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

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April 6, 1994

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Carcinogenicity Peer Review Meeting on **DIFENOCONAZOLE**

FROM: Esther Rinde, Ph.D. E.R.
Manager, Carcinogenicity Peer Review
Health Effects Division (7509c)

TO: Addressees

Attached for your review is a package on **DIFENOCONAZOLE** prepared by Jess Rowland.

A meeting to consider the carcinogenicity classification of this chemical is scheduled for **Wednesday April 27, 1994**, at **10:00 am in Room 817, CM2.**

Addressees

P. Fenner-Crisp
R. Engler
W. Burnam
K. Baetcke
M. Van Gemert
K. Dearfield
H. Pettigrew
B. Fisher
L. Brunsman
E. Doyle
J. Rowland
C. Swentzel
R. Hill
Y. Woo
R. DiLavore/L.Brennecke

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OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

DATE: **April 6, 1994**

MEMORANDUM

SUBJECT: **DIFENOCONAZOLE:** Peer Review by the Health Effects Division Carcinogenicity Peer Review Committee.

FROM: Jess Rowland, M.S, Toxicologist Jess Rowland 4/6/94
Section II, Toxicology Branch II, Health Effects Division (7509C)

TO: Esther Rinde, Ph.D, Manager
HED Carcinogenicity Peer Review Committee
Science Analysis and Coordination Branch, Health Effects Division (7509C)

THROUGH: K. Clark Swentzel, Head K. Clark Swentzel 4/6/94
Section II, Toxicology Branch II, Health Effects Division (7509C)

and

Marcia Van Gemert, Ph.D, Chief Marcia Van Gemert 4/6/94
Toxicology Branch II, Health Effects Division (7509C)

The Health Effects Division Carcinogenicity Peer Review Committee is requested to evaluate the carcinogenic potential of Difenoconazole. A data package consisting of Data Evaluation Records of critical studies as well as summary of toxicology studies submitted to the Office of Pesticide Programs are attached.

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DIFENOCONAZOLE----CARCINOGENICITY PEER REVIEW

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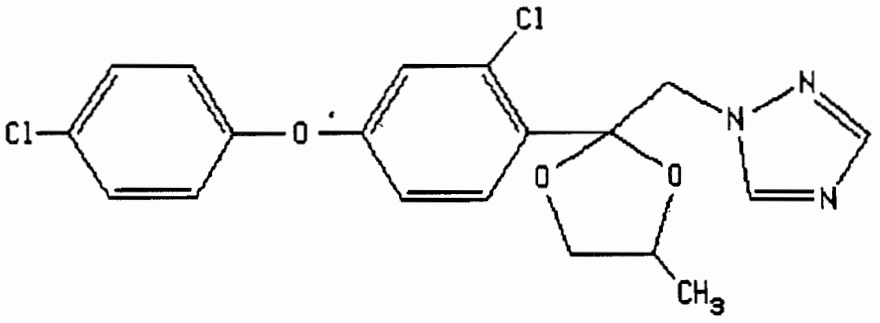
I. BACKGROUND INFORMATION

Difenoconazole [CGA-169374 Technical]; 1-[2-(2-chloro-4-(4-chlorophenoxy)phenyl)-4-methyl-1,3-dioxolan-2-yl)-methyl]-1H-1,2,4-triazole] is a triazole fungicide. The P.C Code is 128847 and the Caswell Number is 955. The Registrant, Ciba-Geigy, is requesting tolerances allowing import of wheat, barley, and rye grain harvested from Difenoconazole treated seed as well as domestic tolerances of this fungicide in straw and forage of wheat and barley. The structure of Difenoconazole is provided below:



II. EVALUATION OF THE CARCINOGENIC POTENTIAL

1. Carcinogenicity Study in Mice:

Reference: Cox, R.H. Oncogenicity Study in Mice. Project Report 483-250. Hazleton Laboratories, Report issued, April 3, 1989. MRID # 420900-15 & 427100-06. HED Document # 009689 & 010588

A. Experimental Design

Groups of 60-70 male and 60-70 female Crl:CD-1 mice were fed diets containing difenoconazole at 0, 10, 30, 300, 2500 or 4500 ppm [males only] for 78 weeks. The mean daily test article intake was 1.5, 5, 46, 423 and 819 mg/kg/day in males and 2, 6, 58 and 512 mg/kg/day in females, respectively. Ten animals per sex from each group were sacrificed after 52 weeks of treatment. Ten additional animals, allocated to the control, 2500 ppm and 4500 ppm dose groups were kept for a four week recovery period after one year on the study.

2. Discussion of Tumor Data

As presented in Table 1, statistical analyses of tumor data showed significant increases in liver tumors in both sexes of mice.

Male mice exhibited significant [p <0.05] increasing trends in hepatocellular adenomas, carcinomas, and combined adenomas/carcinomas. Pair-wise comparison showed a significant [p <0.05] increase in hepatocellular adenomas at 300 ppm [9/56,16%] when compared to controls [4/68, 6%]. Pair-wise comparison showed significant [p <0.05] increases in hepatocellular adenomas [13/70, 19%] and combined adenomas/carcinomas [16/70, 23%] at 2500 ppm when compared to controls [adenomas: 4/68, 6%; combined adenomas/ carcinomas, 5/68, 7%]. Pair-wise comparison showed significant [p <0.01] increases in adenomas [20/56, 36%], carcinomas [13/56, 23%] and combined adenomas/ carcinomas [28/56, 50%] at 4500 ppm when compared to controls [adenomas: 4/68, 6%; carcinomas, 1/68, 1%; and combined adenomas/ carcinomas, 5/68, 7%].

Female mice exhibited significant [p <0.01] dose-related increasing trends in hepatocellular adenomas, carcinomas, and combined adenomas/carcinomas. Pair-wise comparison showed a significant increase in hepatocellular adenomas at 2500 ppm [16/59, 27%; p<0.01], carcinomas [4/39, 10%; p<0.05], and combined adenomas/ carcinomas [17/59, 29%; p<0.01] when compared to none of each in the controls.

Table 1. Hepatocellular Tumor Rates[+] and Peto's Prevalence Test Results in MALE MICE

ppm	0	10	30	300	2500	4500
mg/kg/day	0	1.51	4.65	46.29	423.16	818.87
Adenomas	4/68	10/57	8/58	9/56	13[a]/70	20/56
%	6	18	14	16	19	36
p=	**0.000****	0.053	0.078	**0.035***	**0.036***	**0.000****
Carcinomas	1/68	0/57	1/58	0/56	5/70	13[b]/56
%	1	0	2	0	7	23
p=	**0.000****	--	0.546	--	0.093	**0.000****
Combined	5/68	10/57	9/58	9/56	16[c]/70	28[c]/56
%	7	18	16	16	23	50
p=	**0.000****	0.114	0.128	0.061	**0.023***	**0.000****

[+] No. of tumor bearing animals / No. of animals examined [excluding those that died or were sacrificed before observation of first tumor.

[a] First adenoma observed at week 53, dose 2500 ppm.

[b] First carcinoma observed at week 62, dose 4500 ppm.

