18-MONTH DIETARY ADMIX CARCINOGENICITY STUDY IN MICE WITH CGA-248757

ppm =	0		1		10		100		300	
	Val	%	Val	%	Val	%	Val	%	Val	%
Males										
Body wt-g	45	-	44	97	42	93	43	96	42	92
Absolute-g	2.0	-	1.9	96	1.9	94	2.2	112	2.2*	114
Rel BW-%	4.4	-	4.3	99	4.4	101	5.1*	116	5.4**	122
Rel Br-%	363	-	355	97	347	95	412	113	430	118
Females										
Body wt-g	36	-	35	97	36	99	37	104	36	102
Absolute-g	1.5	-	1.5	98	1.6	107	1.7**	114	1.8**	115
Rel BW-%	4.3	-	4.3	101	4.6	108	4.7*	110	4.8**	113
Rel Br-%	281	-	279	99	300	107	324**	115	328**	116

Val = value (absolute or relative weight)-
% = percent of control
Rel = Relative Rel BW = Relative-to-Body Weight Rel Br = Relative-to-Brain Weight
Statistical Significance: * = $p<0.05$; ** = $p<0.01$
NOTE: All values have been "rounded off" by this Reviewer.
Data extracted from Report Tables 9.19 and 9.20, pages 90 and 91.

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2. Macroscopic Pathology (Report Tables 9.21-9.28, pages 92-132)

Livers, kidneys and thymuses appeared to be the only tissues which may have been grossly effected by test article administration. Table 7 summarizes the macroscopic observations in these tissues.

Table 7

INCIDENCES OF SELECTED MACROSCOPIC OBSERVATIONS IN AN 18-MONTH DIETARY ADMIX CARCINOGENICITY STUDY IN MICE WITH CGA-248757

Tissue Observation	Males (ppm)					Females (ppm)				
	0	1	10	100	300	0	1	10	100	300
Liver No. Mice	50	50	50	50	50	50	50	50	50	50
Discoloration,focal	4	1	4	12	7	1	2	8	7	5
Discoloration,focal, raised	3	1	2	1	7	1	0	1	1	4
Masses	12	15	11	23	30	2	1	4	4	2
Kidney No. Mice	50	50	50	50	50	50	50	50	50	50
Cyst(s)	16	16	26	16	22	5	8	5	10	10
Thymus No. Mice	50	50	50	50	50	50	50	50	50	50
Enlarged	2	0	2	0	0	3	2	4	6	9

Data extracted from Report Tables 9.24 and 9.28, pages 106-113 and 126-132.

3. Microscopic Pathology (Report Tables 9.29-9.38, pages 133-383)

a. Non-Neoplastic Lesions (See Table 8)

Treatment-related lesions were primarily noted in the liver. Some of these lesions were observed at dose levels of 10 ppm and greater. The incidence and statistical significance is noted in Table 8. The liver effects did not affect survival in either sex. In addition, the incidences and/or severity of amyloidosis were greater in the 300 ppm females (findings in duodenum, ileum, jejunum, kidney, thyroid and uterus). Report page 30 stated that this was not considered to be treatment related. There was no correlation between the macroscopic and microscopic observations in the kidneys and thymuses.

b. Neoplastic Lesions (See Table 9)

The liver was the only tissue which showed a test article effect on the incidence of microscopic neoplasia. At 100 and 300 ppm, males had a greater incidence ($p<0.05$) of carcinomas than the control group.

Although not significant, the incidence of adenomas was greater in the 100 and 300 ppm males than in the control or two lower dose groups (19 and 20 versus 12, 9 and 10 in the 0, 1 and 10 ppm groups) For carcinomas and/or adenomas, a significant ($p<0.01$) increase was observed at 300 ppm compared with the control (31 versus 15), although the incidence at 100 ppm was elevated but not significant (26).

For females, there were no statistically significant differences between treated and control incidences regarding carcinomas, adenomas or adenomas/carcinomas. However, for adenomas at 100 and 300 ppm, the incidences in these two groups were 7 compared with two controls. In addition, incidences of adenomas/carcinomas at 100 and 300 ppm were 9 and 8, respectively, compared with 3 controls.

TABLE 8 (INCIDENCE OF MICROSCOPIC NON-NEOPLASTIC LIVER FINDINGS) APPEARS ON PAGES 17 AND 18

TABLE 8

INCIDENCES OF MICROSCOPIC NON-NEOPLASTIC LIVER FINDINGS IN A 18-MONTH DIETARY ADMIX CARCINOGENICITY STUDY IN MICE WITH CGA-248757

Observation	Males (ppm)					Females (ppm)				
	0	1	10	100	300	0	1	10	100	300
No. of Tissues =	50	50	50	50	49	50	50	50	50	50
Focus, basophilic cell 1	1	1	0	4	1	0	1	0	1	0
2	1	1	1	1	2	0	0	1	1	0
3	0	0	1	4	3	0	0	0	1	2
4	0	0	0	2	2	0	0	0	0	0
5	0	0	0	0	1	0	0	0	0	0
	2	2	2	11**	9**	0	1	1	3	2
Centrilobular necrosis, individual cell 1	0	1	14	10	8	0	0	11	14	12
2	1	0	7	16	18	1	2	1	1	5
3	0	0	1	9	12	0	0	1	0	0
4	0	0	0	0	2	0	0	0	0	0
	1	1	22**	35**	40**	1	2	13	15	17**
Centrilobular cell degeneration 1	0	0	10	6	7	0	1	10	11	9
2	2	1	10	27	16	0	0	3	7	10
3	1	0	1	5	12	0	1	1	0	0
4	0	0	0	0	2	0	0	0	0	0
	3	1	21**	38**	37**	0	2	14	18	19
Histiocytic pigment 1	8	12	13	5	12	9	9	6	9	11
2	5	6	18	23	23	7	8	17	15	23
3	0	0	3	13	10	4	6	9	11	6
4	1	0	0	3	1	0	0	1	0	2
	14	18	34**	44**	46**	20	23	33	35	42
Centrilobular cell hypertrophy 1	9	11	13	4	1	3	6	7	2	1
2	9	2	10	12	4	0	0	4	2	1
3	1	0	0	1	0	0	0	0	0	0
	19	13	23	17	5*	3	6	11**	4	2

Observation	Males (ppm)					Females (ppm)				
	0	1	10	100	300	0	1	10	100	300
No. of Tissues =	50	50	50	50	49	50	50	50	50	50
Karyomegaly 1	11	14	13	15	18	6	2	14	15	13
2	10	8	17	17	19	1	2	8	8	12
3	1	3	8	13	8	0	0	1	0	2
	22	**25**	**38****	**45****	**45****	7	**4**	**23****	**23****	**27****
Syncytial cell formation 1	0	0	1	6	3	1	0	0	0	0
2	0	0	0	1	0	0	0	0	1	0
	0	**0**	**1**	**7****	**3**	**1**	**0**	**0**	**1**	**0**
Chronic-active inflammation 1	19	15	19	26	9	16	16	12	11	14
2	4	10	9	10	14	12	12	17	13	16
3	1	0	2	2	1	4	8	11	17	7
4	1	0	0	0	0	0	0	0	0	0
	25	**25**	**30**	**38**	**24**	**32**	**36**	**40***	**41****	**37**
Bile duct hyperplasia 1	1	0	0	0	7	0	0	0	0	0
2	0	0	0	1	2	0	0	0	0	0
3	1	0	0	0	0	0	0	1	0	0
4	0	0	0	0	0	1	0	0	0	0
	2	**0**	**0**	**1**	**9**	**1**	**0**	**1**	**0**	**0**
Centrilobular fatty change 1	1	3	0	1	0	1	0	0	3	7
2	0	0	1	0	0	0	0	0	2	7
3	0	1	0	0	0	0	0	0	2	5
	1	**4**	**1**	**1**	**0**	**1**	**0**	**0**	**7**	**19****
Focus,eosinophilic cell 1	0	0	0	3	0	0	0	0	1	1
2	0	0	0	4	6	1	0	1	1	2
3	0	1	0	1	2	0	0	1	0	0
4	0	0	0	0	1	0	0	0	0	0
	0	**1**	**0**	**8**	**9**	**1**	**0**	**2**	**2**	**3**

bold numbers = total number of animals with the observation

1, 2, 3, 4 or 5 = "severity" of observations: 1 = minimum; 2 = mild; 3 = moderate; 4 = marked; 5 = severe [Detailed explanations presented on Report page 21]

Statistical Significance: * = $p<0.05$; ** = $p<0.01$

Data extracted from Report Tables 9.29-9.36, pages 133-383.

Table 9

INCIDENCES OF MICROSCOPIC NEOPLASTIC LIVER FINDINGS IN AN 18-MONTH DIETARYADMIX CARCINOGENICITY STUDY IN MICE WITH CGA-248757

Observation	Males (ppm)					Females (ppm)				
	0	1	10	100	300	0	1	10	100	300
No. of Tissues =	50	50	50	50	49	50	50	50	50	50
Adenomas	12	9	10	19	22	2	0	1	7	7
	24	**18**	**20**	**38**	**45**	**4**	**0**	**2**	**14**	**14**
Carcinomas	3	5	6	12*	13*	1	0	1	2	2
	6	**10**	**12**	**24**	**27**	**2**	**0**	**2**	**4**	**4**
Adenomas/Carcinomas	15	13	14	26	31*	3	0	2	9	8
	30	**26**	**28**	**52**	**63**	**6**	**0**	**4**	**18**	**16**

Non-bold numbers = number of mice **Bold numbers** = % of mice
Statistical significance: * = $p<0.05$; ** = $p<0.01$; ANOVA/Dunnett's Test or Fisher Exact Probability Test
Data extracted from Report Tables 9.37 and 9.38, pages 372-383.

Historical Control Hepatocellular Tumor Incidence (Appendix 10.13.7, pages 1511 and 1512).

For males, the Registrant's 9 studies indicated a maximum of 31.9% (15/47) of the mice (in one study) with combined adenomas/carcinomas. The maximum incidence regarding Charles River male animals (as presented in the Report) was 16.3% for adenomas and 6.0% for carcinomas (no indication of combined incidence). The current 18-month study showed an increased incidence (not statistically significant) of adenomas at 100 and 300 ppm (38 and 45%, respectively) compared with 24% in the control group (18 or 20% in the 1 and 10 ppm groups). For carcinimas only, control males had 6% and the 100 and 300 ppm groups had 24 and 27% ($p<0.05$), respectively. Adenomas/carcinomas had an incidence of 52 and 63% at 100 and 300 ppm, respectively ($p<0.05$ at 300 ppm) compared with 30, 26 and 28% for the 0, 1, and 10 ppm groups. Because of the increased incidence of tumors at 100 and 300 ppm in males compared with concurrent controls as well as historical controls, it is indicated that the findings are due to CGA-248757 administration.

In females, there is a non-statistically significant increase in the percent of 100 and 300 ppm mice in the current 18-minth study when compared with concurrent controls. The maximum incidence in any study (Registrant or Charles River data) was 3.3%. The maximum for the concurrent controls was 6% with the 100 and 300 ppm groups having 14% for adenomas only and 4% for carcinomas only. Therefore, there is the suggestion that the increase in tumors in female mice was due to test article administration.

Table 10

HISTORICAL CONTROL HEPATOCELLULAR TUMOR DATA FROM 78-WEEK MOUSE STUDIES CONDUCTED AT THE CIBA-GEIGY ENVIRONMENTAL HEALTH CENTER AND FROM THE CHARLES RIVER LABORATORY

Study	Males				Females			
	Adenoma	Carcinoma	Combined	%	Adenoma	Carcinoma	Combined	%
A	5	1	6/55	10.9	1	0	1/60	1.7
B	5	0	5/59	8.5	2	0	2/60	3.3
C	4	1	5/57	8.8	0	0	0/60	0
D	5	3	8/50	16.0	1	0	1/51	2.0
E	14	1	15/47	31.9	1	0	1/50	2.0
F	8	5	13/59	22.0	0	0	0/60	0
G	7	0	7/60	11.7	0	0	0/60	0
H	4	2	5/60	8.3	0	0	0/60	0
I	8	0	8/50	16.0	0	0	0/50	0
CHAR. RIVER	Males: adenomas = 0-16.3% Males: carcinomas = 0-6.0%				Females: adenomas = 0-2.7% Females: carcinomas = 0-0.7%			

III. DISCUSSION

AUTHOR'S COMMENTS (Report page 12)

1. There were no test article effects on: survival, clinical signs, food consumption or feed efficiency.
2. In 300 ppm males only, body weight gains were decreased slightly during the last weeks of the study.
3. After 18 months, there was a slight, but treatment-related decrease in mean corpuscular volume and mean corpuscular hemoglobin in males ≥100 ppm. At 300 ppm in

females only, there was almost a 30% decrease in RBC counts, hemoglobin and hematocrit.

4. Absolute and relative liver weights were greater than controls ≥100 ppm in males and females. At the same doses, an increased incidence of mice with liver masses and foci were reported for males only. The incidence of female livers with discolored foci was slightly increased ≥10 ppm.

5. The incidences of mice with hepatocellular adenomas and/or carcinomas were increased in males and females ≥100 ppm. Non-neoplastic liver lesions seen primarily in the centrilobular region, included: cell necrosis and degeneration; histiocytic pigmentation; and karyomegaly. These were increased in incidence and/or severity in both sexes at ≥10 ppm. Other lesions in males included: basophilic foci (≥100 ppm), bile duct hyperplasia (300 ppm) and syncytial cell formation (≥100 ppm). In females, there were: fatty changes (≥100 ppm), centrilobular hypertrophy (10 ppm) and chronic active inflammation (10 and 100 ppm).

REVIEWER'S DISCUSSION

Analytical data for stability, concentration and homogeneity were considered to have been within acceptable limits.

There were no clinical signs which were considered to have been the result of test article administration. Survival in treated groups of both sexes was equal to or greater than respective control values.

There was a significantly ($p<0.01$) lower group mean body weight in 300 ppm males at the week 78 weighing (8% lower). The group mean male body weight gain over the course of the study was 17% below controls (15 g versus 18 g, $p<0.05$). These statistically significant differences were noted only at the week 78 weighing. No decrease in body weight gain was observed in females.

Group mean food consumption was slightly less (statistically significant) in all treated male (not female) groups (except at 100 ppm), but only during the first week of the study. No consistent effect on food consumption was reported for females. Food efficiency did not appear to be affected (calculated only through week 40).

Test article intake (mg/kg/day) over the course of the study was as follows (1, 10, 100 and 300 ppm): males = 0.1, 1.0, 10 and 32; females = 0.1, 1.2, 12 and 37.

Ophthalmic examinations on 0 and 300 ppm mice did not reveal any test article related findings and, therefore, examinations were not performed on the 1, 10 or 100 ppm animals.

The mean corpuscular volume and mean corpuscular hemoglobin for the 100 and 300 ppm males only were lower ($p<0.01$) than the respective control values. Group mean erythrocyte, hemoglobin and hematocrit values for 300 ppm females were below ($p<0.01$) respective control values.

Generally, though not always statistically significant, there were increases in male and female absolute and relative (to-body weight and to-brain weight) liver weights at 100 and 300 ppm. Group mean relative (to-body weight) liver weights in 100 and 300 ppm males were greater ($p<0.05$ or 0.01) than controls with absolute weights being greater ($p<0.05$) at 300 ppm. In females, absolute and relative (to body weight and brain weight) liver weights were heavier than controls ($p<0.05$ or 0.01) at 100 and 300 ppm.

Macroscopic findings indicated an increase in the incidence of liver focal discoloration (also raised) at 100 and/or 300 ppm in males and at 10, 100 and 300 ppm in females. The number of mice with liver masses appeared to be increased at 100 and 300 ppm in males only. There was the suggestion of an increased incidence of kidney cysts in males (10 and 300 ppm) and females (100 and 300 ppm). Enlarged thymuses appeared in more 100 and 300 ppm females only than in the control group.

The only tissue with consistent non-neoplastic microscopic test article related findings was the liver. Statistically significant ($p<0.01$) or biologically suggestive increases in the incidences in males and/or females at 10, 100 and 300 ppm occurred for the following: centrilobular necrosis, centrilobular cell degeneration, histiocytic pigmentation and karyomegaly. Bile duct hyperplasia was noted at a higher incidence (not statistically significant) in 300 ppm males. High dose (300 ppm) females showed a higher incidence ($p<0.01$) of centrilobular fatty change. The incidences of animals with tumors had statistical significance ($p<0.05$ or 0.01) only in males at the following: carcinomas at 100 and 300 ppm and adenomas/carcinomas at 300 ppm. However, regarding adenomas only, the number of males with this finding were (0, 1, 10, 100 and 300 ppm): 12, 9, 10, 19 and 22. Though not statistically significant, in females, there were greater incidences in 100 and 300 ppm groups (compared with controls) for adenomas (2, 0, 1, 7 and 7) and adenomas/carcinomas (3, 0, 2, 9 and 8). The tumor incidences were greater than those presented in the historical control data.