[c] Includes animals with more than one tumor type [i.e., adenomas + carcinomas]

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 2. Hepatocellular Tumor Rates[+]/Exact Trend Test/Fisher's Exact Test Results in FEMALE MICE

ppm	0	10	30	300	2500
mg/kg/day	0	1.90	5.63	57.79	512.61
Adenomas	0/57	0/56	0/56	1/56	16[a]/59
%	0	0	0	2	27
p=	**0.000****	1.000	1.000	0.496	**0.000****
Carcinomas	0/47	0/45	1/44	0/45	4[b]/39
%	0	0	2	0	10
p=	**0.002****	1.000	0.484	1.000	**0.039***
Combined	0/57	0/56	1/56	1/56	17[c]/59
%	0	0	2	2	29
p=	**0.000****	1.000	0.496	0.496	**0.000***

[+] No. of tumor bearing animals / No. of animals examined [excluding those that died or were sacrificed before observation of first tumors, Week 53 for adenomas and combined, and Week 58 for carcinomas].

[a] First adenoma observed at week 53, dose 2500 ppm.
[b] First carcinoma observed at week 72, dose 2500 ppm.
[c] Includes animals with more than one tumor type [i.e., adenomas + carcinomas]

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 $p < 0.01$.

Historical control data for hepatocellular adenomas and carcinomas observed in Charles River CD-1 mice from 10 studies conducted at the testing laboratory [Hazleton Labs] are presented in Table 3. Historical control data were not available for combined adenomas/carcinomas.

In males, the incidence of hepatocellular adenomas at 300 ppm [9/56, 16%], at 2500 ppm [13/70, 19%] and at 4500 ppm [20/56, 36%] exceeded both the weighted average of the historical control incidences [7.4%] from all studies and the historical control range [0 - 11%]. Hepatocellular carcinomas [13/56; 23%] at the 4500 ppm also exceeded the weighted average of the historical control incidences [3%] from all studies and the historical control range [0 - 8.2%]. The incidence of hepatocellular adenomas and carcinomas in the concurrent untreated males was 6% and 1%, respectively.

In females, the incidence of hepatocellular adenomas [16/59; 27%] at 2500 ppm [HDT] also exceeded the weighted average of the historical control incidences [1.4%] from all studies and the historical control range [0 - 6.1%]. Hepatocellular carcinomas [17/59; 29%] at the HDT exceeded both the weighted average of the historical control incidences [0.6%] from all studies and the historical control range [0 - 2.1%]. No liver tumors were seen in the concurrent untreated females.

Table 3. Historical Control: Hepatocellular Adenomas and Carcinomas in CD-1 Mice[a]

Study Identification	Adenomas [%]	Carcinomas [%]
MALES		
5DE	4/51 [7.8]	1/51 [2.0]
6DE	2/49 [4.1]	2/49 [4.1]
1DE	1/49 [2.0]	4/49 **[8.2]**
2DE	4/49 [8.2]	1/49 [2.0]
10DE	6/55 **[11.0]**	3/55 [5.5]
7DE	3/47 [6.4]	0/47 [0.0]
11DE	4/50 [8.0]	0/50 [0.0]
8DE	5/50 [10.0]	1/50 [2.0]
4DE	4/50 [8.0]	2/50 4.0]
26DE	4/49 [8.2]	1/49 2.0]
Weighted Average	**[7.4]**	**[3.0]**
FEMALES		
5DE	0/40 [0.0]	0/49 [0.0]
6DE	0/48 [0.0]	1/48 **[2.1]**
1DE	1/48 [2.1]	0/48 [0.0]
2DE	3/49 **[6.1]**	0/49 [0.0]
10DE	0/55 [0.0]	0/55 [0.0]
7DE	1/49 [2.0]	1/49 [2.0]
11DE	0/50 [0.0]	0/50 [0.0]
8DE	0/49 [0.0]	0/49 [0.0]
4DE	1/50 [2.0]	0/50 [0.0
26DE	1/48 [2.1]	1/48 [2.1]
Weighted Average	**[1.4]**	**[0.6]**

[a] Historical control data were obtained from 10 chronic/carcinogenicity studies with Charles River CD-1 mice conducted at Hazleton Laboratories between April 1984 and April 1987. All studies were 78 weeks in duration.

C. Non-neoplastic Lesions

Treatment-related non-neoplastic histopathological changes were confined to the liver and included: necrosis of individual hepatocytes; focal/multifocal necrosis; hepatocellular hypertrophy; inflammation; bile stasis; and fatty changes. These lesions were observed at the interim and terminal sacrifices in males at 300 ppm and above and in the females at 300 and 2500 ppm. Hepatic lesions are summarized below in Table 4.

Table 4. Non-Neoplastic Lesions in the Liver of Mice fed Difenoconazole.

Hepatic Lesions	0 ppm		10 ppm		30 ppm		300 ppm		2500 ppm		4500 ppm	
Sex No. Examined	M 70	F 60	M 60	F 60	M 60	F 60	M 70	F 70	M 70	F 70	M 70	F#
Necrosis of individual hepatocytes	5	3	5	0	2	0	**13***	6	**52****	**27****	**53****	
Focal/multifocal necrosis	4	4	2	2	4	0	6	7	**11***	6	**16****	
Hypertrophy	17	2	16	7	15	2	**26***	7	**61****	**53****	**57****	
Inflammation	18	13	5	13	8	7	12	18	21	12	7	
Bile stasis	1	0	0	0	0	0	3	3	**56****	**50****	**50****	
Fatty changes	2	0	1	0	0	2	4	2	**13****	**9****	**32****	

Females were not dosed at this level.
* = $P \leq 0.05$; $P \leq 0.01$; significantly different from the controls.

D. Toxicological Effects

The statistical evaluation of mortality indicated a significant increasing trend in mortality with increasing doses of Difenoconazole in male mice [Table 5]. Females showed no significant incremental changes in mortality with increasing doses of the test compound [Table 6]. At 4500 ppm, 11/70 males and all females died within the first two weeks of the study. Prior to death, clinical signs of toxicity included thinness, hunched posture and rough haircoat in the high-dose females. These signs were also seen with increased incidence throughout the study in both sexes at 2500 ppm and in the remaining males at 4500 ppm. In spite of comparable food consumption, cumulative body weight gain at 13 weeks was approximately 16%, 19% and 64% lower [statistically significant at $p < 0.05$] than the weight gain of the control males at 300, 2500 and 4500 ppm, respectively [Table 7]. At termination, the values for male mice were 12%, 10% and 34% at 300, 2500 and 4500 ppm, respectively. In females, at 13 weeks, reductions in body weight gain were 16% at 300 ppm and 33% at 2500 ppm. At termination, the values were 7% at 300 ppm and 22% at 2500 ppm [Table 7]. Alterations in clinical chemistry were manifested by elevations in alanine aminotransferase, SAP and SDH in males at 2500 and 4500 ppm and in females at 2500 ppm.

Table 5. Mortality Rates[+] In Male Mice and Cox or Generalized K/W Test Results

	Study Weeks					
Dose (mg/kg/day)	**1-52**	**53[i]**	**53-56**	**57[i]**	**57-80[f]**	**Total (%)**
0	2/70	10/68	1/58	9/57	17/48	20/51 (39)**
1.51	2/60	10/58	0/48	0/48	16/48	18/50 (36)
4.65	2/60	10/58	3/48	0/45	18/45	23/50 (46)
46.29	4/60	10/56	0/46	0/46	22/46	26/50 (52)
423.16	0/70	10/70	0/60	10/60	16/50	16/50 (32)
818.87	13[#]/69[a]	10/56	0/46	10/46	20/36	33/49 (67)**

[+] No. of animals that died during interval / No. of animals alive at the beginning of the interval.

[#] All 13 of these animals died within the first 25 weeks of the study; 11 of 13 died before week 4.

[a] One accidental death at week 39, dose 818.87 mg/kg/day.

[i] Interim sacrifices at weeks 53 and 57.

[f] Final sacrifice at week 79.

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

	Study Weeks					
Dose (mg/kg/day)	**1-52**	**53[i]**	**53-56**	**57[i]**	**57-81[f]**	**Total (%)**
0	2/59[a]	10/57	0/47	0/47	23/47	25/49 (51)
1.90	2/59[b]	10/57	1/47	0/46	11/46	14/49 (29)[*]
5.63	3/59[c]	10/56	2/46	0/44	15/44	20/49 (41)
57.79	4/60	10/56	1/46	0/45	10/45	15/50 (30)
512.61	10[#]/69[d]	10/59	10/49	10/49	10/39	20/49 (41)

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

[#] All 10 of these animals died within the first 16 weeks of the study; 9 of 10 died before week 4.

[a] One accidental death at week 6, dose 0 mg/kg/day.

[b] One accidental death at week 51, dose 1.90 mg/kg/day.

[c] One accidental death at week 19, dose 5.63 mg/kg/day.

[d] One accidental death at week 7, dose 512.61 mg/kg/day.

[i] Interim sacrifices at weeks 53 and 57.

[f] Final sacrifice at week 79 for all animals except the 10 animals in the 512.61 mg/kg/day dose group that were originally in the control group which were sacrificed at week 81.

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. Selected Cumulative Body Weight Gain [G] in Mice Fed Difenoconazole

Study Week	Males						Females				
ppm	0	10	30	300	2500	4500	0	10	30	300	2500
1	1.2	1.1	1.0	0.3	-2.8	-5.5	0.6	0.6	0.7	0.3	-4.4
4	3.2	3.2	3.1	1.9*	0.9*	-1.3*	2.6	2.7	2.5	2.1	0.9*
8	4.4	4.6	4.5	4.0	2.9*	-0.1*	4.7	4.2	4.5	4.3	2.9*
13	5.8	5.8	5.6	4.9*	4.7*	2.1*	5.8	5.4	5.8	4.9*	3.9*
40	8.3	8.8	7.9	6.7*	6.3*	5.5*	8.6	8.1	8.3	8.4	6.5*
52	8.5	8.9	8.1	7.2*	6.7*	5.8*	10.0	9.2	9.8	9.2	7.7*
60	8.8	9.1	9.3	7.8	7.5	7.1	10.3	9.9	10.0	10.5	8.1
76	8.5	9.0	8.0	7.5	7.7	5.6*	11.6	11.0	10.2	10.8	9.0*

* Significantly different from control at $p \leq 0.05$

E. Adequacy of Dosing for Assessment of Carcinogenic Potential

The highest dose tested [2500 ppm in females and 4500 ppm in males] induced **toxicological** [mortality, $\geq$ 10% reductions in body weight/body weight gain and clinical signs], **pharmacological** [changes in liver enzymes indicative of liver damage], and **histopathological** [non-neoplastic and neoplastic hepatic lesions] **changes** in both sexes of mice. Therefore, it is concluded that the 2500 ppm was adequate to assess the chronic toxicity and the carcinogenicity in both sexes and that the 4500 ppm dose tested in male mice was excesive due to the high mortality rate.

2. Chronic Toxicity/Carcinogenicity Study in Rats:

Reference: Cox, R.H. Combined Chronic Toxicity and Oncogenicity Study of CGA 169374 Technical in Rats. Project Report 483-249. Hazleton Laboratories, Report issued, March 31, 1989. MRID # 420900-19 & 427100-10. HED Document # 009689 & 010588.

A. Experimental Design

Groups of 80 male and 80 female Sprague-Dawley rats were fed diets containing Difenoconazole at 0, 10, 20, 500 or 2500 ppm for 104 weeks. The mean daily test article intake was 0.48, 0.96, 24.1 and 124 mg/kg/day in males and 0.64, 1.27, 32.8 and 170 mg/kg/day in females, respectively. Ten animals per sex from each group and were sacrificed after 52 weeks of treatment. Ten additional animals, allocated to the control and 2500 ppm dose groups were kept for a four weeks recovery period after one year on the study.

B. Discussion of Tumor Data

Difenoconazole did not increase the neoplastic lesions commonly seen in this strain/age of rats. Statistical analyses of tumor data showed no significant treatment-related or dose-related tumors in either sex of rats.

C. Non-neoplastic Lesions

As shown in Table 8, treatment-related non-neoplastic histopathological changes were limited to an increased incidence of hepatocytic hypertrophy [centrilobular to diffuse to focal/mutifocal] in both sexes at 500 and 2500 ppm. The lesions were characterized by the presence of lightly stained enlarged hepatocytes with poorly distinguishable cell borders.

Table 8. Non-Neoplastic Lesions in the Liver of Rats fed Difenoconazole.

Hepatic Lesions	0 ppm		10 ppm		20 ppm		500 ppm		2500 ppm	
No. Examined: 70/Dose	M	F	M	F	M	F	M	F	M	F
Hepatocytic # hypertrophy [%]	7 [10]	4 [6]	5 [7]	0 [0]	8 [11]	0 [0]	29 [41]	17 [24]	39 [56]	36 [51]

D. Toxicological Effects

Treatment caused no adverse effect on survival. Over the first year of treatment, body weight gain of the 500 and 2500 ppm groups was 94% and 80% of the control values in males and 90% and 59% in the females [statistical significance at $p < 0.05$], respectively. The mean body weights of the rats at 2500 ppm remained consistently below the control values reaching 89% of the control values in the males and 63% in the females, respectively at termination [Table 9]. The only remarkable changes seen in clinical chemistry parameters were consistently [at Weeks 28, 53, 79 and 105] increased albumin and decreased globulin concentrations in the males at 2500 ppm which resulted in an increased albumin/globulin ratio. At both the interim and terminal sacrifices, absolute and relative liver weights were elevated at 2500 ppm. After 52 weeks of treatment, the liver-to-body weight ratio amounted to 114% of the control value in males and 148% in females, respectively. The values were in the same range at terminal sacrifice.