In conclusion, the primary target tissue is the liver. This is evidenced by non-neoplastic as well as neoplastic changes. In addition, the erythropoietic system also appears to be affected.

Guideline Series 84: **MUTAGENICITY**

Reviewed by: Byron T. Backus, Ph.D. Byron T. Backus 12/22/94
Section II, Toxicology Branch II (7509C)
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Section II, Tox Branch II (7509C)

DATA EVALUATION REPORT IX

STUDY TYPE: Lethal DNA Damage in E. coli.

CHEMICAL: CGA-248757 Technical; 9-(4-chloro-2-fluoro-5-methoxycarbonylmethylthiophenylimino)-8-thia-1,6-diazabicyclo [4.3.0]nonan-7-one; [(2-chloro-4-fluoro-5-[(tetrahydro-3-oxo-1H,3H-[1,3,4-thiadiazol[3,4a]pyridazin-1-ylidene)amino]phenyl]thio] acetic acid methyl ester.

PC Code: 108803

MRID NUMBER: 433484-28

SYNONYMS/CAS No.: KIH-9201

SUBMITTER: Ciba-Geigy Corporation
P.O. Box 18300
Greensboro, NC 27419

TESTING FACILITY: Life Science Research Ltd.
Suffolk, England

TITLE OF REPORT: KIH-9201: Assessment of its Ability to Cause Lethal DNA Damage in Strains of Escherichia coli.

AUTHORS: May, K.

LABORATORY STUDY NUMBER: 90/KCI110/0536

STUDY COMPLETION DATE: February 9, 1990

EXECUTIVE SUMMARY: Three different strains of E. coli (WP2, WP67, and CM871), varying in DNA repair capabilities, were exposed to concentrations of KIH-9201 ranging from 32 - 10,000 µg/ml (2-hr and 18-hr exposures). There were no indications of any differences in cell survival between the three strains. **Under the assay conditions there was no indication that exposure to KIH-9201 was associated with lethal DNA damage in these strains of E. coli.** The positive controls elicited appropriate responses.

STUDY CLASSIFICATION: Acceptable. This study and its negative findings can be used as supporting data for purposes of registration and/or reregistration. While the March, 1991 Subdivision F Addendum 9 Mutagenicity Guidelines do not specify a lethal DNA damage study in E. coli as part of the initial battery of mutagenicity data requirements, it is stated that: "If tests for end-points that may be predictive of mutagenicity are performed in addition to the initial battery, the results of such tests shall be submitted to the OPP along with results from the initial battery."

A. MATERIALS

1. Test Material: KIH-9201 (also known as CGA 248757)
   Description: White powder
   Batch number: G26-04
   Stated purity: 97.8%
   Receipt date: December 8, 1989
   Stability: reported as stable for 1 year under refrigeration at <5°C.
   Contaminants: not reported
   Solvent used: DMSO

2. Control Materials:
   Vehicle: DMSO
   Negative: From p. 17: "Ampicillin (25 µg/ml), dissolved in distilled water, was included in the main assay as a negative control; this antibiotic is toxic to bacterial cells by a replication-dependent mechanism not involving DNA damage. Equal toxicity to all strains (or greater toxicity to the WP2 strain which is faster-growing) demonstrates that chemicals which are lethal by non-genetic mechanisms do not give positive results in the E. coli assay system."
   Positive: From p. 17: Mitomycin C (0.05 µg/ml) in the absence of S-9 mix.
   2-Aminoanthracene (5 µg/ml) "was examined in the absence and presence of S-9 mix." (2-AA would be active only in the presence of S-9 mix).

3. Activation: (refer to p. 32) S9 derived from

_X_ Aroclor 1254	_X_ induced	_X_ rat	_X_ liver
___ phenobarbital	___ non-induced	___ mouse	___ lung
___ none		___ hamster	___ other
___ mixed phenobarbital and 3-methylcholanthrene			

S9 mix composition:

0.1M NADP, Na salt	0.4 ml
0.1M glucose-6-phosphate, Na salt	0.5 ml
0.4M $MgCl_2.6H_2O$/1.65M KCl	0.2 ml
Supernatant from liver homogenate	1.5 ml
0.1M KH_2PO_4-Na_2HPO_4 buffer, pH 7.4 to a final volume of 10.0 ml	

4. Test cells: From p. 13: "Cultures of the WP2 strain of Escherichia coli, and its derivatives, WP67 and CM871, were derived from cultures provided by Dr. Michael Green, Cell Mutation Unit, Sussex University. The strains are virtually isogenic except for the possession or lack of specific DNA repair systems...

WP2 - is a tryptophan-dependent auxotroph. This strain is fully proficient in DNA repair.

WP67 - derived from WP2; it is deficient in uvrA and polA repair systems.

CM871 - derived from WP2; it is deficient in uvrA, recA and lexA repair systems.

"Cultures of all organisms were prepared by overnight incubation of nutrient broth (Oxoid No. 2), freshly inoculated from a frozen culture stock."

"New batches of frozen culture stocks, stored at -80°C, are prepared at intervals from a central stock held in a liquid nitrogen refrigerator. Samples from each new batch are thawed ...and checked for growth inhibition on nutrient agar plates spread with Mitomycin C solutions at various concentrations up to 25 µg/plate: growth of strain WP67 is inhibited at lower Mitomycin C concentrations than strain WP2, and strain CM871 is inhibited at even lower concentrations. The strains are also checked for tryptophan requirement, characteristic spontaneous reversion rate and increased reversion in the presence of MNNG."

Results obtained with the culture stocks used in this assay are shown in appended page 1.

5. On p. 6 of the report there is a signed and dated declaration that the study was performed under the principles of Good Laboratory Practices; on p. 8 there is a signed and dated Quality Assurance Statement.

B. TEST PERFORMANCE AND RESULTS:

1. Dose selection and preliminary toxicity test: From p. 15: "An overnight broth culture of E. coli strain WP2 was diluted in 0.1M phosphate buffer to approximately 2 x 10^8 cells/ml. Aliquots (0.1 ml) of each of 6 concentrations of KIH-9201 solution (in DMSO) were added to sterile bottles containing the bacterial; suspension (2 ml); in this way, test concentrations over the range 32 µg/ml to 10 mg/ml were established. More buffer or S-9 mix (0.2 ml) was then added. All cultures were prepared in duplicate... After incubation at 37°C for 2 hours in a shaking water bath, and again after 18 hours, aliquots (0.1 ml) were removed and dilutions (10^{-2}, 10^{-4}, 10^{-5} and 10^{-6}) plated on nutrient agar plates...three 20 µl drops of each dilution were plated."

"Bottles were also set up containing the diluted bacterial culture (2 ml) with buffer (0.3 ml) alone, and the diluted bacterial culture (2 ml) with solvent (0.1 ml) and S-9 mix or buffer, to provide data on the survival of both untreated cells and cells treated with the solvent. Checks for sterility were performed on the KIH-9201 solution and the S-9 mix, by spreading on nutrient agar."

"All plates were incubated for 1 day at 37°C. Toxicity was assessed by counting the numbers of colonies per 20 µl spot after incubation. The concentration of KIH-9201 selected as the maximum exposure level for use in the main assay is normally the lowest level at which anti-bacterial effects are shown. In the absence of such effects, a maximum level of 10 mg per ml was selected..."

Results: Refer to appended pages 2 and 3 for plate counts. On the basis of these findings the concentrations of KIH-9201 used in the subsequent assay were 100, 316, 1000, 3160 and 10,000 µg/ml.

2. Main assay: From p. 16: "Overnight broth cultures of the E. coli strains were diluted in 0.1M phosphate buffer to approximately 2 x 10^8 cells/ml. Aliquots (0.1 ml) of each level of KIH-9201 solution were added to sterile bottles containing the bacterial suspension (2 ml). Where appropriate, S-9 mix or buffer (0.2 ml) was added. All cultures were prepared in duplicate, and the bottles were inverted to mix. After incubation at 37°C for 2 hours in a shaking water bath, and again after 18 hours, aliquots (0.1 ml) were removed and dilutions (10^{-2}, 10^{-4}, 10^{-5} and

10^{-6}) plated on nutrient agar plates...three 20 µl drops of each dilution were plated. Test were conducted in this way using five concentrations of KIH-9201 in the incubation medium: 100, 316, 1000, 3160 and 10000 µg per ml. The solvent (DMSO) was also tested, to confirm its inability to show differential lethality towards the three strains."

"Bottles were also set up containing diluted bacterial culture (2 ml) with buffer (0.3 ml) alone. These cultures were also sampled after 2 and 18 hours, to provide data on the survival of untreated cells."

"Checks for sterility were performed on the KIH-9201 solution and the S-9 mix, by spreading on nutrient agar."

"All plates were incubated for 1 day at 37°C. Differential lethality was assessed by counting the numbers of colonies per 20 µl spot after incubation."

Results: Refer to appended pages 4 (2-hr exposure) and 5 (18-hr exposure) for cell survivals (expressed as percentage survivals relative to untreated controls), and to appended p. 6 for the coefficients of survival. There were no indications of any consistent differential survival rates associated with exposure to KIH-9201 in either the presence or absence of S-9 mix. The positive controls elicited the appropriate responses.

C. DISCUSSION AND CONCLUSIONS:

There were no indications of any differences in cell survival between the three strains of E. coli (WP2, WP67, CM871) used in this assay. Under the assay conditions then there was no indication that exposure to KIH-9201 was associated with lethal DNA damage in these strains of E. coli. The positive controls elicited the appropriate responses.

This study and its negative findings are acceptable, and can be used as supporting data for purposes of registration and/or reregistration. While the March, 1991 Subdivision F Addendum 9 Guidelines do not specify a lethal DNA damage assay in DNA-repair deficient strains of E. coli as part of the initial battery of mutagenicity data requirements, it is stated (p. 7) that: "If tests for endpoints that may be predictive of mutagenicity are performed in addition to the initial battery, the results of such tests shall be submitted to the OPP along with results from the initial battery."

Page_____ is not included in this copy.

Pages 74 through 79 are not included.

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 quality control procedures.

_____ Identity of the source of product ingredients.

_____ Sales or other commercial/financial information.

_____ A draft product label.

_____ The product confidential statement of formula.

_____ Information about a pending registration action.

X FIFRA registration data.

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Guideline Series 84: MUTAGENICITY

Reviewed by: Byron T. Backus, Ph.D. Byron T. Backus 12/22/94
Section II, Toxicology Branch II (H7509C)
Secondary reviewer: K. Clark Swentzel
Section II, Tox Branch II (H7509C) K. Clark Swentzel 12/22/94

DATA EVALUATION REPORT I

STUDY TYPE: Salmonella typhimurium/mammalian microsome mutagenicity (Ames) assay

CHEMICAL: CGA-248757 Technical; 9-(4-chloro-2-fluoro-5-methoxycarbonylmethylthiophenylimino)-8-thia-1,6-diazabicyclo [4.3. 0]nonan-7-one; [(2-chloro-4-fluoro-5-[(tetrahydro-3-oxo-1H,3H-[1,3,4-thiadiazol[3,4a]pyridazin-1-ylidene)amino]phenyl]thio] acetic acid methyl ester.

PC Code: 108803

MRID NUMBER: 433484-29

SYNONYMS/CAS No.: KIH-9201

SPONSOR: Ciba-Geigy Corporation
P.O. Box 18300
Greensboro, NC 27419

TESTING FACILITY: Life Science Research Ltd.
Suffolk IP23 7PX
England

TITLE OF REPORT: KIH-9201: Assessment of Mutagenic Potential in Amino-Acid Auxotrophs of Salmonella typhimurium and Escherichia coli (The Ames Test).

AUTHOR: May, K.

STUDY NUMBER: LSR Report No. 90/KCI111/0358

STUDY COMPLETION DATE: March 2, 1990

CONCLUSION(S) - Executive Summary:

KIH-9201 (CGA-248757) was tested at 50 to 5000 µg/plate (initial assay) with S. typhimurium strains TA 98, TA 100, TA 1535, TA 1537 and TA 1538 and with E. coli WP2 uvrA, and at the same concentra-

tions with S. typhimurium strains TA 98, TA 100, TA 1535 and TA 1537 (confirmatory assay). **KIH-9201 was negative for inducing reverse gene mutations in S. typhimurium strains TA 1535, TA 1537, TA 1538, TA 98 or TA 100, or in E. coli WP2 uvrA either in the presence or absence of S9 activation.** There was little (if any) cytotoxicity at the highest (limit) dose. Analytical results demonstrate that the actual dosage levels of test material were sufficiently close to the nominal levels, so that the test material was tested at up to the limit dose. The data adequately demonstrate that KIH-9201 (CGA-248757 Technical) is negative in this assay.

STUDY CLASSIFICATION: Acceptable. The study satisfies Guideline requirements (§84-2) for an Ames assay, and is acceptable as supporting data for purposes of registration and/or reregistration.

A. MATERIALS

1. Test Material: KIH-9201
   Description: white powder
   Lot number: G26-04
   Purity: 97.8% (from p. 41)
   Receipt date: 8 December, 1989
   Stability: stable for 1 year under refrigeration (<5°C).
   Contaminants: Not reported
   Solvent used: Dimethyl sulfoxide (DMSO)
   Other provided information: The test material was stored at about 4°C and protected from light until required.

2. Control Materials:
   Solvent/final concentration: 0.1 ml/plate
   Positive: Non-activation:

Sodium azide	0.5	µg/plate TA 1535, TA 100
2-Nitrofluorene	1.0	µg/plate TA 98
2-Nitrofluorene	2.0	µg/plate TA 1538
9-Aminoacridine	80.0	µg/plate TA 1537
2-Aminoanthracene	2.0	µg/plate TA 1535
2-Aminoanthracene	20.0	µg/plate WP2 uvrA
N-Ethyl-N'-nitro-N-nitrosoguanidine (ENNG)	2.0	µg/plate WP2 uvrA
Benzo[a]pyrene	5.0	µg/plate TA 1537, TA 100, TA 98, TA 1538

   Activation:

2-Aminoanthracene	2.0	µg/plate TA 1535
2-Aminoanthracene	20.0	µg/plate WP2 uvrA
Benzo[a]pyrene	5.0	µg/plate TA 1537, TA 100, TA 98, TA 1538

"All positive control compounds were prepared as solutions in DMSO, except sodium azide, which was dissolved in distilled water."

3. Activation: S9 derived from

X Aroclor 1254	X induced	X rat	X liver
___ phenobarbital	___ non-induced	___ mouse	___ lung
___ none		___ hamster	___ other

___ mixed phenobarbital and 3-methylcholanthrene

S9 mix composition:

0.1M NADP solution	0.4 ml
0.1M glucose-6-phosphate	0.5 ml
0.4M $MgCl_2.6H_2O$/1.65M KCl	0.2 ml
Supernatant from liver homogenate	1.5 ml
0.1M KH_2PO_4-Na_2HPO_4 buffer, pH 7.4 to a final volume of 10.0 ml	

4. Test organisms: S. typhimurium strains

___ TA97 X TA98 X TA100 ___ TA102 ___ TA104
X TA1535 X TA1537 X TA1538 ; list any others:
E. coli WP2 uvrA
Properly maintained? Yes
Checked for appropriate genetic markers (rfa mutation, R factor)? Yes

5. Test compound concentrations used (µg/plate): From p. 12: "The maximum concentration of test material employed was selected with the aid of a preliminary toxicity test with strain TA 98 and WP2 uvrA." From information on p. 16 eight different concentrations of test material (2.5 µg to 5 mg/plate) were tested.

In both the initial and confirmatory assays the concentrations assayed were 50, 158, 500, 1580 and 5000 µg/plate ±S9.

6. Criteria for a valid assays: Not stated.

7. Criteria for a negative response: Not stated.

8. Criteria for a positive response: Not stated.

9. There is a signed and dated Good Laboratory Practice statement (from the performing laboratory) on p. 6 of the report. There is a signed and dated "Certification of Good Laboratory Practices" from the sponsor on p. 3 of the report. There is a signed and dated Quality Assurance Statement (giving the dates of inspects and reports to study director and/or management) on p. 8 of the report.

B. TEST PERFORMANCE AND RESULTS:

1. Type of Salmonella assay:
   - x standard plate test
   - ___ pre-incubation (___ minutes)
   - ___ "Prival" modification (i.e. azo reduction method)
   - ___ spot test

2. Preliminary cytotoxicity assay: In the preliminary range-finding assay there was a slight thinning of the background lawn in S. typhimurium strain TA 98 at the highest concentration tested (5000 µg/plate), along with a decrease in the mean number of revertant colonies (a mean of 16 at 5000 µg/plate, compared with a mean of 31.3 in the solvent control plates). For E. coli WP2 uvrA there was a slight decrease in number of revertants at the highest dose (5000 µg/plate: mean of 12.3 vs. a solvent control mean of 20), with, however, no effect noted on the background lawn.

3. Mutagenicity assays: In the initial and confirmatory assays the concentrations tested were 50, 158, 500, 1580 and 5000 µg/plate both with and without metabolic (S9) activation.

   Refer to appended pages 1 through 10 for the results of the initial and confirmatory assays. There was no indication of any increased number of revertants associated with exposure to the test chemical. The positive controls elicited appropriate responses (from p. 21: "Appropriate positive control chemicals (with S-9 mix where required) induced marked increases in revertant colony numbers with all strains, confirming sensitivity of the cultures and activity of the S-9 mix.").

C. CONCLUSIONS:

The results of this study adequately demonstrate that there is no indication of a mutagenic response at the histidine locus in Salmonella typhimurium strains TA 98, TA 100, TA 1535, TA 1537 or TA 1538, or at the tryptophan or transfer RNA loci in E. coli WP2 uvrA following exposure to the test material at up to the limit dose of 5000 µg/plate both in the presence and absence of rat S-9.

It is concluded then that the study, with its negative findings, is acceptable in satisfying the 84-2 Guideline requirement for an Ames assay for KIH-9201 (also known as CGA-248757) for purposes of registration and/or reregistration.

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Pages 84 through 93 are not included.

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The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

Guideline Series 84: **MUTAGENICITY**

Reviewed by: Byron T. Backus, Ph.D. Byron T. Backus 12/22/94
Section II, Toxicology Branch II (7509C)
Secondary Reviewer: K. Clark Swentzel K. Clark Swentzel 12/22/94
Section II, Tox Branch II (7509C)

DATA EVALUATION REPORT VIII

STUDY TYPE: In vitro Autoradiographic DNA Repair Test on Rat Hepatocytes (Unscheduled DNA Synthesis Assay)

CHEMICAL: CGA-248757 Technical; 9-(4-chloro-2-fluoro-5-methoxycarbonylmethylthiophenylimino)-8-thia-1,6-diazabicyclo [4.3. 0]nonan-7-one; [(2-chloro-4-fluoro-5-[(tetrahydro-3-oxo-1H,3H-[1,3,4-thiadiazol[3,4a]pyridazin-1-ylidene)amino]phenyl]thio] acetic acid methyl ester.

PC Code: 108803

MRID NUMBER: 433484-30

SYNONYMS/CAS No.: KIH-9201

SUBMITTER: Ciba-Geigy Corporation
P.O. Box 18300
Greensboro, NC 27419

TESTING FACILITY: Ciba-Geigy Ltd.
Genetic Toxicology
Basle, Switzerland

TITLE OF REPORT: CGA-248757 Technical Autoradiographic DNA Repair Test on Rat Hepatocytes

AUTHORS: Hertner, T.

LABORATORY STUDY NUMBER: 921082

STUDY COMPLETION DATE: December 15, 1992

EXECUTIVE SUMMARY: In an in vitro rat hepatocyte UDS study performed with both initial and confirmatory assays, there was no indication of any induction of UDS at doses ranging from 3.7 to 200 µg/ml. Cytotoxicity precluded evaluation at 400 µg/ml, and a precipitate occurred at concentrations ≥100 µg/ml. In a preliminary assay, there was no indication of DNA synthesis inhibition at concentrations ranging from 3.7 to 200 µg/ml.

(94)

STUDY CLASSIFICATION: Acceptable. This study and its negative findings can be used as supporting data for purposes of registration and/or reregistration. While the March, 1991 Subdivision F Addendum 9 Mutagenicity Guidelines do not specify an in vitro DNA repair test (UDS assay) as part of the initial battery of mutagenicity data requirements, it is stated that: "If tests for end-points that may be predictive of mutagenicity are performed in addition to the initial battery, the results of such tests shall be submitted to the OPP along with results from the initial battery."

A. MATERIALS

1. Test Material: CGA 248757 tech. (also known as KIH-9201)
   Description: White powder
   Batch number: FL-920346
   Stated purity: 97.7%
   Receipt date: March 16, 1993
   Stability: reported as "stable"
   Contaminants: not reported
   Solvent used: DMSO; from p. 17: "CGA 248757 tech. was dissolved in DMSO at room temperature. The solution was filtered through a 0.22 µm filter. The highest attainable concentration of CGA 248757 tech. was determined in a preliminary solubility test to be 167 mg/ml".

2. Control Materials:
   Vehicle: DMSO; from p. 17: "The final concentration of DMSO in the culture medium was 1%."
   Positive: From p. 12: 2-Acetylaminofluorene (2-AAF at 45 µmol/l)

3. Test cells: From p. 16: "Primary hepatocytes were isolated from adult male rats (Tif.RAIf (SPF), weight 170-350 g) by in situ-collagenase perfusion... The liver was perfused in situ through the portal vein for eight to twelve minutes with calcium-free Hank's solution (BSS) which was aerated with carbogen (95% O_2, 5% CO_2). Its temperature was about 37°C and the Ph about 7.4. After insertion of a canula into the thoracic part of the vena cava, the perfusion was continued for [a] further 10-18 minutes by recirculation of BSS, which was supplemented with 0.05% collagenase and 5 Mm $CaCl_2$. The liver was then carefully excised and placed into a dish containing calcium-free BSS supplemented with 100 U/ml penicillin, 100 µg/ml streptomycin and 2.5 µg/ml amphotericin (4°C). After opening the Glisson's capsule, the cells were dispersed by gently shaking the liver in the solution. The cells were then filtered (mesh width of 61 µm) and washed twice with BSS (sedimentation rate of 50 g for 3 minutes at 2°C). Finally, the cells were suspended in Williams' medium E and analysed for viability by trypan-blue exclusion. The viability of hepatocytes prepared in this was generally greater than 80%."

4. On p. 7 of the report there is a signed and dated sheet titled "Statement of Compliance with Good Laboratory Practice;" there is also a "Certification of Good Laboratory Practice and Verification of a Complete and Unaltered Copy of the Report by the Study Sponsor" on p. 6, and a signed and dated Quality Assurance Statement on p. 9.

B. TEST PERFORMANCE AND RESULTS:

1. Dose selection and preliminary assays: Part I. From p. 18: "A toxicity test was firstly performed to determine the highest concentration to be used in the DNA repair assay."

"The toxicity-test was initiated four to five hours after having removed the unattached cells, by supplementing the medium in the compartments with the test material and the vehicle control in order to give the desired concentrations. The test substance was dissolved in DMSO and eleven stock solutions were prepared by serial dilution with the vehicle. From each, a volume of 20 µl was added to two compartments containing 2 ml medium. The highest of the eleven compartments was 1670 µg/ml, the lowest 1.6 µg/ml. In addition, a negative control containing the vehicle only was run. The treatment period with the test substance lasted for 16-18 hours."

"After treatment, the medium was removed and the cells were washed twice with BSS and stained with Trypan blue solution (0.2%) for five minutes. After washing with BSS, the cells were fixed. The percentage of unstained (viable) cells was evaluated by counting 100 cells and the morphological quality of the viable cells was judged."

"The concentration best suited as the highest to be used in the DNA repair test was determined by reference to the following three criteria:

1. A sufficiently large number of cells must adhere to the coverslips.
2. At least 25% of the cells must show viability upon examination by means of the vital-staining technique.
3. A sufficient number of viable cells must be in good condition upon morphological examination.

"If no toxic effect was observed at any concentration, the highest concentration to be used in the DNA-repair assay was determined according to the solubility limit of the test substance..."

Part II: "Another toxicity test was carried out which allows the detection of a possible DNA synthesis inhibiting activity of the test substance in the critical range of concentrations tested in the DNA repair test. A strong inhibition of replicative DNA synthesis by the test substance at non-toxic concentrations can be indicative of an inhibition of DNA repair synthesis."

"Rat hepatocytes were treated with the test substance, one positive control (10 mmol/l hydroxyurea) and one negative control (DMSO). Treatment and culture conditions were the same as described above. One hour after addition of the test substance, 50 µCi (5 µCi/ml) ^{3}H-thymidine was added to each culture..."

"After washing the cells, nuclei were isolated and washed. Half of the nuclear suspension was used for determination of radio-activity by TCA (Trichloroacetic acid) precipitation on glass fibre filters. The precipitated radioactivity was measured in a liquid scintillation counter using an adequate dpm-program. From the other half on the suspension the content of DNA was determined by measuring the fluorescence after staining with the Hoechst dye 33258."

"Relative DNA synthesis rates were calculated by dividing the ^{3}H-dpm values by the corresponding fluorescent values. The DNA synthesis rates were tabulated."

"A significant reduction of DNA synthesis rate in combination with an unaltered or only slightly reduced viability was considered as a specific, substance related, inhibitory effect. The interpretation of the particular results was based on the experience of the Study Director and on the respective test results as a whole."

Results: Part I (cytotoxicity): Refer to appended page 1. From p. 25: "The concentration of 208.8 µg/ml was the highest one yielding a sufficient number of a adhered, viable cells which were of sufficient quality for scoring. Concentrations of 417.5 µg/ml and higher resulted in an inadequate number of adhered cells. From the results obtained...400 µg/ml was decided to be the highest in the original DNA repair test. ...concentrations of 104.4 µg/ml and higher gave rise to precipitates when added to the culture medium."

Part II (possible DNA synthesis inhibition): Refer to appended p. 2. From p. 25: "The results...indicate a nearly complete inhibition of...replicative DNA synthesis activity...at the highest concentration of 400 µg/ml. This correlates with the cytotoxicity observed in the two [subsequent] DNA repair experiments with the same concentration... No inhibition of the replicative DNA synthesis activity, which could be of any relevance for DNA repair synthesis, was observed at the five lower concentrations tested...concentrations of 100 µg/ml and higher gave rise to precipitates when added to the culture medium."

2. First DNA repair assay: From p. 25: "The original experiment was carried out with concentrations of 3.7, 11.1, 33.3, 100, 200 and 400 µg/ml...concentrations of 100 µg/ml and higher gave rise to precipitates when added to the culture medium. The highest concentration of 400 µg/ml was not scorable due to toxicity..."

   Refer to appended p. 3 and 4 for the results from the first DNA repair assay.

3. Confirmatory DNA repair assay: From p. 26: "The confirmatory experiment was carried out with concentrations of 3.7, 11.1, 33.3, 100, 200 and 400 µg/ml...concentrations of 100 µg/ml and higher gave rise to precipitates when added to the culture medium. Again the highest concentration of 400 µg/ml was not scorable due to toxicity."

   Refer to appended pages 5 and 6 for the results from the confirmatory DNA repair assay.

4. Statistics: Refer to appended pages 7-14 for the statistical analysis, to appended page 15 for historical vehicle control data, and to appended page 16 for historical positive control data.

5. Individual grain count data: Because the data from the reporting laboratory appear to indicate considerably higher percentages of nuclei exposed to CGA 248757 being in repair as compared to their negative (vehicle) controls, this reviewer has examined the individual data, and determined the incidences of cells with ≥5 net nuclear grains/dosage level:

First DNA repair assay:

Treatment	# Cells examined	Number of cells ≥ 5 net nuclear grains	Incidence of cells ≥5 net nuclear grains
Vehicle control	150	1	0.0067
Positive control: 2-AAF (45 µM)	150	118	0.7867
CGA 248757 - 3.7 µg/ml	150	0	0.0000
11.1 µg/ml	150	3	0.0200
33.3 µg/ml	150	2	0.0133
100 µg/ml	150	0	0.0000
200 µg/ml	150	2	0.0133

Confirmatory (Second) DNA repair assay:

Treatment	# Cells examined	Number of cells ≥ 5 net nuclear grains	Incidence of cells ≥5 net nuclear grains
Vehicle control	150	0	0.0000
Positive control: 2-AAF (45 µM)	150	134	0.8933
CGA 248757 - 3.7 µg/ml	150	2	0.0133
11.1 µg/ml	150	1	0.0067
33.3 µg/ml	150	1	0.0067
100 µg/ml	150	0	0.0000
200 µg/ml	150	1	0.0067

It is concluded that there is no indication of an increased incidence of cells in UDS associated with exposure to CGA 248757.

C. DISCUSSION AND CONCLUSIONS:

The laboratory reports statistical significance associated with some slight elevations in mean net nuclear grain counts relative to their control values. However, the slight elevations are not biologically significant. This reviewer accepts the explanation (p. 27 of the report) that: "The occasional occurrence of statistically significant results is explainable by the low variance of the test values and the relatively low negative control values, whereby just a small deviation from the mean of the negative control has been evaluated by the statistics as a significant difference." The only question this reviewer has is why these statistical methods were applied to an assay in which a positive result is usually indicated by something like a $\geq$5 increase in mean net nuclear grain counts (or increases in incidences of cells with $\geq$5 and/or $\geq$20 net nuclear grains).

Under the assay conditions then, there was no indication of a biologically significant increase in mean net nuclear grains associated with in vitro exposure of rat hepatocytes to concentrations of CGA 248757 ranging from 3.7-200 µg/ml. The positive control (2-AAF at 45 µM) elicited the appropriate response.

This study and its negative findings are acceptable, and can be used as supporting data for purposes of registration and/or reregistration. While the March, 1991 Subdivision F Addendum 9 Guidelines do not specify an in vitro UDS assay in rat hepatoctyes as part of the initial battery of mutagenicity data requirements, it is stated (p. 7) that: "If tests for endpoints that may be predictive of mutagenicity are performed in addition to the initial battery, the results of such tests shall be submitted to the OPP along with results from the initial battery."

Page_____ is not included in this copy.

Pages 101 through 116 are not included.

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 quality control procedures.

_____ Identity of the source of product ingredients.

_____ Sales or other commercial/financial information.

_____ A draft product label.

_____ The product confidential statement of formula.

_____ Information about a pending registration action.

X FIFRA registration data.

_____ The document is a duplicate of page(s) _____.

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The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

Guideline Series 84: **MUTAGENICITY**

Reviewed by: Byron T. Backus, Ph.D. Byron T. Backus 12/22/94
Section II, Toxicology Branch II (7509C)
Secondary Reviewer: K. Clark Swentzel K. Clark Swentzel 12/22/94
Section II, Tox Branch II (7509C)

DATA EVALUATION REPORT VII

STUDY TYPE: In vitro Cytogenetic Test on CHL Cells

CHEMICAL: CGA-248757 Technical; 9-(4-chloro-2-fluoro-5-methoxycarbonylmethylthiophenylimino)-8-thia-1,6-diazabicyclo [4.3.0]nonan-7-one; [(2-chloro-4-fluoro-5-[(tetrahydro-3-oxo-1H,3H-[1,3,4-thiadiazol[3,4a]pyridazin-1-ylidene)amino]phenyl]thio] acetic acid methyl ester.

PC Code: 108803

MRID NUMBER: 433484-31

SYNONYMS/CAS No.: KIH-9201

SUBMITTER: Ciba-Geigy Corporation
P.O. Box 18300
Greensboro, NC 27419

TESTING FACILITY: Life Science Research Institute
Kumiai Chemical Industry Co., Ltd.
Shizuoka, Japan

TITLE OF REPORT: KIH-9201: Chromosome Aberration in Cultured CHL Cells.

AUTHORS: Mizuhashi, F., Murata, K. and Ishikawa, K.

LABORATORY STUDY NUMBER: None reported

STUDY COMPLETION DATE: January 10, 1991

EXECUTIVE SUMMARY: CHL cell cultures were exposed for 24 and 48 hours to concentrations of 50, 100, and 200 µg/ml KIH-9201 in the absence of S9, and for 6 hours (followed by 18-hr recovery) to concentrations of 375, 750 and 1500 µg/ml in the absence and presence of S9. At 200 µg/ml -S9 there were significant increases in structural chromosomal aberrations, but there was no indication of a response at lower concentrations (50 and 100 µg/ml). There were significant increases in numbers of cells with chromosomal aberrations following 6-hr exposure to 750 and 1500 µg/ml both in the presence and absence of S-9.