Table 9. Selected Cumulative Body Weight Gain [G] in Rats Fed Difenoconazole

Study Week	Males					Females				
ppm	0	10	20	500	2500	0	10	20	500	2500
1	52	49	49	47	25	20	19	20	18	6
4	160	152	147	143	116	61	61	62	55	38
8	237	225	225	222	183	93	91	91	84	62
13	287	276	272*	265*	222*	115	113	115	104*	77*
40	399	393	391	374*	321	196	201	205	189	128
52	423	417	416	398*	339*	232	234	237	209*	139*
60	425	417	415	407	349	242	237	239	219	141
76	410	405	398	397	350	252	262	254	237	159
88	395	379	377	394	345	256	269	259	239	158
104	332	340	352	359	294*	251	270	269	238	158*

* Significantly different from control at $p \leq 0.05$

E. Adequacy of Dosing for Assessment of Carcinogenic Potential

The highest dose tested [2500 ppm] induced **toxicological** [$\geq$ 10% reductions in body weight/body weight gain], **pharmacological** [liver effects indicative of enhanced functional load], and **histopathological** [non-neoplastic hepatic lesions] **changes.** Therefore, it is concluded that the dose levels employed in this study were adequate to assess the chronic toxicity and the carcinogenicity potential of difenoconazole in rats.

III. ADDITIONAL TOXICOLOGY DATA ON DIFENOCONAZOLE

1. Metabolism

Reference: MRID # 420900-28/29/30/31; 427100-13/14]
HED Document # 009689 & 010588

The biotransformation of Difenoconazole is shown in Figure 1. The compound undergoes successive oxidation and conjugation reactions. One of its metabolites, CGA-205375, accounts for 6-24% of the applied dose and is found only in urine and feces of oral high-dose (300 mg/kg) rats. The presence of this intermediate in excreta of only high-dose rats, suggests that its rate of further biotransformation has reached saturation at the high dose. Additionally, excretion of radioactivity in bile, feces and urine of rats orally dosed with [^{14}C]-difenoconazole is consistent with saturation g.i. absorption of the chemical at 300 mg/kg.

2. Mutagenicity

Reference: MRID # 420900-25; 427100-11 & 427100-12
HED Document # 09689 & 010588

Difenoconazole was nonmutagenic with or without metabolic activation, when tested at concentrations ranging from 340 to 5447 μg/plate in two independently performed microbial/mammalian microsome plate incorporation assays using *Salmonella typhimurium* strains TA1535, TA1537, TA98 and TA100 and *Escherichia coli* strain WP2uvrA. In an *in vivo*, micronucleus assay, no increase in micornucleated polychromatic erythrocyte counts were seen in the bone marrow cells of mice given oral administration of difenoconazole at 0, 400, 800 or 1600 mg/kg/day. Difenoconazole was negative in an *in vitro* UDS assay with primary rat hepatocytes at concentrations up to 50.0 μg/mL.

3. Acute, Subchronic and Chronic Toxicity

i. Acute Toxicity The acute toxicity data are summarized below:

Reference: MRID # 420900-06 thru 11 HED Document No. 009689

Type of Study	Species	Results	Toxicity Category
Oral LD_{50}	Rat	1453 mg/kg/	III
Dermal LD_{50}	Rabbit	>2010 mg/kg	III
Inhalation LC_{50}	Rat	>3300 mg/m^3	III
Primary Eye Irritation	Rabbit	Moderate irritant	III
Primary Dermal Irritation	Rabbits	Slight irritant	IV
Dermal Sensitization	Guinea pigs	Non-sensitizer	Not applicable

ii. Sub chronic Toxicity

Reference: 13-Week Feeding Study in Mice; MRID# 420900-21; HED Document # 009689

In a subchronic toxicity study CD-1 mice [15/sex/dose] were fed diets containing Difenoconazole at 0, 20, 200, 2500, 7500 or 15,000 ppm for 13 weeks. These levels were equivalent to approximately 0, 3, 30, 375, 1125 or 2250 mg/kg/day, respectively. All but 2 mice fed 7500 ppm and all mice fed 15,000 ppm died during the first three weeks of the study. Clinical signs seen prior to death were thinness, hunched posture, languidness and tremors. The cumulative body weight gains at 2500 ppm were 9% for males and 41% for females. Absolute and relative liver weights were significantly increased in both sexes of mice at 2500 ppm. Histopathology revealed erosion/ulceration of the glandular stomach and hyperkeratosis of the non-glandular stomach as well as diffused hepatocellular enlargement and necrosis in many of the mice that died. Histopathology showed hypertrophic and cytotoxic changes in the liver at feeding levels $\geq$ 2500 ppm; hepatocellular enlargement was seen in all mice [10/sex] while vacuolation was seen in 7 males and 7 females at 2500 ppm. Results indicate that the MTD is lower than 2500 ppm. **The LOEL of 200 ppm [30 mg/kg/day is based on mortality, reductions in body weight gain, and histopathologic alterations in the liver. The NOEL is 20 ppm [3 mg/kg/day].**

Reference: 13-Week Feeding Study in Rats; MRID # 420900-22; HED Document # 009689

In a subchronic toxicity study Sprague-Dawley rats [15/sex/dose] were fed diets containing Difenoconazole at 0, 20, 200, 750, 1500 or 3000 ppm for 13 weeks. These levels were equivalent to 0, 1.23, 11.3, 47.8, 99.3, and 189 mg/kg/day in males and 0, 1.43, 15.5, 61.8, 124 and 194 mg/kg/day in females, respectively. The body weight gains were significantly reduced in males at 3000 ppm and in females at 200 ppm and above. At the high dose, the overall body weight gain amounted to 85% of the control value in males and 62% in females, respectively. The absolute and relative liver weights were significantly increased in males at 750 and above and in females at 200 ppm and above. Although no changes in key liver marker enzymes were observed, histopathology revealed a dose-related increased incidence of diffuse hepatocellular enlargement in both sexes treated at 1500 ppm and above. Results indicate that the MTD was exceed at 3000 ppm due to severe depression in body weight gain. **The LOEL of 200 ppm [11.3 mg/kg/day in males and 15.5 mg/kg/day in females] is based on reductions in body weight gain and increased liver weights. The NOEL is 20 ppm [1.23 mg/kg/day in males and 1.43 mg/kg/day in females].**

iii. Chronic Toxicity

Reference: 52-Week Feeding Study in Dogs; MRID #420900-14 & 427100-05 HED Document # 009689 & 010588

In a chronic toxicity study, beagle dogs [4/sex/dose] were fed diets containing Difenoconazole at 0, 20, 100, 500 or 1500 ppm for 52 weeks. These levels were equivalent to 0, 0.71, 3.4, 16.4 or 51.2 mg/kg/day in males and 0, 0.63, 3.7, 19.4 or 44.3 mg/kg/day in females, respectively. The body weight gain was consistently diminished in females at 500 and 1500 ppm; at termination the body weight gain was 60% and 69% of the control values, respectively. No reductions in body weight gain were seen in male dogs at any dose level. Male dogs exhibited dose-related increases in serum alkaline phosphatase activity on Days 85, 175 and 359 with the increase reaching statistical significance at the high dose. No treatment-related histopathologic changes were seen. **The LOEL of 500 ppm [16.4 mg/kg/day in males and 19.4 mg/kg/day in females] is based on reductions in body weight gain. The NOEL is 100 ppm [3.4 mg/kg/day in males and 3.7 mg/kg/day in females]**

Reference: 78-Week Feeding Study in Mice; MRID # 420900-15 & 427100-06. HED Document # 009689 & 010588