STUDY CLASSIFICATION: Acceptable. This study and its positive findings can be used as supporting data for purposes of registration and/or reregistration. While the March, 1991 Subdivision F Addendum 9 Mutagenicity Guidelines do not specify an in vitro mammalian cell cytogenetic study by itself as part of the initial battery of mutagenicity data requirements, it is stated that: "If tests for end-points that may be predictive of mutagenicity are performed in addition to the initial battery, the results of such tests shall be submitted to the OPP along with results from the initial battery."

A. MATERIALS

1. Test Material:
   Description: White powder
   Lot number: 6
   Stated purity: 99.6%
   Receipt date: October 1, 1987
   Stability: not reported
   Contaminants: not reported
   Solvent used: DMSO; reported to be soluble in DMSO up to a maximum concentration of 300 mg/ml.

2. Control Materials:
   Vehicle: DMSO
   Positive: From p. 10: without S9: N-methyl-N'-nitro-N-nitroso-guanidine (MNNG))
   With S9: Benzo(a)pyrene, (B(a)P)

3. Activation: From p. 23 "S-9...derived from rat liver was purchased from Oriental Yeast Co. Ltd., Tokyo, Japan and stored at -80°C."

_?_ Aroclor 1254	_?_ induced	_X_ rat	_X_ liver
_?_ phenobarbital	_?_ non-induced	___ mouse	___ lung
___ none		___ hamster	___ other

S-9 mix (from p. 23):

S-9	3 ml
20 mM HEPES buffer solution (pH 7.2)	2 ml
50 mM $MgCl_2$	1 ml
330 mM KCl	1 ml
50 mM Glucose-6-phosphate	1 ml
40 mM NADP	1 ml
Distilled water	1 ml

4. Test cells: From p. 10: "The CHL cell line derived from Chinese hamster lung fibroblast [cells] was obtained from [the] National Institute of Hygienic Sciences, Tokyo, Japan. It has been maintained in Eagle's minimum essential medium supplemented with 10% calf serum (culture medium). The cells were grown as a monolayer and the cell line has a modal number of 25 chromosomes [note by reviewer: this number appears to be too high and it is indicated on p. 13 that the metaphases selected ranged were 21 ± 2 centromeres]. All assays reported here were conducted using cells at passage numbers 25 to 27."

   It is not indicated whether or not the cells were regularly screened for mycoplasma infection.

5. On p. 5 of the report there is a signed and dated sheet titled "Responsibility" with signatures of the personnel involved in the study. On p. 3 the submitter has stated that: "This study is a preliminary assessment and cannot be certified as being in full compliance with good laboratory practice standards."

B. TEST PERFORMANCE AND RESULTS:

1. Dose selection: From p. 10: "In preliminary screening, the concentration at which cell growth was inhibited was determined for each sample. Cells (1.2×10^4) were seeded in a 3 cm plastic petri dish with 2 ml of culture medium and the sample at different concentrations (usually diluted at a nominal ratio of 2) was added on the 3rd day of culture at 5% CO_2 and 37°C atmosphere. After...2 days the medium was discarded and the cells were washed with sterilized saline. Formalin solution (10%) was added (approximately 10 minutes) to fix the cells which were then stained with 0.1% crystal violet (10 minutes) and dried after washing with water. The amount of inhibition of cell growth at each concentration of the sample was determined by Monocellater ...[with] the proliferation rate of solvent (DMSO) cells...adjusted to 100%. A concentration with approximately 50% inhibition was determined by concentration-inhibition of growth regression line."

   Results: From p. 16: "KIH-9201 was found to be soluble in DMSO at a concentration [of] 300 mg/ml. The median growth inhibition concentration (IC_{50}) was 199 µg/ml in [the] non-activation assay and more than 1500 µg/ml in [the] activation assay..."

   Refer to appended pages 1 and 2 for the growth inhibition observed in the preliminary cytotoxicity assays

2. Mutagenicity assay: "Following the results of preliminary toxicity test[ing]...the chromosome aberration test was performed using the KIH-9201 concentrations [indicated] below:

   - non-activation assay: 50, 100 and 200 μg/ml
   - activation assay: 375, 750 and 1500 μg/ml

   From p. 11: "Cells (2×10^4) were seeded in a 6 cm plastic petri plate (with 5 ml of culture medium). Approximately 72 hours after culture establishment, the culture medium was removed from each culture and replaced with fresh medium with DMSO (at 0.5% of medium) of the test material or S-9 mix where appropriate at 500 μl per 3 ml culture...including the test material. Culture medium alone was added to untreated control cultures. The final volume in each culture was 5 ml (non-activation assay) and 3 ml (activation assay). The cultures in non-activation assay were then gassed with 5% CO_2 and re-incubated at 37°C for 24 and 48 hours after treatment."

   "After 6 hours of incubation in [the] activation assay the medium was removed from each culture. Three milliliters of fresh culture medium was then added to each plate and the cultures re-incubated, again in a 5% CO_2 atmosphere, for a further 18 hours."

   "Two hours prior to termination, cell division was arrested by the addition of...colcemid, to each culture (to a final concentration of 0.2 μg/ml). After 2 hours of incubation, the cells were harvested by low speed centrifugation at 1000 rpm for 5 minutes following trypsinization and resuspension. The pellets of cells...were resuspended in hypotonic potassium chloride solution (75 mM) for 15 minutes at 37°C and then fixed in freshly prepared methanol:glacial acetic acid fixative (3:1 v/v) at 4°C. After two further changes of fixative, the tubes were centrifuged. The supernatant [was] removed and the cell pellet [was] resuspended in a few drops of fresh fixative. Single drops of the cell suspension were [dropped on] clean, moist, grease-free slides and the slides left to air-dry. A maximum of two slides were made from each culture, stained for 10 minutes in Giemsa stain (3% in Sorensen's buffer, pH 6.8), washed in buffer and left to air-dry."

   From p. 16: "One hundred metaphases were scored from each culture, where possible. These were not possible from two cultures at 200 μg/ml in non-activation and at 1500 μg/ml in the absence of S-9 mix in activation assay." [note by reviewer: it took me a while to figure this statement out, but testing with S-9 mix also involved a concurrent set of cultures exposed to the same concentrations].

From 100 metaphases (with 21 ± 2 centromeres), the following characters were recorded:

- chromosome number
- all chromosomes normal or some aberrant
- specific types and numbers of aberrations

"Scoring followed the recommendations of the Ad Hoc committee of the Environmental Mutagen Society and the Institute for Medical Research..."

Results: Refer to appended p. 3 for the results from the non-activation assay (with 24- and 48-hr exposure), and to appended p. 4 for the results from the activated assay (keeping in mind that the latter results involved 6-hr exposure, followed by replacement of the medium and incubation for an additional 18 hours, and include data from a concurrent set of cultures that were run in the absence of S-9 mix).

In the absence of S9, at 200 µg/ml (but not at ≤100 µg/ml) there were significant increases in the numbers of cells with structural aberrations, both with 24 and 48-hour exposure. In the presence of S-9 (exposure to the test material was for 6 hours, followed by replacement of the medium and 18-hour additional incubation) there were significant increases in percentage of cells with aberrations at 750 and 1500 µg/ml, both in the presence and absence of S9.

C. DISCUSSION AND CONCLUSIONS:

Under the assay conditions, there was a statistically significant increase in chromosomal aberrations associated with 24 and 48-hour exposure to 200 µg/ml CGA-248757 technical in the absence of S-9, and a similar increase at 750 and 1500 µg/ml with 6-hr exposure (both with and without S-9). It is concluded that CGA-248757 is positive for clastogenic activity under the conditions of this assay both in the presence and absence of S-9.

This study and its positive findings are acceptable, and can be used as supporting data for purposes of registration and/or reregistration. While the March, 1991 Subdivision F Addendum 9 Guidelines do not specify an in vitro mammalian cell cytogenetic study as part of the initial battery of mutagenicity data requirements, it is stated (p. 7) that: "If tests for endpoints that may be predictive of mutagenicity are performed in addition to the initial battery, the results of such tests shall be submitted to the OPP along with results from the initial battery."

Page_____ is not included in this copy.

Pages 122 through 125 are not included.

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 quality control procedures.

_____ Identity of the source of product ingredients.

_____ Sales or other commercial/financial information.

_____ A draft product label.

_____ The product confidential statement of formula.

_____ Information about a pending registration action.

X FIFRA registration data.

_____ The document is a duplicate of page(s) _____.

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Guideline Series 84: **MUTAGENICITY**

Reviewed by: Byron T. Backus, Ph.D. Byron T. Backus 12/22/94
Section II, Toxicology Branch II (7509C)
Secondary Reviewer: K. Clark Swentzel
Section II, Tox Branch II (7509C) K. Clark Swentzel 12/22/94

DATA EVALUATION REPORT IV

STUDY TYPE: In vitro Cytogenetic Test on Human Lymphocytes

CHEMICAL: CGA-248757 Technical; 9-(4-chloro-2-fluoro-5-methoxycarbonylmethylthiophenylimino)-8-thia-1,6-diazabicyclo [4.3.0]nonan-7-one; [(2-chloro-4-fluoro-5-[(tetrahydro-3-oxo-1H,3H-[1,3,4-thiadiazol[3,4a]pyridazin-1-ylidene)amino]phenyl]thio] acetic acid methyl ester.

PC Code: 108803

MRID NUMBER: 433484-32

SYNONYMS/CAS No.: KIH-9201

SPONSOR: Ciba-Geigy Corporation
P.O. Box 18300
Greensboro, NC 27419

TESTING FACILITY: Ciba-Geigy Limited
Genetic Toxicology
Basel, Switzerland

TITLE OF REPORT: Cytogenetic Test on Human Lymphocytes in vitro.

AUTHORS: Hertner, T.

LABORATORY STUDY NUMBER: 921081

STUDY COMPLETION DATE: December 10, 1993

EXECUTIVE SUMMARY: **in an in vitro assay with human lymphocytes there was no indication that CGA 248757 technical, at doses of up to 300 µg/ml ±S9 metabolic activation, causes clastogenic and/or aneugenic activity. Clastogenic effects were observed at 600 µg/ml +S9 (this dose level was evaluated only on one occasion)** along with considerable cytotoxicity (71.1% reduction in mitotic index), but there was no indication of a similar effect at 150 µg/ml -S9 even though there was a similar reduction (68.8%) in the mitotic index. The highest dose tested in the absence of S9 was 300 µg/ml.

IN VITRO CHROMOSOMAL ABERRATION ASSAY IN HUMAN LYMPHOCYTES

IV-2

STUDY CLASSIFICATION: Acceptable. This study and its findings can be used as supporting data for purposes of registration and/or reregistration. While the March, 1991 Subdivision F Addendum 9 Mutagenicity Guidelines do not specify an in vitro mammalian cell cytogenetic study by itself as part of the initial battery of mutagenicity data requirements, it is stated that: "If tests for endpoints that may be predictive of mutagenicity are performed in addition to the initial battery, the results of such tests shall be submitted to the OPP along with results from the initial battery."

A. MATERIALS

1. Test Material:
   Description: White powder
   Lot number: FL-920346
   Purity: 97.7%
   Receipt date: not reported
   Stability: reported (p. 11) as stable in the vehicle used.
   Contaminants: not reported
   Solvent used: DMSO

2. Control Materials:
   Vehicle: DMSO
   Positive: From p. 12: without S9: Bleomycin, 4 µg/ml
   With S9: Cyclophosphamide, 8 µg/ml

3. Activation: From p. 17: "Rat-liver post mitochondrial supernatant (S9 fraction) was...purchased from CCR (Cytotest Cell Research GmbH & Co. KG, Rossdorf, Germany). It was prepared from Wistar rats which received a single i.p. injection of 500 mg/ml Aroclor 1254 in olive oil 5 days prior to sacrifice..."

X Aroclor 1254	X induced	X rat	X liver
___ phenobarbital	___ non-induced	___ mouse	___ lung
___ none		___ hamster	___ other

"S9 fraction was thawed immediately before use, mixed with NADP and isocitric acid and added to culture medium to give the following final concentrations:

Rat liver S9 fraction	15	µl/ml (1.5%)
NADP	3.14	µmol/ml
Isocitric acid (trisodium salt)	15.3	µmol/ml

4. Test cells: phytohemagglutinin stimulated human lymphocytes in whole blood cultures.

Donor 1: (original experiments 1-2): male, 37 years old, nonsmoker.

Donor 2: (confirmatory experiments 1-4): female, 27 years old, nonsmoker.

5. Assay evaluation criteria: From p. 22:

Criteria for a positive response: "Under the standard conditions of our laboratories, the test substance is generally considered to be active in human lymphocytes if the following conditions are met:

- The percentage of specific aberrations in a treatment group is higher than 6.0 and is statistically significantly different from the value of the negative control; and/or an increased number of exchange figures appears together with a high number of other specific chromosomal aberrations such as breaks and fragments.

- A concentration-related response should be demonstrable."

Criteria for a negative response: "Under the standard conditions of our laboratories, the test substance is generally considered to be inactive in the human lymphocytes if the following conditions are met:

- The percentage of specific aberrations in all treatment groups is less than or equal to 6.0 and does not differ statistically significant from the respective value of the negative control."

Exceptions: "At the limits of the criteria for a positive or for a negative response or if the criteria for a positive response are only partially fulfilled or if effects are obtained at extremely high concentrations or in the toxic range of the test substance only, the Study Director will decide by experience about the interpretation of the results."

Assay acceptance criteria:

- The results of the experiments should not be influenced by a technical error, contamination or a recognized artifact.

- The quality of the slides should allow, at least to a large extent, the chromosomes to be easily identifiable.

- In the negative controls the percentage of metaphases showing specific chromosomal aberrations should be less than 6.0 (based on historical negative control range).

- The results of the positive control experiments should meet the criteria for a positive response.

- The highest concentration to which cells were exposed in the mutagenicity test should exert sufficient toxicity (suppression of mitotic activity by 50% or more), represent the limit of solubility of the test material, or be at least 5 mg/ml (or 10 mMol/l).

6. There is a signed and dated "Certification of Good Laboratory Practice and Verification of a Complete and Unaltered Copy of the Amendment by the Study Sponsor" on page 6 of the report, along with a signed and dated "Statement of Compliance with Good Laboratory Practice" on page 7. There is also a "Certification of Good Laboratory Practices" statement on page 3 of the report from the submitter. There is a signed and dated Quality Assurance Statement on page 9, giving dates of QA activity.

B. TEST PERFORMANCE AND RESULTS:

1. Dose selection: This was apparently dependent on the solubility of the test material: From p. 16: "CGA 248757 tech. was dissolved in DMSO at room temperature and sterilized by filtration through a 0.2 µm Acrodisc-CR filter. The highest concentration soluble in DMSO (stock solution) was about 143 mg/ml. This solution caused the formation of strong precipitates after 100 fold dilution with culture medium. The highest concentration of CGA 248757 tech. in DMSO resulting in a tolerable homogeneous turbidity after 100 fold dilution with culture medium was about 86 mg/ml. The highest concentration in DMSO (stock solution) selected for the study, was 60 mg/ml. At this concentration no precipitates were observed after 100 fold dilution with culture medium..."

2. Cytotoxicity/Mutagenicity test: From p. 17: "The cytotoxicity test was performed as an integral part of the mutagenicity test. A series of tissue culture flasks were seeded with 0.5 ml fresh blood diluted with 4.5 ml culture medium. The preincubation time before treatment was about 72 hours. In the cultures without metabolic activation, culture medium was replaced immediately before addition of the test substance. The substance in DMSO was added (1:100) to the cells in culture medium. Cells were exposed to the substance during the whole incubation period (20 or 42 hours). In the experiments in which the substance was metabolically activated, 0.55 ml of the activation mixture and the substance in DMSO...were added to 5 ml of culture. Cells were exposed to the substance for three hours... Thereafter, culture medium was replaced by fresh medium and incubation was continued for a further 17 or 39 hours. Bleomycin, 4 µg/ml, a mutagen not requiring S9 activation, and cyclophosphamide, 8 µg/ml, which requires metabolic activation, were used as positive controls. In addition a negative control was set in each experiment, supplemented with

the respective volume of the vehicle. Duplicate cultures were prepared for each group in each assay. The detailed treatment conditions were as follows:"

Original study, first experiment: "The cells were exposed for 20 hours without metabolic activation to eight concentrations of the test substance. The cells were harvested immediately after treatment. Concentration range: 4.69 - 600 µg/ml."

Original study, second experiment: "The cells were exposed for three hours with metabolic activation to eight concentrations of the test substance. After removal of the test substance the cells were washed and incubated in new complete culture medium for 17 hours. Concentration range: 4.69 - 600 µg/ml."

Confirmatory study, first experiment: "The cells were exposed for 20 hours without metabolic activation to five concentrations of the test substance. The cells were harvested immediately after treatment. Concentration range: 18.75 - 300 µg/ml."

Confirmatory study, second experiment: "The cells were exposed for three hours with metabolic activation to five concentrations of the test substance. After removal of the test substance the cells were washed and incubated in new complete culture medium for 17 hours. Concentration range: 37.5 - 600 µg/ml."

Confirmatory study, third experiment: "The cells were exposed for 42 hours without metabolic activation to eight concentrations of the test substance. The cells were harvested immediately after treatment. Concentration range: 2.34 - 300 µg/ml."

Confirmatory study, fourth experiment: "The cells were exposed for three hours with metabolic activation to eight concentrations of the test substance. After removal of the test substance the cells were washed and incubated in new complete culture medium for 39 hours. Concentration range: 4.69 - 600 µg/ml."

In the groups treated with test substance or with vehicle alone, a third culture was run. These cultures were exposed to 10 µg/ml BrdU during treatment and recovery.

"2.5 hours prior to harvesting, all cultures were treated with colcemid...0.4 µg/ml to arrest cells in metaphase." This was followed by hypotonic treatment in 0.075 M KCl solution and fixation in 3:1 methanol:acetic acid. Drop preparations were made and air-dried. "Slides for chromosome analysis were stained with orcein. Slides from BrdU-labelled cultures were UV-irradiated, heated in 2xSSC and stained with Giemsa."

Selection of concentrations for analysis: The highest concentration administered, or the lowest concentration suppressing mitotic activity by 50-80% (compared to controls) was selected as the highest concentration analyzed for chromosome aberrations. The next two lower concentrations were also analyzed. "For the determination of the mitotic index (M.I.) the preparations from the various cultures were examined first, uncoded. The percentages of mitotic suppression in comparison with the controls were evaluated by counting at least 2000 cells from one slide [from] each of the treatment groups and the negative control group... From the results of [the] corresponding original run, five suitable concentrations were determined for the first and second experiment of the confirmatory study."

Scoring of BrdU slides: "From the original experiments, BrdU-labelled slides were analysed to determine the distribution of first, second and third post-treatment mitosis..."

Scoring of slides for chromosome aberrations: "Prior [to] analysis the selected slides were coded, likewise...vehicle [controls]...as well as the positive control. Whenever possible two hundred well spread metaphase figures with 46 centromeres from two cultures (100 metaphases per replicate culture) in the vehicle control and in the treated groups were scored. At least fifty metaphases were scored in the positive controls (25 per replicate culture)..."

Results: There was some variability between different assays with respect to the highest dose selected for analysis. These were as follows:

Original study, first experiment: 20-hour exposure without metabolic activation: 150 µg/ml (68.8% suppression of mitotic activity).

Original study, second experiment: 3-hour exposure with metabolic activation followed by 17 hours recovery: 600 µg/ml (highest dose tested; 71.1% suppression of mitotic activity).

Confirmatory study, first experiment: 20-hour exposure without metabolic activation: 300 µg/ml (highest dose tested; 63.9% suppression of mitotic activity).

Confirmatory study, second experiment: 3-hour exposure with metabolic activation followed by 17 hours recovery: 300 µg/ml (22.6% suppression; at the next highest dose, 600 µg/ml, there was 96.8% suppression and this concentration was not scorable).

Confirmatory study, third experiment: 42-hour exposure without metabolic activation: 150 µg/ml (40.4% suppression of mitotic activity).

Confirmatory study, fourth experiment: 3-hour exposure with metabolic activation followed by 39 hours recovery: 300 µg/ml (48.0% suppression of mitotic activity).

Refer to appended pages 1-3 for mitotic index values, to appended page 4 for the frequencies of cells in M1 and M2 (as determined by BrdU labelling) in the original (first) study, to appended page 5 for the explanation of abbreviations used in the reporting of chromosomal aberrations, to appended pages 6-8 for the percentages of cells with aberrations and the total numbers of cells with specific aberrations, and to appended pages 9-10 for historical negative control data. In the original (first) study, with metabolic activation, at 600 µg/ml, a highly significantly elevated percentage (13.0%) of the cells had aberrations. At this dose level the mitotic index was reduced by 71.1%; and 97% of the metaphases scored were M1 (as compared to 46% for the solvent control). The report states (p. 15) that: "By using a high concentration, which provoked a reduction of the mitotic index by more than 70%, clastogenic effects were noted in the presence of metabolic activation. However, these effects are rather attributed to the high level of cytotoxicity, than to a direct clastogenic property of CGA 248757 tech."

C. DISCUSSION AND CONCLUSIONS:

Under the assay conditions, there was no indication that CGA 248757 technical, at doses of up to 300 both with and without S9 metabolic activation, causes clastogenic and/or aneugenic activity. The report ascribes the clastogenic effects at 600 µg/ml with metabolic activation to the high level of cytotoxicity (71.1% reduction in mitotic index), but there was no indication of a similar effect at 150 µg/ml in the absence of metabolic activation even though there was a similar reduction (68.8%) in the mitotic index. While it is then appropriate to consider the test material as clastogenic at 600 µg/ml in the presence of activation, it is difficult to envision circumstances under which this concentration could be attained under normal physiological conditions.

IN VITRO CHROMOSOMAL ABERRATION ASSAY IN HUMAN LYMPHOCYTES

IV-8

This study and its findings are acceptable, and can be used as supporting data for purposes of registration and/or reregistration. While the March, 1991 Subdivision F Addendum 9 Guidelines do not specify an in vitro mammalian cell cytogenetic study as part of the initial battery of mutagenicity data requirements, it is stated (p. 7) that: "If tests for endpoints that may be predictive of mutagenicity are performed in addition to the initial battery, the results of such tests shall be submitted to the OPP along with results from the initial battery."

Page_____ is not included in this copy.

Pages 134 through 143 are not included.

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 quality control procedures.

_____ Identity of the source of product ingredients.

_____ Sales or other commercial/financial information.

_____ A draft product label.

_____ The product confidential statement of formula.

_____ Information about a pending registration action.

X FIFRA registration data.

_____ The document is a duplicate of page(s) _____.

_____ The document is not responsive to the request.

The information not included is generally considered confidentia by product registrants. If you have any questions, please contac the individual who prepared the response to your request.

Guideline Series 84: **MUTAGENICITY**

Reviewed by: Byron T. Backus, Ph.D. Byron T. Backus 12/22/94
Section II, Toxicology Branch II (7509C)
Secondary Reviewer: K. Clark Swentzel K. Clark Swentzel 12/22/94
Section II, Tox Branch II (7509C)

DATA EVALUATION REPORT II

STUDY TYPE: In vitro Mammalian Cell Forward Gene Mutation Assay in Chinese Hamster V79 Cells at the HGPRT Locus.

CHEMICAL: CGA-248757 Technical; 9-(4-chloro-2-fluoro-5-methoxycarbonylmethylthiophenylimino)-8-thia-1,6-diazabicyclo [4.3.0]nonan-7-one; [(2-chloro-4-fluoro-5-[(tetrahydro-3-oxo-1H,3H-[1,3,4-thiadiazol[3,4a]pyridazin-1-ylidene)amino]phenyl]thio] acetic acid methyl ester.

PC Code: 108803

MRID NUMBER: 433484-33

SYNONYMS/CAS No.: KIH-9201

SPONSOR: Ciba-Geigy Corporation
P.O. Box 18300
Greensboro, NC 27419

TESTING FACILITY: Ciba-Geigy Limited
Genetic Toxicology
Basel, Switzerland

TITLE OF REPORT: Gene Mutation Test with Chinese Hamster Cells V79

AUTHORS: Geleick, D.

LABORATORY STUDY NUMBER: 921080

STUDY COMPLETION DATE: October 9, 1992

EXECUTIVE SUMMARY: CGA 248757 technical was tested without metabolic (S9) activation in an initial V79/HGPRT gene mutation assay at 3.3, 10, 30 and 90 µg/ml and in a confirmatory assay at 3.7, 11.1, 33.3 and 100 µg/ml. It was tested with metabolic activation at 31.7, 95.2, 285.7 and 857 µg/ml in both the initial and confirmatory assays. In the initial (but not confirmatory)

assay, slightly elevated increases in mutants/surviving cells following treatment at 857 µg/ml +S9 were due to considerably fewer surviving cells rather than increases in numbers of mutants. Under the conditions of this assay, CGA 248757 is negative.

STUDY CLASSIFICATION: Acceptable. This study satisfies the 1991 Subdivision F Addendum 9 mutagenicity Guideline requirement [84-2(b)(2)] for a mammalian cells in culture forward gene mutation assay for CGA 248757 technical and can be used as supporting data for purposes of registration and/or reregistration.

A. MATERIALS

1. Test Material:
   Description:
   Lot number: FL-920346
   Purity: 97.7%
   Receipt date:
   Stability: Reported (p. 12) as "stable."
   Contaminants: Not reported
   Solvent used: DMSO
   Other provided information: From p. 17: "CGA 248 757 tech. was dissolved in dimethylsulfoxide at room temperature and sterilised by filtration through a 0.2 µm filter. The highest concentration...was determined in a preliminary test to be 142.8 mg/ml soluble in dimethylsulfoxide. This concentration produced non tolerable precipitates in the culture medium. However, at a concentration of 85.7 mg/ml a homogeneous turbidity was observed after 100 fold dilution with culture medium."

2. Control Materials:
   Solvent/final concentration: 1%
   Positive: Non-activation:
   From p. 19: Ethylmethanesulfonate (EMS) at a concentration of 300 nl/ml.

   Activation:
   From p. 19: N-nitroso-dimethylamine (DMN) at a concentration of 1.0 µl/ml.

3. Activation: From p. 17: "Rat-liver post mitochondrial supernatant (S9 fraction) was prepared...from male RAI rats... The animals (150-250 g) were treated with Aroclor 1254 (500 mg/kg, i.p.) 5 days prior to sacrifice."

X Aroclor 1254	X induced	X rat	X liver
___ phenobarbital	___ non-induced	___ mouse	___ lung
___ none		___ hamster	___ other

S9 mix composition: from p. 18:

Rat liver S9 fraction	250 µl/ml
Glucose-6-phosphate	10 µmol/ml
NADP	8 µmol/ml
$CaCl_2$	20 µmol/ml
$MgCl_2$	20 µmol/ml
Na_2HPO_4	1 µmol/ml
FCS (presumably fetal calf serum)	30 µl/ml

From p. 18: "The S9 mixture was immediately filter-sterilised by passage through a 0.45 µm filter. The activation mixture was added to the medium at a concentration of 10% in both the cytotoxicity test and the mutagenicity test and the final concentration of S9 fraction was 2.5% during the treatment."

4. Test cells: V79 Chinese hamster cells originally derived from embryonic lung tissue. Obtained from Dr. D. Wild, Freiburg, Germany. "Large stocks of the V79 cell line have been stored in liquid nitrogen allowing the repeated use of the same cell culture batch in experiments. Consequently, the parameters of the experiments remain similar... All stock cells were cultured in cleansing medium for three days to purge the cultures of existing hgprt- mutants. Cleansing medium was growth medium supplemented with 3 µM aminopterin. The cells have a stable karyotype with a modal chromosome number of 22 ± 1. All stock cells were checked for mycoplasma contamination, using the Hoechst-Dye staining method, before being frozen. Thawed stock culture cells are kept for twelve passages (three months) in culture."

   From p. 16: "The cells were cultured in Ham's F10 medium supplemented with 10% pre-tested foetal calf serum, 100 U/ml penicillin and 100 µg/ml streptomycin in tissue culture (plastic) flasks. The humidity in the incubator was adjusted to >85% rH, the air was enriched to 5 ± 1.0 Vol% CO_2 and the temperature was 37 ± 1°C. Twice per week the growth medium was replaced by fresh one. The laboratory cultures were passaged weekly in low number (about 5 x 10^4 cells per 175 cm^2) to keep the level of spontaneous mutants low and to prevent the cells from reaching a stationary phase of cell growth..."

5. Assay acceptance criteria: From p. 20:

   1. The results of the experiments should not be influenced by a technical error, contamination of a recognized artifact.

   2. From each experiment, at least three concentrations of the test substance, one positive and one solvent control should be evaluated.

3. The mutant frequency of the solvent controls (spontaneous mutant frequency) should not exceed $35x10^{-6}$.

4. The positive control should fulfill the criteria for a mutagenic substance.

5. The highest concentration of the test substance applied in the mutagenicity test should either reduce the viable cells by about 50-90% or correspond to the test substance's solubility limit (precipitates in culture). In case of non-toxic compounds the highest tested concentration will be 5 mg/ml. In special cases the highest concentration can be determined by the sponsor.

6. Criteria for a positive response: From p. 21: "The test substance will be considered mutagenic in this test system, if either:
   - The mutant frequency of the treated culture exceeds that of the solvent controls by a mutant factor of 2.5 and there is a dose-dependent increase of the mutant frequency.

   or:
   - The mutant frequency in a treated culture exceeds that of the solvent control by a mutant factor of 3.0 at any concentration tested and reported and the absolute number of clones in the treated and untreated cultures differ by more than 20 clones per 10^6 cells plated."

7. Criteria for a negative response: From p. 22: "The test substance will be considered to be inactive in this test system:
   - If there is no concentration-dependency of the mutant-frequency values determined and the highest mutant frequency of a treated culture exceeds that of the solvent controls by a factor lower than 3.0 or the absolute number of clones in the treated and untreated culture with the highest mutant frequency value differs by less than 20 clones per 10^6 cells plated.

   or
   - If there is a concentration-dependency of the mutant frequency values determined and the mutant frequency in a treated culture exceeds that of the solvent controls only by a factor lower than 2.5."

8. There is a signed and dated "Certification of Good Laboratory Practice and Verification of a Complete and Unaltered Copy of the Amendment by the Study Sponsor" on page 6 of the report, along with a signed and dated "Statement of Compliance with Good Laboratory Practice" on page 7. There is also a "Certification of Good Laboratory Practice" statement on page 3 of the report from the submitter. There is a signed and dated Quality Assurance Statement on page 9.

B. TEST PERFORMANCE AND RESULTS:

1. Dose selection: From p. 18: "A cytotoxicity test was performed on V79 cells as a preliminary test to determine the highest concentration of the test substance to be applied in the mutagenicity assay. For each concentration and the untreated controls, 2.5×10^3 cells were seeded in 5 ml growth medium into a 25 cm^3 tissue culture flask and incubated overnight. The cultures were exposed to the test substance for five hours in the presence and for 21 hours in the absence of a metabolic activation system. In the two parts of the experiment, 12 concentrations of the test substance and two vehicle (DMSO) controls were tested." From information on p. 23: "The cytotoxicity test was performed with concentrations of 0.42-857.0 µg/ml. 857.0 µg/ml represents the solubility limit of the test chemical. Higher concentrations produced non tolerable precipitates in the culture medium." From p. 18: "The treatment was terminated by washing the cultures with phosphate buffered saline (PBS). Compound-induced cytotoxicity was estimated by cloning efficiency immediately after treatment. The cultures were counted and diluted so that 100 cells were seeded per 9.6 cm^2, in 3 ml of growth medium. After seven to eight days of growth the cultures were fixed and stained with Giemsa and the [number of] surviving colonies determined... The concentration selected as the highest for the mutagenicity assay was the one causing about 50-90% reduction of viable cells in comparison with the mean of the two negative controls or corresponds to the substance's solubility limit (precipitates in culture)."

2. Mutagenicity assays: From p. 19: "Depending on the toxicity of the test compound $2.5\text{-}5.0 \times 10^6$ cells of passage 28 (original experiment) and passage 25 (confirmatory experiment) were plated in 30 ml growth medium into 175 cm^2 flasks and incubated overnight. The growth medium was replaced for five hours by 27 ml treatment medium and 3.0 ml S9 activation mixture, or for 21 hours by 30 ml treatment medium alone."

"In each assay, cultures were treated in duplicate with four test chemical combinations, a positive and a negative (DMSO) control..."

"The treatment was terminated by washing the cell layer extensively with PBS. After washing the cells were suspended by trypsinisation, pelleted, resuspended in fresh growth medium and counted..., diluted with fresh growth medium and replated into flasks at 2×10^6 cells. The cultures were incubated at 37°C for seven to eight days during which the cells could...recover and divide to express the mutant phenotype. The cultures were subcultured after the second or third day transferring 2×10^6 cells to a fresh flask to maintain exponential growth during the

expression phase."