In a carcinogenicity study [discussed in detail on Page ...], CD-1 mice [60-70/sex/dose] were fed diets containing Difenoconazole at 0, 10, 30, 300, 2500 or 4500 ppm [males only] for 78 weeks. **The systemic LOEL of 300 ppm [46.29 mg/kg/day in males and 57.79 mg/kg/day in females] is based on reductions in cumulative body weight gain. The NOEL is 30 ppm [4.65 mg/kg/day in males and 5.63 mg/kg/day in females.**

Reference: 104-Week Feeding Study in Rats MRID # 420900-15 & 427100-06. HED Document # 009689 & 010588

In a chronic toxicity/carcinogenicity study [discussed in detail on Page .....], Sprague-Dawley rats were fed diets containing difenoconazole at 0, 10, 20, 500 or 2500 ppm for 104 weeks. **The systemic LOEL of 500 ppm [24.12 mg/kg/day in males and 32.79 mg/kg/day in females] is based on reductions in cumulative body weight gain. The NOEL is 20 ppm [0.96 mg/kg/day in males and 1.27 mg/kg/day in females.**

4. Registrant's Rebuttal Concerning Carcinogenicity of Difenoconazole in Mice.

Reference: Position Document entitled: "Assessment of the Liver Tumors Observed in CD-1 Mice Fed Excessive Levels of Difenoconazole [CGA 169374]: A Mitogenic Response". Submitted by Ciba-Geigy Corporation. MRID # 429728-03

The Registrant contends that the carcinogenic response was noted 1) at excessive dietary levels where the hepatocytotoxic maximum tolerated dose was exceeded; 2) liver tumors were accompanied by a clear sequela of non-neoplastic changes which clearly support a hepatotrophic effect; and 3) liver damage is most likely to result in mitogenesis [cell replication] in the liver. The Registrant, therefore, asserts that the liver tumors observed in the mouse study are not germane to the toxicological assessment of human exposure to difenoconazole.

The weight of evidence for a mitogenic response is as follows:

- Subchronic studies in mice, rats, and dogs showed that difenoconazole is well tolerated at relatively high dietary levels [greater than 50 mg/kg/day selectively results in hepatocyte damage];

- In rats and dogs, liver damage is manifested by liver enlargement, hepatocyte hypertrophy and elevation of key liver marker enzymes. These may be described as adaptive reactions of the liver and reflect functional overload of this organ at high levels. **These changes were reversible and there were no corroborative histopathological changes in the liver in these species.**

- Similar to rats and dogs, liver was the target organ in mice. Mouse liver exhibited the same pattern of liver enlargement, hepatocyte hypertrophy, elevation of liver marker enzyme. **But, these alterations were corroborated with a clear profile of liver pathology characterized by necrosis, fatty infiltration, and vacuolation.**

- The cross-species hepatotrophic and hepatotoxic effects can partially be explained by the fact that Difenoconazole is a mixed phenobarbital-type and steroidal microsomal enzyme inducing agent. A similar profile of cellular hypertrophy accompanied by cytotoxicity is seen with other hepatic enzyme inducing agents

- In the absence of specific cell proliferation studies, to determine an association exists between the degenerative process and the presence of a tumor, odds ratios were calculated. The odds ratios expressed the increased likelihood of tumor presence when a specific degenerative process was present. As shown below, a clear association was seen between the formation of liver tumors and the *apriori* cytotoxic event. Therefore, the tumor response can be ascribed to a mitogenic error that results from the homeostatic regeneration of hepatocytes to replace damaged tissue. The odds ratio showing the likelihood of tumor presence when a specific degenerative process is present below:

Degenerative Process	Odds Ratio	P-Value	95% Confidence Bounds	
			Lower Bound	Upper Bound
Necrosis	1.862*	0.002	1.251	2.773
Inflammation	0.749	0.320	0.418	1.343
Hypertrophy	2.023**	0.001	1.318	3.105
Fatty Changes	2.438**	<0.001	1.621	3.665
Bile Stasis	2.752**	<0.001	1.865	4.060

* Significantly different from the control at $P \leq 0.05$
** Significantly different from the control at $P \leq 0.01$

- Metabolism data provide evidence that Difenoconazole is absorbed from the intestinal tract, is rapidly metabolized and is quickly eliminated. No accumulation of the compound in individual tissues or animal products has been detected.

- No evidence of mutagenicity was seen in a battery of tests which covered different endpoints in procaryotes an eucaryotes *in vivo* and *in vitro.*

IV. STRUCTURE-ACTIVITY CORRELATIONS

Difenoconazole is structurally related to Azaconazole, Baycor, Bayleton, Baytan, Cyproconazole, Etaconazole, Fenbuconazole, Hexaconazole, Propiconazole, Tebuconazole, and Uniconazole. The structural formulas of these compounds are shown in Table 10. The tumor types and cancer classifications of these compounds are provided below:

Azaconazole: **Non carcinogenic** in male and female mice [strain not specified]; the doses used may not have been adequate to assess the carcinogenic potential of this compound.

Baycor: **Non carcinogenic** in male and female mice and male and female rats at doses up to and including 500 ppm.

Bayleton: **Group "C" (nq)** carcinogen; based on hepatocellular adenomas in male and female NMRI mice and a dose-related trend for thyroid follicular cell adenomas in males and cystic hyperplasia in both sexes.

Baytan: **"Weak C"** carcinogen; based on hepatocellular adenomas and hyperplastic nodules in female CF1-W74 mice.

Cyproconazole: **Group B2** carcinogen; based on hepatocellular adenomas and carcinomas in male and female CD-1 mice and the absence of an acceptable carcinogenicity in rats.

Etaconazole: **No classification;** increased the incidence of liver adenomas and carcinomas; registration voluntarily withdrawn.

Fenbuconazole: **Group C (Q)** carcinogen; based on thyroid follicular cell adenomas and/or combined adenomas/carcinomas in male Sprague-Dawley rats in two studies. Increased incidence of hepatocellular adenomas and/or carcinomas in CD-1 mice fed inadequate doses.

Hexaconazole: **Group C (Q)** carcinogen; based on benign Leydig cell testicular tumors in ALpk:APfSD (Wistar derived) rats. Doses used in the CD-1/Alpk mouse study were not adequate to assess carcinogenicity.

Propiconazole: **Group C (nq)** carcinogen; based on hepatocellular adenomas, carcinomas, and/or adenomas and carcinomas combined in CD-1 mice.

Tebuconazole: **Group C (nq)** carcinogen; based on hepatocellular adenomas, carcinomas, and combined adenomas/carcinomas in both sexes of NMRI mice.

Uniconazole: **Group C (nq)** carcinogen; based on hepatocellular adenomas and carcinomas in male Crl:CD-1(ICR)BR mice. Non carcinogenic in male or female rats.

IV. WEIGHT OF EVIDENCE CONSIDERATIONS

The weight-of-the-evidence for the determination of the carcinogenic potential of Difenoconazole is presented below:

1. Difenoconazole increased the incidence of hepatocellular adenomas, carcinomas, and/or combined adenomas/carcinomas in male CD-1 mice at doses at 300 ppm and above and in female mice at the dose of 2500 ppm. There were statistically significant increases in tumor incidence by pair-wise comparisons with concurrent controls as well as significant increasing trends.

2. In male mice, there was a significant [p=0.000] increasing trend in hepatocellular adenomas, carcinomas, and combined adenomas/carcinomas. At 300 ppm, hepatocellular adenomas [16%] were significantly [p=0.035] increased when compared to controls [6%]. At 2500 ppm, hepatocellular adenomas [19%; p=0.036] and combined adenomas/carcinomas [23%; p=0.023] were significantly increased when compared to controls [adenomas: 6%; combined adenomas/ carcinomas: 7%]. At 4500 ppm, adenomas [36%], carcinomas [23%] and combined adenomas/ carcinomas [50%] were significantly [p <0.000] increased when compared to controls [adenomas: 6%; carcinomas: 1%; and combined adenomas/ carcinomas:7%].

3. In female mice, there was a significant [p <0.01] dose-related increasing trend in hepatocellular adenomas, carcinomas, and combined adenomas/carcinomas. At 2500 ppm, hepatocellular adenomas [27%; p=0.000], carcinomas [10%; p=0.039], and combined adenomas/ carcinomas [29%; p=0.000] when compared to none in the controls.

4. When compared to historical control data, in male mice, the dose-related increases in the incidence of hepatocellular adenomas [16% at 300 ppm, 19% at 2500 ppm and 36% at 4500 ppm] exceeded the concurrent controls [6%] as well as both the weighted average of the historical control incidence [7.4%] and the historical control range [0 - 11%]. Similarly, hepatocellular carcinomas [23%] at the HDT also exceeded the concurrent controls [1%] as well as the weighted average of the historical control incidence [3%] and the historical control range [0 - 8.2%].

5. When compared to historical control data, in female mice, the incidence of hepatocellular adenomas [27%] at the HDT exceeded the concurrent controls [0%] and the weighted average of the historical control incidence [1.4%] and the historical control range [0 - 6.1%]. Similarly, hepatocellular carcinomas [29%] at the HDT exceeded the concurrent controls [0%] and both the weighted average of the historical control incidence [0.6%] and the historical control range [0 - 2.1%].

6. Difenoconazole has been shown to be nonmutagenic both *in vivo* and *in vitro*. It was negative for gene mutations in a *Salmonella*/microsomal assay, did not cause an increase in micronucleated polychromatic erythrocytes in a mouse micronucleus assay, and was negative in an UDS assay with primary rat hepatocytes.

7. Dose-dependent changes in metabolite patterns (e.g. the intermediate CGA-205375 was found only at the high-dose) were seen at the high dose in rats. These changes may result from saturation of metabolite oxidation and/or conjugation in going from the low dose (0.5 mg/kg) to the high dose (300 mg/kg).

8. Difenoconazole is structurally related to eight other triazole pesticides [Baytan, Bayleton, Cyproconazole, Ectaconazole, Fenbuconazole, Propiconazole, Tebuconazole, and Uniconazole] known to have induced hepatocellular tumors in several strains [CD-1, Swiss, NMRI, and CF1-W74] mice.

Table 10. Structurally Related Triazole Pesticides.

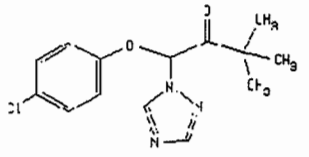
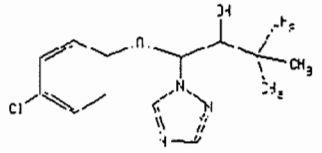
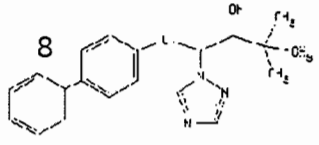
Compound	*Structure*	*Carcinogenic Effect*	*Carcinogen Classification*
Bayleton PC Code 109901 Tox. Chem. # 862AA		**NMRI Mouse,** 50, 300, & 1800 ppm. Only hepatocellular adenoma, at 1800 ppm in (22%)♂ & (18%)♀, $p<0.05$ for trend and paired comparisons. Hist. Conts.:18.4% ♂ & 2.0% ♀. **Wistar Rat,** 50, 500 & 5000 ppm. Dose related trend in TFC adenomas in ♂ & comb. w. cystic hyperplasia in ♂ & ♀; pairwise comparisons not significant.	**C NQ**
Baytan PC Code: 127201 Tox. Chem # 074A		**CF1-W74 Mouse,** 2000 ppm: Hepatocellular adenomas and hyperplastic nodules ($p<0.01$) in ♀. No increase in ♂. Adrenal adenomas noted in ♀ LDT and HDT but not in hist. conts. No elevation in carcinomas.	**Weak C** SAP 12/23/87.
Baycor PC Code: 112403 Tox Chem.# 087AA		**Mouse:** up to 500 ppm: (-) **Rat:** up to 500 ppm : (-)	

Table 10. Structurally Related Triazole Pesticides (Continued)

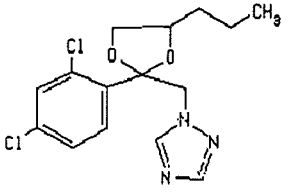
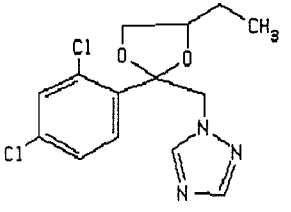
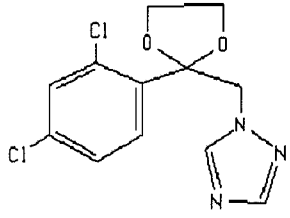
Compound	*Structure*	*Carcinogenic Effect*	*Carcinogen Classification*
Propiconazole PC Code: 122101 Tox. Chem. # 323EE		**CD-1 Mouse,** 100, 500 & 2500 ppm. Statistically significant trend and pairwise comparisons in liver adenomas and combined at 2500 ppm. For carcinomas only there were statistically significant trend and pairwise comparisons at the HDT for data from 2 of 3 pathologists; for the data from the third pathologist only the trend was significant ($p=0.028$), the pairwise comparison HDT vs. control had a $p = 0.050$.	**C NQ**
Etaconazole PC: None		**Swiss Mouse** increased incidence of liver adenomas and carcinomas in both sexes. Registration voluntarily withdrawn.	None
Azaconazole PC Code: 128882 Tox. Chem. # 321A		**Mouse,** 25,100 &400 ppm. There is the question of whether the MTD was reached. No carcinogenicity effect.	

Table 10. Structurally Related Triazole Pesticides (Continued).

Table 10. Structurally Related Triazole Pesticides (Continued).