"In parallel cytotoxicity of the compound was estimated from the cloning efficiency immediately after treatment. The counted cell suspension of each concentration level was further diluted so that 100 cells were seeded per 9.6 cm^2 in 2.5 ml of growth medium and incubated at 37°C. The number of colonies which developed with seven to eight days in these cultures reflected the viability at the end of treatment (Survivor I values)."

"At the end of the expression period the cultures were trypsinised, pelleted, resuspended in fresh growth medium and counted... The cell suspension of each culture was diluted with fresh growth medium and an aliquot replated into two flasks (75 cm^2 growth area) each containing $1x10^6$ cells for the mutant selection. This cell density is assumed not to allow cell-cell cooperation and thus should not influence the number of the mutant clones. The high-density cultures were subjected to the mutant selection procedure by supplementing the growth medium with 8 µg/ml 6-thioguanine. Only cells mutated at the hgprt locus could survive the 6-thioguanine treatment. The number of colonies formed in these flasks during the following days reflected the overall number of mutations induced by the treatment with the test substance or the mutagen (positive control). After seven to eight days incubation at 37°C, the cultures were fixed with methanol and stained with Giemsa. The mutant clones were counted..."

"In parallel the viability at the end of the expression period was estimated from the cloning efficiency. The remaining cell suspensions from the various expression cultures were further diluted such that 100 cells were seeded per 9.6 cm^2 in 2.5 ml of growth medium and were incubated at 37°C. The number of colonies which developed within these low-density cultures reflected the viability at the end of the expression period (Survivor II values)."

Results:

Refer to appended pages 1, 2, 3 and 4 for the summary results of the initial assay with S9 activation, the initial assay without S9 activation, the confirmatory assay with S9 activation and the confirmatory assay without S9 activation, respectively. and to appended pages 5, 6, 7 and 8 for the numbers of 6-TG resistant (mutant) clones counted. The report also includes (appended pages 9 and 10) historical control data.

C. DISCUSSION AND CONCLUSIONS:

In the original experiment the average "mutant factor" following exposure to 857 µg/ml +S9 was 2.87 (average of 3.22 and 2.52), meeting one of the laboratory's criteria for a positive response ("The test substance will be considered mutagenic...if the mutant frequency of the treated culture exceeds that of the solvent controls by a mutant factor of 2.5 and there is a dose-dependent increase of the mutant frequency."). However, as noted on p. 23 of the report, a similar increase at 857 µg/ml +S9 did not occur in the confirmatory assay. From p. 24: "The increase in the absolute mutant number was less than 20 for both cultures..." From information on page 36 the average number of 6-thioguanine resistant clones/flask at 857 µg/ml was 10.25; for the negative controls it was 12.25. The higher "mutant factor" was due to lower numbers of survivors following treatment with 857 µg/ml +S9. The mutant frequencies at 857 µg/ml +S9 in the first assay were 22.85 and 17.88 x 10^{-6}; values as high as 32.9 x 10^{-6} are reported in the historical control data.

Since the increased "mutant factor" at 857 µg/ml +S9 was observed only in one assay and not the other, since it was also marginal (close to the value of 2.5) and since it did not involve an actual increased number of 6-thioguanine resistant clones/flask, it can be considered incidental. We can accept the study and its findings as indicating that CGA 248757 technical is negative for mutagenic effects under the conditions of this assay.

This study satisfies the 1991 Subdivision F Addendum 9 mutagenicity Guideline requirement [84-2(b)(2)] for a mammalian cells in culture forward gene mutation assay for CGA 248757 and can be used as supporting data for purposes of registration and/or reregistration.

Page_____ is not included in this copy.

Pages 151 through 160 are not included.

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 quality control procedures.

_____ Identity of the source of product ingredients.

_____ Sales or other commercial/financial information.

_____ A draft product label.

_____ The product confidential statement of formula.

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011508

Guideline Series 84: MUTAGENICITY

Reviewed by: Byron T. Backus, Ph.D. Byron T. Backus 12/22/94
Section II, Toxicology Branch II (7509C)
Secondary Reviewer: K. Clark Swentzel K. Clark Swentzel 12/22/94
Section II, Tox Branch II (7509C)

DATA EVALUATION REPORT V

STUDY TYPE: In vitro Cytogenetic Test on CHO-K1 Cells

CHEMICAL: CGA-248757 Technical; 9-(4-chloro-2-fluoro-5-methoxycarbonylmethylthiophenylimino)-8-thia-1,6-diazabicyclo [4.3.0]nonan-7-one; [(2-chloro-4-fluoro-5-[(tetrahydro-3-oxo-1H,3H-[1,3,4-thiadiazol[3,4a]pyridazin-1-ylidene)amino]phenyl]thio] acetic acid methyl ester.

PC Code: 108803

MRID NUMBER: 433484-34

SYNONYMS/CAS No.: KIH-9201

SPONSOR: Ciba-Geigy Corporation
P.O. Box 18300
Greensboro, NC 27419

TESTING FACILITY: Life Science Research Ltd.
Suffolk, England

TITLE OF REPORT: In Vitro Assessment of the Clastogenic Activity of KIH-9201 in Cultured Chinese Hamster Ovary (CHO-K1) Cells

AUTHORS: Edwards, C.

LABORATORY STUDY NUMBER: 90/KCI109/0380 (and addendum no. 92/KCI109/1091)

STUDY COMPLETION DATE: July 16, 1990

EXECUTIVE SUMMARY: CHO-K1 cells were exposed for 3 hours ±S9 (with a subsequent recovery period of 21 hours before harvest). Dose levels evaluated (+ and -S9) were 50, 100, and 200 µg/ml. A white precipitate was observed at 250 µg/ml in a preliminary cytotoxicity

study. **There was a statistically significant (and dose related) increase in chromosomal aberrations associated with exposure to CGA-248757 technical in the absence of S9. There was a similar (but not so pronounced) effect in the presence of S9**, statistically significant only when gaps (not normally considered an aberration) were included in the analysis.

There was a dose-related increased incidence of endoreduplication associated with exposure to CGA-248757 ± S9 suggesting (but not conclusively demonstrating) some type of aneugenic (spindle poison) activity. Endoreduplication was not included in the statistical analysis for chromosomal aberrations.

STUDY CLASSIFICATION: Acceptable. This study and its positive findings can be used as supporting data for purposes of registration and/or reregistration. While the March, 1991 Subdivision F Addendum 9 Mutagenicity Guidelines do not specify an *in vitro* mammalian cell cytogenetic study by itself as part of the initial battery of mutagenicity data requirements, it is stated that: "If tests for end-points that may be predictive of mutagenicity are performed in addition to the initial battery, the results of such tests shall be submitted to the OPP along with results from the initial battery."

A. MATERIALS

1. Test Material:
   Description: White powder
   Lot number: G26-04
   Purity: 97.8%
   Receipt date: December 8, 1989
   Stability: not reported
   Contaminants: not reported
   Solvent used: DMSO; reported to be soluble in DMSO up to a maximum concentration of approximately 270 mg/ml.

2. Control Materials:
   Vehicle: DMSO
   Positive: From p. 13: without S9: Mitomycin-C
   With S9: Cyclophosphamide, 8 µg/ml

3. Activation: From p. 32: "Young male CD rats, c. 200 g...were obtained from Charles River Breeding Laboratories (U.K.)... Aroclor 1254 (500 mg/kg bodyweight in corn oil) was administered as a single intraperitoneal injection to induce microsomal enzyme activity."

"Four days after treatment, the animals were fasted overnight and then killed... The livers were removed, washed in cold 0.15M KCl, then homogenised with one volume of the same medium... Homogenates were centrifuged at 9000 G for ten minutes, and supernatants collected and stored at -196°C until required for preparation of the S-9 mix. Supernatant is used within six months of preparation."

X Aroclor 1254	X induced	X rat	X liver
___ phenobarbital	___ non-induced	___ mouse	___ lung
___ none		___ hamster	___ other

S-9 mix:

0.1M KH_2PO_4-Na_2HPO_4 buffer (pH 7.4)	7.4 ml
0.4 $MgCl_2.6H_2O$/1.65M KCl aqueous solution	0.2 ml
0.1M NADP, sodium salt, aqueous solution	0.4 ml
0.1M glucose-6-phosphate, sodium salt, aqueous solution	0.5 ml
Supernatant from liver homogenate	1.5 ml

4. Test cells: From p. 14: "The Chinese hamster ovary (CHO-KI) cell line was obtained from Menarini Laboratories, Rome, Italy in March 1989. It has been maintained at LSR in Ham's nutrient F-10 medium, supplemented with 10% Foetal calf serum and antibiotics. The cells were grown as a monolayer and the cell line has a modal number of 21 chromosomes. All assays reported here were conducted using cells at passage numbers 7 to 9."

"Cells...are regularly screen for mycoplasma infection."

5. On p. 5 of the report there is a signed and dated declaration that includes the statement: "The aspects of the study conducted by Life Science Research were performed in accordance with the principles of Good Laboratory Practice Standards or Guidelines..." On p. 7 there is a signed and dated Quality Assurance Statement. On p. 3 there is a "Certification of Good Laboratory Practices" from the submitter.

B. TEST PERFORMANCE AND RESULTS:

1. Dose selection: There was a preliminary cytotoxicity test with concentrations of 2, 10, 50, 250 and 1250 µg/ml of the test material ±S9. "Approximately 24 hours before treatment, sufficient 25 cm^2 culture flasks were seeded with CHO-KI cells, the cultures gassed with 5% CO_2, and incubated at 37°C."

After 24 hours: "...the medium was removed from each culture and replaced with fresh culture medium containing the test material solution or solvent (at 25 µl per 5 ml culture) and S-9 mix where appropriate (at 500 µl per 5 ml culture...). The final volume in each culture was 5 ml. All control and test material exposures were established in duplicate cultures. The cultures were then gassed with 5% CO_2 and re-incubated at 37°C."

"After three hours, the medium (containing test or control solution and S-9 mix where added) was removed from all cultures and the cell sheet was washed three times with Hanks balanced salts solutions (HBSS) to remove the test compound and/or S-9 mix. Culture medium (5 ml) was then added to each flask and the cultures re-incubated, again in a 5% CO_2 atmosphere, for a further 21 hours (including a 2 hour Colcemid treatment...)."

"After 2 hours of incubation [with Colcemid at 0.4 µg/ml] the cells were harvested by low speed centrifugation...following trypsinisation and resuspension. The pellets of cells...were resuspended in hypotonic potassium chloride solution (0.56% w/v) for 4 minutes and then fixed in...methanol:glacial acetic acid fixative (3:1 v/v).

For the preliminary toxicity test: "At least 1000 cells per culture were examined...and the mitotic index calculated as the percentage of cells examined that were in mitosis (metaphase). A concentration of KIH-9201 that was expected to produce depression of mitotic activity was then chosen as the highest level for the main cytogenetic test."

"Mitotic indices were scored for all cultures."

Results (preliminary cytotoxicity test): From p. 24:

Dose level KIH-9201	S9 Mix	Mean Mitotic Index
0 (DMSO only)	-	5.6
2 µg/ml	-	7.5
10 µg/ml	-	7.3
50 µg/ml	-	7.2
250 µg/ml	-	2.6
1250 µg/ml	-	0.4
0 (DMSO only)	+	2.2
2 µg/ml	+	1.9
10 µg/ml	+	2.2
50 µg/ml	+	2.2
250 µg/ml	+	a
1250 µg/ml	+	b

a = Few cells and no metaphases present; not scored
b = No cells and no metaphases present; not scored

IN VITRO CYTOGENETIC ASSAY IN CHO-KI CELLS

V-5

2. Mutagenicity assay: Cell cultures were treated in the same way as in the preliminary cytotoxicity study. "At least two slides from each culture were randomly assigned code numbers by a person not subsequently engaged in the study. Care was taken to ensure that all unique identifications remained concealed to eliminate bias. The slides were examined under a low power (x 10 objective) and those areas judged to be of sufficient technical quality to permit scoring were located and examined under high power (x 100, oil immersion objective).

From 100 metaphases (with 21 ± 2 centromeres), the following characters were recorded:

- chromosome number
- all chromosomes normal or some aberrant
- specific types and numbers of aberrations

"Scoring followed the recommendations of the Ad Hoc committee of the Environmental Mutagen Society and the Institute for Medical Research..."

Results: Refer to appended pages 1 and 2 for the mitotic indices of the treated cultures, and to appended pages 3 and 4 for number of types of chromosomal aberrations. Refer to appended pages 5 and 6 for summaries of the chromosomal aberration data.

In the absence of S9, there was a well-defined (and statistically significant) relationship involving increased numbers of cells with aberrations and concentration of the test material (refer to appended p. 5). From p. 10: "...dose-related increases in the mean frequency of aberrant metaphases were apparent both including and excluding gap-type aberrations. These increases were statistically significant at 100 and 200 μg/ml ($0.05 > p > 0.01$ and $p < 0.001$ respectively)."

"In the presence of S-9 mix, solvent control cultures gave a mean aberrant cell frequency of 11.0% (2.0% excluding gaps); untreated controls showed a mean frequency of 10.3% (1.7%). Mean frequencies for cultures treated with KIH-9201 were 14.3% (1.7%), 13.3% (2.3%) and 16.7% (3.0%) at 50, 100 and 200 μg/ml respectively."

"Thus, a small, but statistically significant ($0.05 > p > 0.01$) increase in the mean frequency of aberrant metaphases was apparent for cultures treated with KIH-9201 at 200 μg/ml, but only when gaps were included in the analysis."

Examination of summary data for specific aberrations (see report pages 31 and 32; appended pages 7 and 8) shows a strong correlation between dose level and incidence of endoreduplication:

Dose level KIH-9201	S9 Mix	Incidence of cells with endoreduplication
0 (DMSO only)	-	0/300
0 (no treatment)	-	0/300
50 µg/ml	-	0/300
100 µg/ml	-	4/300
200 µg/ml	-	7/300
Mitomycin-C (0.1)	-	0/300
0 (DMSO only)	+	1/300
0 (no treatment)	+	0/300
50 µg/ml	+	2/300
100 µg/ml	+	9/300
200 µg/ml	+	15/300
Cyclophosphamide (0.1)	+	0/300

By Fisher's Exact Test, comparing incidences of 1/300 (DMSO controls +S9) and 9/300 (100 µg/ml KIH-9201 +S9) yields a $p = 0.0102$; comparing 1/300 (DMSO controls +S9) and 15/300 (200 µg/ml +S9) gives $p = 0.000222$.

The dose-related increased incidence of endoreduplication associated with exposure to KIH-9201 ± S9 suggests (but does not conclusively demonstrate) some type of aneugenic (spindle poison) activity. It is noted (see appended pages 3 and 4) that endoreduplication was "not included in those analysed for aberrations." It is also stated for endoreduplication (p. 17): that: "these are recorded separately and excluded from the total of cells scored."

C. DISCUSSION AND CONCLUSIONS:

Under the assay conditions, there was a statistically significant (and dose-related) increase in chromosomal aberrations associated with exposure to CGA-248757 technical in the absence of S9. There was a similar (although not so pronounced effect) in the presence of S9, statistically significant only when gaps (not normally considered an aberration) were included in the analysis.

A noteworthy finding was a dose-related increased incidence of endoreduplication associated with exposure to KIH-9201 ± S9 suggesting (but not conclusively demonstrating) some type of aneugenic (spindle poison) activity. Endoreduplication was not included in the analysis for chromosomal aberrations (see appended pages 3 and 4 stating that endoreduplication was "not included in those [findings?] analysed for aberrations.").

This study and its positive findings are acceptable, and can be used as supporting data for purposes of registration and/or reregistration. While the March, 1991 Subdivision F Addendum 9 Guidelines do not specify an in vitro mammalian cell cytogenetic study as part of the initial battery of mutagenicity data requirements, it is stated (p. 7) that: "If tests for endpoints that may be predictive of mutagenicity are performed in addition to the initial battery, the results of such tests shall be submitted to the OPP along with results from the initial battery."

Page_____ is not included in this copy.

Pages 168 through 177 are not included.

The material not included contains the following type of information:

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011508

Guideline Series 84: MUTAGENICITY

Reviewed by: Byron T. Backus, Ph.D. Byron T. Backus 12/22/94
Section II, Toxicology Branch II (7509C)
Secondary Reviewer: K. Clark Swentzel K. Clark Swentzel 12/22/94
Section II, Tox Branch II (7509C)

DATA EVALUATION REPORT III

STUDY TYPE: In vivo Micronucleus Test on Rat Hepatocytes

CHEMICAL: CGA-248757 Technical; 9-(4-chloro-2-fluoro-5-methoxycarbonylmethylthiophenylimino)-8-thia-1,6-diazabicyclo [4.3.0]nonan-7-one; [(2-chloro-4-fluoro-5-[(tetrahydro-3-oxo-1H,3H-[1,3,4-thiadiazol[3,4a]pyridazin-1-ylidene)amino]phenyl]thio] acetic acid methyl ester.

PC Code: 108803

MRID NUMBER: 433484-35

SYNONYMS/CAS No.: KIH-9201

SPONSOR: Ciba-Geigy Corporation
P.O. Box 18300
Greensboro, NC 27419

TESTING FACILITY: Ciba-Geigy Limited
Genetic Toxicology
Basel, Switzerland

TITLE OF REPORT: In vivo Micronucleus Test on Rat Hepatocytes

AUTHORS: Hertner, T.

LABORATORY STUDY NUMBER: 921079

STUDY COMPLETION DATE: February 10, 1993

EXECUTIVE SUMMARY: CGA 248757 technical was administered orally to groups of 4 male rats at doses of 1250, 2500, and 5000 mg/kg. One set of 3 groups was dosed with the mitotic stimulating agent 4-AAF 3 days after treatment; the other set of 3 groups was treated with 4-AAF about 30 hours before exposure to CGA 248757. Additional groups were treated with the vehicle alone, or with the positive controls dimethylnitrosamine (10 mg/kg) or cyclophosphamide (20 mg/kg). Three days after the last treatment (mitotic stimulus or

dosage of CGA 248757) slides of hepatocytes from 3 animals/group were prepared and scored for presence of micronuclei. There were slight (but usually statistically significant) increases in numbers of micronucleated hepatocytes associated with exposure to CGA 248757 at 5000 mg/kg (but not at 2500 or 1250 mg/kg). **The results are consistent with a weakly positive clastogenic and/or aneugenic response associated with dosage of CGA 248757 at 5000 mg/kg under the conditions of this assay.**

STUDY CLASSIFICATION: Acceptable. This study satisfies the 1991 Subdivision F Addendum 9 mutagenicity Guideline requirement [84-2(b)(3)] for an *in vivo* mammalian cells cytogenetics assay for CGA 248757 technical and can be used as supporting data for purposes of registration and/or reregistration.

A. MATERIALS

1. Test Material:
   Description: White powder
   Lot number: FL-920346
   Purity: 97.7%
   Receipt date: not reported
   Stability: reported (p. 11) as "stable."
   Contaminants: not reported
   Solvent used: DMSO
   Other provided information: From p. 18: "A solubility test was performed to determine the highest applicable dose level (solution/suspension) of the test substance for the tolerability test (up to a top dose level of 5000 mg/kg body weight). CMC, 0.5% was found to be the best suited vehicle, yielding the highest applicable dose level of 5000 mg/kg."

2. Control Materials:
   Vehicle (negative control): CMC, 0.5%
   Positive: From p. 19: dimethylnitrosamine (DMN 10 mg/kg), dissolved in distilled water and administered IP (used in part I, which involved administration of the mitogenic agent 4-AAF 3 days after dosage); cyclophosphamide (CPA 20 mg/kg, dissolved in distilled water and administered IP (used in part II, which involved administration of 4-AAF about 30 hours before dosage of the test substance).

3. Test animal: Male rats (Tif:RAIf [SPF]), reared by Ciba-Geigy, Sisseln, Switzerland.

4. Assay acceptance criteria: From p. 22:

   1. The results of the experiments should not be influenced by a significant technical error or a recognized artifact.

2. The high dose of the test substance applied should be the maximum tolerated dose causing no death in a group of two male animals in the range-finding test. In case of missing toxicity the high dose should be up to a maximum of 5000 mg/kg body weight or the highest applicable dose due to the limited solubility of the test substance.

3. At least three male animals per dose and control group should be evaluated.

4. The positive control should fulfill the criteria for a mutagenic substance.

5. Assay evaluation criteria: From p. 22: "Assessing a possible mutagenic property of the test substance was based on statistical analysis of the test data and on scientific judgement by the study director."

6. There is a signed and dated "Certification of Good Laboratory Practice and Verification of a Complete and Unaltered Copy of the Amendment by the Study Sponsor" on page 6 of the report, along with a signed and dated "Statement of Compliance with Good Laboratory Practice" on page 7. There is also a "Certification of Good Laboratory Practice" statement on page 3 of the report from the submitter. There is a signed and dated Quality Assurance Statement on page 9, giving dates of QA activity.

B. TEST PERFORMANCE AND RESULTS:

1. Dose selection: From information on p. 18 CMC 0.5% was found to be "the best suited vehicle." Also from p. 18: "Two tolerability tests, designated as part 1 and part 2 respectively, were performed to determine the maximum tolerated dose level of the test substance, which is the highest causing no death in a group of two male animals during the observation period; that is the interval between administration and sacrifice of the animals in the respective part of the micronucleus test, plus one day. Two male rats were treated with a dose of 5000 mg/kg. In part 1... the treatment was performed with one single application three days before 4-AAF gavage; the entire observation period of the animals was seven days. In part 2 of the tolerability test the treatment was performed with one single application about 30 hours after 4-AAF-gavage; the entire observation period of the animals was five days."

Results: From p. 23:

Part 1: "A dose of 5000 mg/kg CGA 248757 tech. was administered to two rats. Both animals survived the treatment. They showed diarrhea and reduced locomotor activity. Based on these results the dose of 5000 mg/kg was chosen as the highest dose to be administered in part 1 of the micronucleus test."

Part 2: "A dose of 5000 mg/kg CGA 248757 tech. was administered to two rats. Both animals survived the treatment. With one of them reduced motor activity was noted. Based on these results the dose of 5000 mg/kg was chosen as the highest dose to be administered in part 2 of the micronucleus test."

2. Micronucleus assays:

Part 1: From information on p. 19 the vehicle (CMC, 0.5%) was used as negative control (oral gavage; dosage volume 10 ml/kg) and the positive control was dimethylnitrosamine (DMN 10 mg/kg, dissolved in distilled water, administered by IP with a dosing volume of 5 ml/kg). "In the negative and positive control group and in the groups treated with the test substance, each four male animals were treated. The treatment was performed with one single application three days before 4-AAF-gavage. The preparation of the hepatocytes (liver perfusion) was performed three days after 4-AAF-application."

Part 2: The vehicle (CMC 0.5%) was used as negative control as in Part 1; cyclophosphamide (CPA 20 mg/kg, dissolved in distilled water, administered IP in a dosing volume of 5 ml/kg) was used as positive control. From p. 20: "The treatment was performed with one single application 29 hours after 4-AAF-gavage." Hepatocytes preparations were performed three days after administration of the control or test material.

Mitogenic stimulus: From p. 20: "4-AAF enhances the mitotic activity of hepatocytes. This compound was administered to rats via oral gavage (1000 mg/kg body weight) suspended in arachis oil; dosing volume 10 ml/kg).

Isolation of hepatocytes by liver perfusion: "Three animals per dose group were perfused, serving for the isolation of the hepatocytes...additional animals were kept in reserve."

"Primary hepatocytes were isolated by in situ-collagenase perfusion... The animal was anesthetized and killed by a cut through the vena cava inferior and the liver was perfused in situ through the portal vein for about 8-12 minutes with HBSS

(Hank's balanced salt solution, without Ca^{2+} and Mg^{2+}). This solution was aerated with carbogen (95% O_2, 5% CO_2), its temperature was about 37°C and the pH about 7.4. After insertion of a canule into the thoracic part of the vena cava, the perfusion was continued for further 12-18 minutes by recirculation of HBSS, which was supplemented with 0.05% collagenase and 5 mM $CaCl_2$. The liver was then carefully excised and placed into a dish containing HBSS. After opening the Glisson's capsule, the cells were dispersed by gentle shaking of the liver in the solution. The cells were then filtered (mesh width of 61 µm) and washed twice with HBSS (sedimentation rate of 50 g for 3 minutes at 2°C). Finally, the cells were suspended in HBSS."

Preparation of slides: "From the hepatocyte suspension...0.3 - 0.5 ml were transferred with a pipette to a regular microscope slide. The slides were then fixed on a holder of a Hemaspinner® slide centrifuge and smears of hepatocytes were prepared. After fixation of the cells with ethanol/glacial acetic acid/ formaldehyde (37%) 85:10:5 v/v, the hepatocytes were stained with Feulgen, which stains specifically for DNA and with Light Green as a counterstain for visualization of the cytoplasm and the cell boundary."

Criteria for scoring micronuclei: "Prior to analysis the slides were coded. From each animal (three animals per dose-group), the incidence of micronucleated hepatocytes were calculated by counting a total of 1000 hepatocytes. In an additional scoring of two groups of part 2 1600 to 2000 additional hepatocytes were evaluated."

"The selection and scoring of hepatocytes for micronucleated hepatocytes was made according to the following criteria:

- micronuclei were only scored in hepatocytes with intact cellular and nuclear membranes.
- cells with pyknotic nuclei were neglected.
- no distinction was made between mono- and binucleated hepatocytes.
- hepatocytes with two or more micronuclei were scored as single micronucleated hepatocytes.
- the diameter of the micronucleus was equal or smaller than one-third of the diameter of the nucleus.
- The color of the micronucleus was similar to the color of the nucleus.

IN VIVO CYTOGENETICS - RAT HEPATOCYTE MICRONUCLEUS

III-6

Results:

Part 1: From p. 23: "Evaluation of the numbers of micronucleated hepatocytes of the different groups dosed with CGA 248757 tech. revealed slightly increased values at the low dose (0.97%) and the high dose (0.87%) when compared to the negative control group (0.37%)... No statistical significance was obtained using the Cochran-Armitage trend test. However the ANOVA revealed significant differences for the low and high dose groups... The historical control data...show that frequencies of micronucleated hepatocytes up to 0.9% may also occur in negative control animals. In addition, the effects observed in this experiment showed no dose-dependency. Therefore, they are considered to be purely fortuitous events and not treatment-related."

Part 2: From p. 24: "The two lower doses of CGA 248757 tech. revealed no statistically significant increase in the number of micronucleated hepatocytes in comparison with the negative control group... A slightly increased value of 1.10% was obtained with the high dose group compared to the negative control value of 0.67%. To check the significance of this difference, additional slides from the same animals of the negative control and the high dose groups were evaluated. Four to five slides were scored, 400 hepatocytes per slide. From this additional scoring a frequency of 0.75% micronucleated hepatocytes was obtained for the high dose group compared to a frequency of 0.47% for the control group... Statistical Analysis revealed a slightly significant trend (Cochran-Armitage trend test) for both, the originally and additionally scored values. The group comparison test (ANOVA) showed a significance for the additional data only..."

"As already mentioned above, negative control animals showed also frequencies of up to 1.2% micronucleated hepatocytes [Note by reviewer: this refers to historical negative control data] which are in the range of the values found in this experiment for the high dose group (0.75-1.1%). Therefore, the difference obtained is considered to be purely fortuitous and not treatment-related. The statistical significances found can be explained by the exceptionally low inter animal variations obtained in the respective groups of this experiment."

Refer to appended pages 1-4 for the group and individual data, as well as statistical analyses. Refer to appended p. 5-6 for the historical negative controls.

C. DISCUSSION AND CONCLUSIONS:

In part 1 the following group incidences of micronucleated hepatocytes were obtained:

Test Group	Incidence	p value*
Negative controls	11/3000	-
1250 mg/kg CGA 248757	29/3000	0.00313
2500 mg/kg CGA 248757	18/3000	0.13188
5000 mg/kg CGA 248757	26/3000	0.00985
Positive controls	337/3000	$< 10^{-8}$

*By Fisher's Exact Test; comparison is with negative controls.

In part 2 the following group incidences of micronucleated hepatocytes were obtained as a result of the first counting:

Test Group	Incidence	p value*
Negative controls	20/3000	-
1250 mg/kg CGA 248757	17/3000	0.37103
2500 mg/kg CGA 248757	16/3000	0.30833
5000 mg/kg CGA 248757	33/3000	0.04844
Positive controls	187/3000	$< 10^{-8}$

*By Fisher's Exact Test; comparison is with negative controls.

Subsequently, additional counts were made from slides from the controls and 5000 mg/kg animals of part 2:

Test Group	Incidence	p value*
Negative controls	28/6000	-
1250 mg/kg CGA 248757	42/6000	0.06#

*By Fisher's Exact Test; comparison is with negative controls.
#Approximation, due to computational overflow.

The historical negative control range (group/experiment) is from 6/3000 to 12/2000, with a mean of 3.95/1000 (application of the vehicle before 4-AAF gavage) and from 2/3000 to 23/2000. with a mean of 3.95/1,000 (application of the vehicle after 4-AAF gavage). The incidence observed for high-dose animals in part 1 was outside the historical control range (26/3000 or 8.67/1000 vs. 2/1000 to 6/1000); in part 2 the incidences were within the historical control range (first count: 33/3000 or 11/1000; second count 42/6000 or 7/1000 vs. 0.67/1000 to 11.5/1000). However, the high historical control value of 11.5/1000 was obtained in August 1988 (the earliest date; designated as a "validation study"), and since that date the historical control range has been 0.67/1000 to 4.33/1000.

From the data then there were consistently elevated incidences of micronucleated hepatocytes following 5000 mg/kg dosage, and these elevations were either statistically significant or close to statistically significant with respect to the negative control values. In addition, there is some indication that these values were at least somewhat elevated with respect to the negative historical control data. It is concluded then that the results of the study are consistent with (and should be interpreted as) a weakly positive clastogenic and/or aneugenic response following dosage of CGA 248757 at 5000 mg/kg (but not at 2500 or 1250 mg/kg) under the conditions of this assay.

This study satisfies the 1991 Subdivision F Addendum 9 mutagenicity Guideline requirement [84-(3)] for an in vivo cytogenetics assay for CGA 248757 and can be used as supporting data for purposes of registration and/or reregistration.

Page _____ is not included in this copy.

Pages 186 through 191 are not included.

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_____ Identity of product impurities.

_____ Description of the product manufacturing process.

_____ Description of quality control procedures.

_____ Identity of the source of product ingredients.

_____ Sales or other commercial/financial information.

_____ A draft product label.

_____ The product confidential statement of formula.

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the individual who prepared the response to your request.

011508

Guideline Series 84: **MUTAGENICITY**

Reviewed by: Byron T. Backus, Ph.D. Byron T. Backus 12/22/94
Section II, Toxicology Branch II (7509C)
Secondary Reviewer: K. Clark Swentzel K. Clark Swentzel 12/22/94
Section II, Tox Branch II (7509C)

DATA EVALUATION REPORT VI

STUDY TYPE: In vivo Micronucleus Test in Mouse Erythrocytes

CHEMICAL: CGA-248757 Technical; 9-(4-chloro-2-fluoro-5-methoxycarbonylmethylthiophenylimino)-8-thia-1,6-diazabicyclo [4.3.0]nonan-7-one; [(2-chloro-4-fluoro-5-[(tetrahydro-3-oxo-1H,3H-[1,3,4-thiadiazol[3,4a]pyridazin-1-ylidene)amino]phenyl]thio] acetic acid methyl ester; KIH-9201

PC Code: 108803

MRID NUMBER: 433484-36

SYNONYMS/CAS No.: KIH-9201

SUBMITTER: Ciba-Geigy Corporation
P.O. Box 18300
Greensboro, NC 27419

TESTING FACILITY: Life Science Research Institute
Kumiai Chemical Industry Co., Ltd.
Shizuoka, Japan

TITLE OF REPORT: KIH-9201: Preliminary Micronucleus Study in Mice

AUTHORS: Mizuhashi, F., Murata, K., and Ishikawa, K.

LABORATORY STUDY NUMBER: Not given

STUDY COMPLETION DATE: January 10, 1991

EXECUTIVE SUMMARY: CGA 248757 (KIH-9201) was administered by oral gavage to groups of 5 male mice/sacrifice time at 5000 mg/kg, with subsequent sacrifice at 24, 48, and 72 hours. Groups of 5 males/sacrifice time received vehicle only, and served as negative controls. **There was no indication of any change in the polychromatic erythrocyte (PCE) to normochromatic cell ratio, nor was there an increase in the incidence of micronucleated PCEs associated with exposure to the test material.**

STUDY CLASSIFICATION: Not acceptable. There is no indication that there were positive controls, females were not tested (and no justification for their exclusion is given) and there are no analytical results from the dosing solutions. In addition, the submitter states that the study cannot be certified as being in full compliance with GLP standards. This study does not satisfy the 1991 Subdivision F Addendum 9 mutagenicity Guideline requirement [84-2(b)(3)] for an in vivo mammalian cells cytogenetics assay for CGA 248757 technical and cannot be used as supporting data for purposes of registration and/or reregistration.