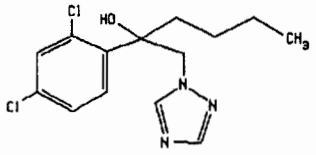
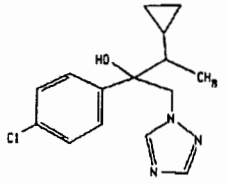
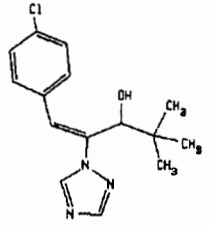
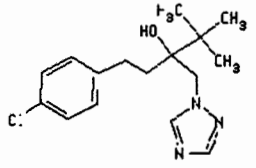
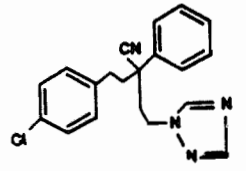
Compound	*Structure*	*Carcinogenic Effect*	*Carcinogen Classification*
Hexaconazole PC Code: 128925 Tox. Chem.# 480G		**CD-1/Alpk Mouse,** 5, 40 & 200 ppm. No carcinogenicity effect. Should be evaluated with caution since an MTD was not reached. **ALpk:APfSD (Wistar derived) Rats,** 10, 100, 1000 ppm. There was a significant ($p<0.01$) dose-related trend and a significant pair-wise comparison with controls at the HDT for benign Leydig cell tumors in the testes. The incidence at HDT (16%) exceeded historical control values of up to 6.0%	**C Q** (Based on rat Study)
Cyproconazole PC Code: 128993 Tox. Chem. # 272E		**CD-1 Mouse,** 5, 15, 100 & 200 ppm. Significant incidence of adenomas & carcinomas at the MDT and HDT in males and at the HDT in females.	**B2**
Uniconazole PC Code: 128976 Tox. Chem. # 207H		**Crl:CD-1(ICR)BR Mouse,** 10, 40, 200 & 1500 ppm. Increased incidence of hepatocellular adenomas & carcinomas in 1500 ppm males only. **Crl:CD-1(ICR)SD Rat,** 10, 40 200 & 1000 ppm. No increase in neoplastic findings.	**C NQ**

Table 10. Structurally Related Triazole Pesticides (Continued).

Compound	*Structure*	*Carcinogenic Effect*	*Carcinogen Classification*
Tebuconazole PC Code: Tox. Chem. # 463P		**NMRI Mice,** 0, 500 & 1500 ppm. Increased incidence of hepatocellular adenomas, carcinomas, and combined adenomas/ carcinomas at HDT	**C NQ**
Fenbuconazole PC Code: 129011 Tox. Chem. # 723Q		**Sprague-Dawley rat,** 0, 8, 80, 800, 1600 ppm. Thyroid follicular cell adenomas and/or carcinomas in male rats in two studies.	**C Q**

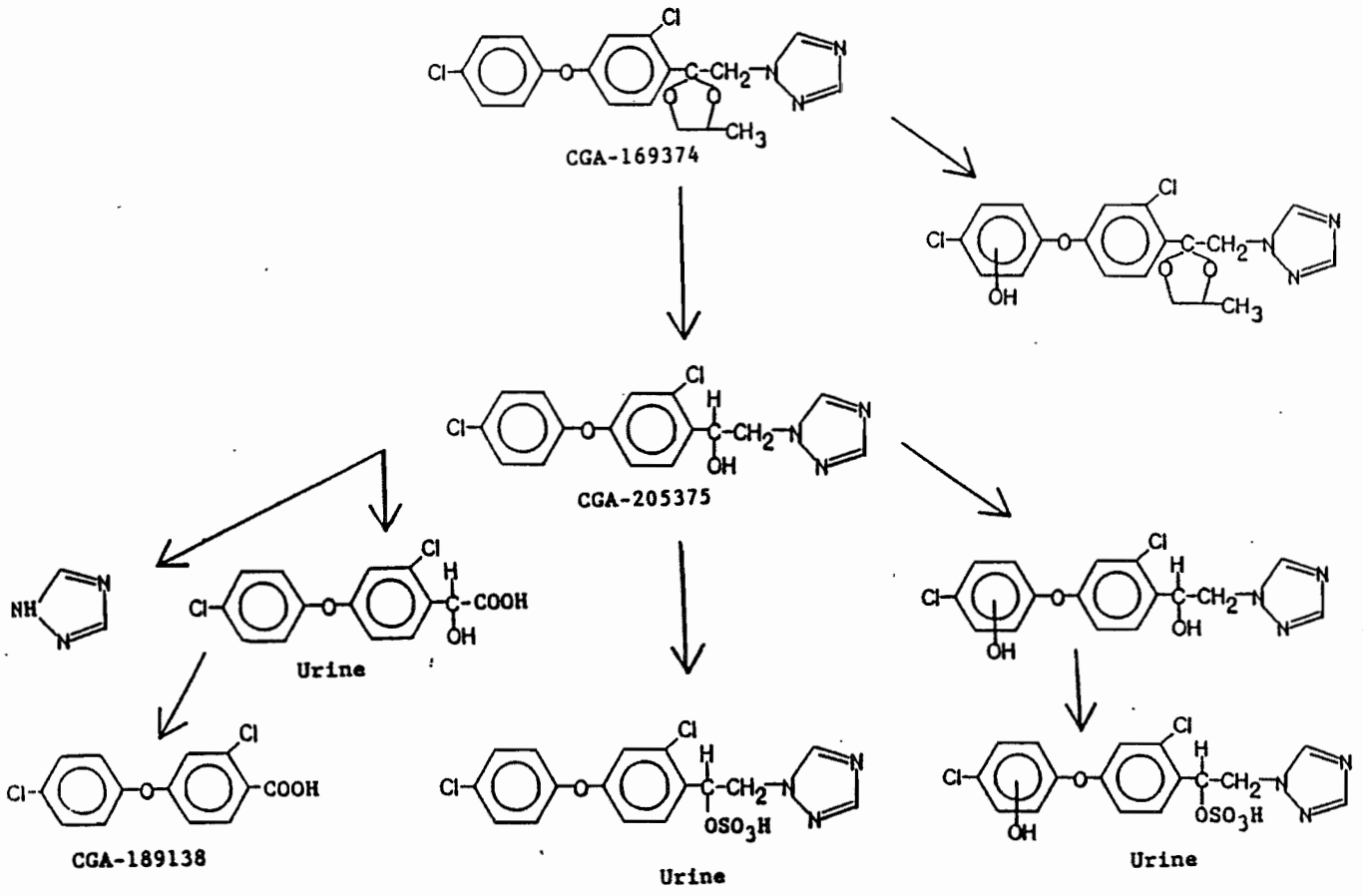


Figure 1. Proposed Metabolic Pathway for CGA 169374 in Rats

Primary Review by: K.E. Whitby, Ph.D. 6/22/92
Toxicologist, Review Section II, Tox. Branch (H7509C)
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JUN 24 1992

DATA EVALUATION REPORT

STUDY TYPE: Carcinogenicity Study in Mice §83-2

HED Project No: 2-0696 MRID NO.: 420900-15

TEST MATERIAL: CGA 169374 SHAUGHNESSY NO: 128847

SYNONYMS: Difenoconazole

LABORATORY STUDY NO.: 483-250

SPONSOR: Agricultural Division
CIBA-GEIGY Corporation
P. O. Box 18300
Greensboro, NC 27419-8300

TESTING FACILITY: Hazelton Laboratories America, Inc.
9200 Leesburg Turnpike
Vienna, VA 22182

TITLE OF REPORT: Oncogenicity Study in Mice

AUTHOR(S): Raymond H. Cox, Ph.D.