A. MATERIALS

1. Test Material:
   Description: White powder
   Lot number: LC9016
   Purity: 96.2%
   Receipt date: February 2, 1989
   Stability: not reported
   Contaminants: not reported
   Vehicle used: 0.5% (w/v) carboxymethylcellulose
   Other provided information (see p. 8); the test material was stored at "4°C, protected from light."

2. Control Materials:
   Vehicle (negative control): CMC, 0.5%
   Positive: none

3. Test animal: From p. 8: "Adult male mice (7 weeks old) of the CD-1 (ICR) strain were obtained from Charles River Japan Inc. They were allowed seven days acclimatization prior to treatment... At commencement of the study they were in the weight range 39 to 42 g (8 weeks old)."

4. Assay acceptance criteria: None given

5. There is a signed and dated page titled "Responsibility" on p. 5 of the report, but there is no indication of a GLP and/or QA statement. On page 3 of the report the submitter states that: "This study is a preliminary assessment and cannot be certified as being in full compliance with Good Laboratory Practice Standards."

B. TEST PERFORMANCE AND RESULTS:

1. Dose selection: It is simply stated on p. 7 that: For the purpose of this preliminary assessment, KIH-9201 was administered by gavage at [a] dose level of 5000 mg/kg, which was the maximum tolerated dose for mice."

2. Micronucleus assay: Groups of 5M/sacrifice time received 0 (vehicle only) or 5000 mg/kg of the test material, with sacrifice at 24, 48 or 72 hours after dosage. From p. 11: "The animals were dosed by the oral route (gavage) at a dose level of 5000 mg/kg (at a volume-dose of 10 ml/kg)... Control animals received the vehicle at the same volume-dose."

   Slide preparation: "Animals were killed by cervical dislocation. Femurs from each animal were rapidly dissected out and cleaned of adherent tissue... Marrow cells were flushed out with 0.6 ml foetal calf serum using a syringe and needle. The recovered cells were centrifuged at 1000 rpm for five minutes. The bulk of the supernatant fluid was discarded and the cell pellet resuspended in the remaining fluid. Single drops of the cell suspension were transferred to clean, dry slides, two smears prepared, and the slides left to air dry. Following fixation in methanol for ten minutes, they were stained manually, using Giemsa..."

   Slide evaluation: "The slides were examined under the light microscope, and regions judged to be of adequate technical quality to permit scoring were selected under low magnification. At high magnification (X 1000, oil immersion) a total of at least 1000 erythrocytes per animal were examined. Each erythrocyte scored was classed as polychromatic or mature: cells stain blue/pink and the older cells stain red/pink. ...each polychromatic cell scored was examined for the presence or absence of micronuclei, and the resultant data were used to calculate the number of micronucleated cells per 1000 polychromatic cells."

   Statistics: "Using the frequency of micronucleated cells per 1000 polychromatic erythrocytes scored, the data were subjected to statistical analysis by the Kastenbaum and Bowman procedure. Data from the ratio of polychromatic to mature cells were analyzed by the Student t-test."

   Results: There were no indications of a shift in the PCE:NCE ratio, nor was there any indication of an increased incidence of micronucleated PCEs associated with exposure to the test material. Refer to appended pages 1-3 for summary and individual data.

C. DISCUSSION AND CONCLUSIONS:

While the test material, KIH-9201 (also known as CGA-248757 technical) showed no evidence of clastogenic and/or aneugenic activity under the conditions of this assay, the following deficiencies are noted:

- There is no indication of any concurrent positive control group.
- Females were not tested (and there is no justification given for their exclusion).
- Dosing solutions were not analyzed.
- The submitter states that the study cannot be certified as being in full compliance with GLP standards.

For these reasons, the study is classified as not acceptable. This study does not satisfy the 1991 Subdivision F Addendum 9 mutagenicity Guideline requirement [84-2(b)(3)] for an in vivo mammalian cells cytogenetics assay for CGA 248757 technical and cannot be used as supporting data for purposes of registration and/or reregistration.

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Pages 196 through 198 are not included.

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X FIFRA registration data.

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The information not included is generally considered confidenti
by product registrants. If you have any questions, please conta
the individual who prepared the response to your request.

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

AUG 31 1998

MEMORANDUM

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

SUBJECT: Action Qualitative Risk Assessment Based On Crl:CD-1 (ICR) BR (Swiss)Mouse and Sprague-Dawley (SD) Rat Dietary Studies

P.C. Code 108803

TO: Alan Levy, Physiologist
Registration Action Branch 2
Health Effects Division (7509C)

FROM: Lori L. Brunsman, Statistician
Science Analysis Branch
Health Effects Division (7509C)

THROUGH: William Burnam, Branch Chief
Science Analysis Branch
Health Effects Division (7509C)

8/31/98

8/31/98

Background

An oncogenicity study in Crl:CD-1(ICR)BR(Swiss)mice was conducted by Ciba-Geigy Corporation, Crop Protection Division, Environmental Health Center, Farmington, Connecticut, for Ciba-Geigy Corporation, Crop Protection Division, Greensboro, North Carolina, and issued June 26, 1995 (Report No. F-00069; MRID No. 438300-15).

The study design allocated groups of 50 mice per sex to dose levels of 0, 1, 10, 100, or 300 ppm of Action for 80 weeks.

A combined chronic/oncogenicity study in Sprague-Dawley (SD) rats was conducted by Ciba-Geigy Corporation, Crop Protection Division, Environmental Health Center, Farmington, Connecticut, for Ciba-Geigy Corporation, Crop Protection Division, Greensboro, North Carolina, and issued September 11, 1995 (Report No. F-00068; MRID No. 438300-17).

The study design allocated groups of 50 rats per sex to dose levels of 0, 5, 50, 3000, or 5000 ppm (males) or 3000 ppm (females) of Action for 105 weeks. An additional 10 rats per sex in the 5, 50, and 3000 ppm groups, and an additional 20 rats per sex in the control and high dose groups, were designated for interim sacrifice at week 53.

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Survival Analyses

The statistical evaluation of mortality indicated no significant incremental changes with increasing doses of Action in male or female mice, or in female rats. Male rats showed a significant decreasing trend for mortality with increasing doses of Action. See Tables 1 and 2 for mouse mortality test results, and tables 5 and 6 for rat mortality test results.

The statistical evaluation of mortality was based upon the Thomas, Breslow and Gart computer program.

Tumor Analyses

Male mice had significant increasing trends for liver adenomas, carcinomas, and adenomas and/or carcinomas combined, as well as significant differences in the pair-wise comparisons of the 300 ppm dose group with the controls for liver carcinomas and adenomas and/or carcinomas combined, all at $p < 0.01$. There were also significant differences in the pair-wise comparisons of the 300 ppm dose group with the controls for liver adenomas, and the 100 ppm dose group with the controls for liver carcinomas and adenomas and/or carcinomas combined, all at $p < 0.05$.

Female mice had significant increasing trends for liver adenomas, and adenomas and/or carcinomas combined, both at $p< 0.01$. There were no significant differences in the pair-wise comparisons of the dosed groups with the controls.

Male rats had significant increasing trends for pancreatic exocrine adenomas at $p < 0.01$, and for pancreatic islet cell adenomas and adenomas and/or carcinomas combined, both at $p < 0.05$. There was a significant difference in the pair-wise comparison of the 5000 ppm dose group with the controls for pancreatic islet cell adenomas at $p < 0.05$.

Female rats had no significant compound-related tumors.

The statistical analyses of the male and female mice were based upon the Exact trend test and the Fisher's Exact test for pair-wise comparisons. The statistical analyses of the male rats were based upon Peto's prevalence test since there were statistically significant negative trends for mortality with increasing doses of Action. See Tables 3 and 4 for mouse tumor analysis results. See Table 7 for male rat tumor analysis results.

Table 1. Action Crl:CD-1(ICR)BR(Swiss) Mouse Study

Male Mortality Rates[+] and Cox or Generalized K/W Test Results

Dose (ppm)	Weeks 1-26	27-52	53-66	67-81[f]	Total
0	0/50	1/50	6/49	8/43	15/50 (30)
1	0/50	1/50	2/49	11/47	14/50 (28)
10	1/50	2/49	2/47	10/45	15/50 (30)
100	0/50	1/50	6/49	8/43	15/50 (30)
300	0/50	1/50	1/49	8/48	10/50 (20)

[+]Number of animals that died during interval/Number of animals alive at the beginning of the interval.

[f]Final sacrifice at week 80.

( )Percent.

Note: Time intervals were selected for display purposes only.

Significance of trend denoted at control.

Significance of pair-wise comparison with control denoted at dose level.

If *, then $p < 0.05$. If **, then $p < 0.01$.

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Table 2. Action Crl:CD-1(ICR)BR(Swiss) Mouse Study

Female Mortality Rates[+] and Cox or Generalized K/W Test Results

Dose (ppm)	Weeks 1-26	27-52	53-66	67-81[f]	Total
0	1/49[a]	0/48	5/48	11/43	17/49 (35)
1	2/50	2/48	3/46	6/43	13/50 (26)
10	0/50	1/50	3/49	9/46	13/50 (26)
100	1/50	2/49	3/47	3/44	9/50 (18)
300	0/50	1/50	4/49	5/45	10/50 (20)

[+]Number of animals that died during interval/Number of animals alive at the beginning of the interval.

[f]Final sacrifice at week 80.

[a]One accidental death at week 25, dose 0 ppm.

( )Percent.

Note: Time intervals were selected for display purposes only.

Significance of trend denoted at control.

Significance of pair-wise comparison with control denoted at dose level.

If *, then $p < 0.05$. If **, then $p < 0.01$.

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Table 3. Action Crl:CD-1(ICR)BR(Swiss) Mouse Study

Male Liver Tumor Rates[+] and Exact Trend Test and Fisher's Exact Test Results (p values)

	Dose (ppm)				
	0	1	10	100	300
Adenomas (%)	12/49 (24)	9/49 (18)	10/47 (21)	19/49 (39)	22[a]/48 (46)
p =	0.001**	0.312[n]	0.448[n]	0.096	0.023*
Carcinomas (%)	3/49 (6)	5/49 (10)	6/47 (13)	12[b]/49 (24)	13/48 (27)
p =	0.002**	0.357[n]	0.223[n]	0.011*	0.005**
Combined (%)	15/49 (31)	13[c]/49 (27)	14[d]/47 (30)	26[e]/49 (53)	31[f]/48 (65)
p =	0.000**	0.412[n]	0.554[n]	0.020*	0.001**

[+]Number of tumor bearing animals/Number of animals examined, excluding those that died or were sacrificed before week 53.

[a]First adenoma observed at week 60, dose 300 ppm.
[b]First carcinoma observed at week 59, dose 100 ppm.

[c]One animal in the 1 ppm dose group had both an adenoma and a carcinoma.
[d]Two animals in the 10 ppm dose group had both an adenoma and a carcinoma.
[e]Five animals in the 100 ppm dose group had both an adenoma and a carcinoma.
[f]Four animals in the 300 ppm dose group had both an adenoma and a carcinoma.

[n]Negative change from control.

Note: Significance of trend denoted at control.

Significance of pair-wise comparison with control denoted at dose level.

If *, then $p < 0.05$. If **, then $p < 0.01$.

Table 4. Action Crl:CD-1(ICR)BR(Swiss) Mouse Study

Female Liver Tumor Rates[+] and Exact Trend Test and Fisher's Exact Test Results (p values)

	Dose (ppm)				
	0	1	10	100	300
Adenomas (%)	2/48 (4)	0/46 (0)	1/49 (2)	7/47 (15)	7[a]/49 (14)
p =	0.003**	0.258[n]	0.492[n]	0.074	0.084
Carcinomas (%)	1/48 (2)	0/46 (0)	1[b]/49 (2)	2/47 (4)	2/49 (4)
p =	0.145	0.511[n]	0.747	0.492	0.508
Combined (%)	3/48 (6)	0/46 (0)	2/49 (4)	9/47 (19)	8[c]/49 (16)
p =	0.004**	0.129[n]	0.490[n]	0.055	0.106

[+]Number of tumor bearing animals/Number of animals examined, excluding those that died or were sacrificed before week 53.

[a]First adenoma observed at week 78, dose 300 ppm.

[b]First carcinoma observed at week 81, dose 10 ppm.

[c]One animal in the 300 ppm dose group had both and adenoma and a carcinoma.

[n]Negative change from control.

Note: Significance of trend denoted at control.

Significance of pair-wise comparison with control denoted at dose level.

If *, then $p < 0.05$. If **, then $p < 0.01$.

Table 5. Action Sprague-Dawley (SD) Rat Study

Male Mortality Rates[+] and Cox or Generalized K/W Test Results

Dose (ppm)	Weeks 1-26	27-52	53[i]	53-78	79-106[f]	Total
0	0/70	2/70	19/68	6/48[a]	26/42	34/50 (68)[**n]
5	1/60	2/59	9/57	4/48	23/44	30/51 (59)
50	2/60	2/58	9/56	8/47	22/39	34/51 (67)
3000	1/60	1/59	10/58	5/48	13/43	20/50 (40)[*n]
5000	0/70	2/70	19/68	1/49	13/48	16/51 (31)[**n]

[+]Number of animals that died during interval/Number of animals alive at the beginning of the interval.

[I]Interim sacrifice at week 53.

[f]Final sacrifice at week 105.

[a]One accidental death at week 70, dose 0 ppm.

[n]Negative trend or negative change from control.

( )Percent.

Note: Time intervals were selected for display purposes only.

Significance of trend denoted at control.

Significance of pair-wise comparison with control denoted at dose level.

If *, then $p < 0.05$. If **, then $p < 0.01$.

Table 6. Action Sprague-Dawley (SD) Rat Study

Female Mortality Rates[+] and Cox or Generalized K/W Test Results

	Weeks					
Dose (ppm)	1-26	27-52	53[i]	53-78	79-107[f]	Total
0	1/70	1/69	20/68	2/48	16/46	20/50 (40)
5	0/60	1/60	10/59	5/49	13/44	19/50 (38)
50	0/60	2/60	9/58	7/49	16/42	25/51 (49)
3000	1/60	1/59	10/58	7/48	14/41	23/50 (46)
7000	3/70	1/67	19/66	7/47	15/40	26/51 (51)

[+]Number of animals that died during interval/Number of animals alive at the beginning of the interval.

[I]Interim sacrifice at week 53.

[f]Final sacrifice at week 106.

( )Percent.

Note: Time intervals were selected for display purposes only.

Significance of trend denoted at control.

Significance of pair-wise comparison with control denoted at dose level.

If *, then $p < 0.05$. If **, then $p < 0.01$.

Table 7. Action Sprague-Dawley (SD) Rat Study

Male Pancreas Tumor Rates[+] and
Peto's Prevalence Test Results

	Dose (ppm)				
	0	5	50	3000	5000
Exocrine Adenomas (%)	1/28 (4)	2/36 (6)	1/31 (3)	5[a]/37 (14)	7/44 (16)
p =	0.007**	0.349	-	0.053	0.107
Islet Cell Adenomas (%)	1/43 (2)	3[b]/46 (7)	2/43 (5)	4/45 (9)	8/47 (17)
p =	0.017*	0.184	0.301	0.105	0.024*
Islet Cell Carcinomas (%)	1/16 (6)	0/21 (0)	0/17 (0)	1/30 (3)	0/35 (0)
p =	0.686	-	-	0.676	-
Islet Cell Tumors Combined (%)	2/43 (5)	3/46 (7)	2/43 (5)	5/45 (11)	8/47 (17)
p =	0.031*	0.385	-	0.202	0.081

[+]Number of tumor bearing animals/Number of animals examined, excluding those that died before observation of the first tumor.

[a]First exocrine adenoma observed at week 93, dose 3000 ppm.

[b]First islet cell adenoma observed at week 71, dose 5 ppm.

[c]First islet cell carcinoma observed at week 106, dose 3000 ppm.

Note: Significance of trend denoted at control.

Significance of pair-wise comparison with control denoted at dose level.

If *, then $p < 0.05$. If **, then $p < 0.01$.

References

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STRUCTURE-ACTIVITY-RELATIONSHIP

NONE

REGISTRANT'S COMMENTS REGARDING CARCINOGENICITY

NONE