REPORT ISSUED: April 3, 1989

STUDY DATES: Initiation of Dosing Period: April 22, 1986
Necropsies Completed: November 4, 1987

CONCLUSION:

CGA 169374 was administered in the diet to male and female mice for 78 weeks at 0, 10, 30, 300, 2500 or 4500 ppm. The NOEL was 30 ppm. The LOEL was 300 ppm based on reductions in cumulative body weight gains in the 300, 2500 and 4500 ppm groups. All females receiving 4500 ppm died or were sacrificed due to moribundity during the first two weeks of the study. Mean liver weight was increased at week 53 at 300 ppm (females only), 2500 ppm (both sexes), and 4500 ppm (males only) and at termination in the 2500 ppm (both sexes) and 4500 ppm (males only) groups (but not in the recovery group at week 57). Histopathological findings were observed in the liver at 300 ppm and above (liver adenoma and/or carcinoma were observed in both sexes at 2500 ppm and in males only at 4500 ppm). Additional findings which indicate that the liver is the target organ were observed in the clinical chemistry data. The dosages appeared to be adequate to test the potential carcinogenicity of CGA 169374. The study may be upgraded after satisfactory review of the Registrant's response to the deficiencies (raw data for the purity of the test material, the stability, homogeneity, and concentration analyses).

Classification: Core Supplementary

NOEL = 30 ppm
LOEL = 300 ppm

A. MATERIALS:

1. Test Compound:

Test Substance: CGA 169374 Technical
Description: dark brown solid
Purity: not stated
Stability: not stated
Storage Conditions: room temperature

Lot No.	Date Required	Purity	Weeks Used
FL851406	November 22, 1985	94.5%	1-20
FL861408	August 4, 1986	95%	21-44
FL861408	February 6, 1987	95%	45-80

Vehicle: Pesticide grade acetone
Source: Fisher Scientific Company
Description: clear liquid
Storage: nonflammable cabinet

Lot No.	Date Received	Weeks Used
856990	March 4, 1986	1-12
860851	May 20, 1986	13-18
862767	July 28, 1986	19-24
862852	September 24, 1986	25-56
865760	March 16, 1987	57-62
862852	April 29, 1987	63-80

Analytical Chemistry

The report indicates (p. 16) that the information pertaining to the synthesis, stability, composition, and other characteristics which define the test material are on file with the Sponsor.

The report indicates (p. 20) that the test material was found to be stable in the diet at 10 ppm for 10 and 16 days at room temperature in this investigation. In pilot studies (HLA 483-241 and 483-242) which were also conducted at Hazelton Laboratories, the test material was also found to be stable at higher concentrations.

The analytical chemistry report (p. 37) provides the results for the stability as a result of the day 16 analysis of the 10 ppm level in HLA 483-249. The amount detected was 97.97 and 97.23% of the target value. Purity is not presented. Homogeneity during week 1 is reported to have ranged (top, bottom, and middle) from 92.89 to 107.1% of the target.

2. Test animals:

Species: Mice
Strain: Crl:CD-1® (ICR)BR
Number Received for Study: 497 ♂
498 ♀
Source: Charles River Laboratories, Inc., Kingston, NJ
Date of Arrival: March 26, 1986
Age: 28 days at receipt
8 weeks at initiation of treatment
Weight: ♂ 28.0 - 33.8 g at initiation of treatment
♀ 21.6 - 27.1 g at initiation of treatment

Not all of the animals received for the purpose of this study were assigned to treatment groups. Animals were selected for participation in the study by the staff veterinarian following examination. Six animals per sex were selected as potential replacement animals and held in the study room for 1 week. An additional 10 animals per sex were randomly selected for serum chemistry. The remaining animals were euthanized.

B. STUDY DESIGN:

1. Animal Husbandry

Purina Certified Rodent Chow® #5002 was used as the basal diet fed ad libitum (during quarantine and study). Tap water was available ad libitum during the quarantine and study periods via an automatic watering system. At receipt two animals were placed in each stainless steel hanging cage. During the conduct of the study the animals were assigned permanent identification numbers and housed singly in stainless steel hanging wire cages. Temperature and relative humidity during the study ranged from 65-77° F and 24-89%, respectively. The animals were maintained on a 12 hour light/dark cycle.

The first step in the assigment of animals to treatment groups was eliminating animals with extreme body weights. The random assignment which produced homogeneity of both variances and means by Bartlett's Test and one-way ANOVA was selected.

Animals were assigned to the following test groups:

Test Group	Dose in diet (ppm)	♂	♀
1	0	70	70
2	10	60	60
3	30	60	60
4	300	60	60
5	2500[a]	70	70
6	4500	70	70

a Animals in this group were treated with a 3000 ppm diet mixture during the first week of the study.

During the first two weeks of exposure, all of the group 6 females and 16 of the group 5 females died. Therefore, the group 5 dose was reduced from 3000 ppm to 2500 ppm. In addition, 10 females from group 1 were moved to group 5 to maintain an adequate number of group 5 animals to the end of the study. In effect, the 10 animals which were removed from the control group were intended as the controls for the recovery study. Therefore, during the recovery study there are no control females to compare with the 2500 ppm (group 5) females. The animals taken from the control group and placed in the 2500 ppm group started to receive the test diet at the beginning of week 3. Survivors were sacrificed during week 81 after 78 weeks of test.

2. Diet Preparation

The test compound was weighed as 100 % a.i.. Test diets were prepared every 2 weeks. The control group received an acetone feed admixture. Prior to the day of admixing in the diet, the test compound was placed overnight (24 hrs) in a water bath at 70° C to achieve a liquid state. The vehicle was used to dissolve and mix the test compound at a ratio of 5 mL vehicle/kg diet. The beaker containing test compound was set on a 100° F hot plate to reach a liquid state, after weighing. A premix was prepared in a mixer (20 min of mixing). The premix was added to the appropriate volume of feed and mixed for 1 min/kg.

3. Observations

All animals were observed twice daily for mortality, and moribundity. Once daily the animals were observed cage side for toxic effects. Once each week animals were palpated for tissue masses.

Ophthalmoscopic examinations were performed by the staff

ophthalmologist for all animals prior to dosing and at 6 month intervals (weeks 27, 53, and 78) for control and high dose animals using indirect ophthalmoscopy.

Body weight was recorded for all animals prior to dosing. Body weight and food consumption were recorded weekly during weeks 1-16, and then once every 4 weeks thereafter.

4. Statistics

The procedures utilized in analyzing the data are included in Appendix I.

5. Compliance

A signed Statement of No Confidentiality Claim was included which was dated 1/18/91 (p. 2).

A signed Statement of Compliance with EPA GLP's was included which was dated 4/3/89 (p. 5).

A signed Quality Assurance Statement was included and dated 4/3/89 (p. 6).

A signed Flagging Criteria Statement was included which was dated 1/16/91 (p. 4). **The study was flagged for adverse effects.** The study meets or exceeds the criteria numbered 1 and 2. There was a statistically significant increase in the number of animals with liver adenoma and/or carcinoma for males fed 2500 and 4500 ppm, and for females fed 2500 ppm.

C. RESULTS:

1. Observations:

Mortality

All females receiving 4500 ppm died or were sacrificed due to moribundity during the first 2 weeks of the study. Eleven males in this group died or were euthanized due to moribundity during the first 3 weeks of the study. Fifteen females died or were sacrificed due to moribundity during the first week in the 3000 ppm group. Therefore, during the second week the dose level for both sexes in this group was reduced to 2500 ppm. Ten females from the control group were placed in the 2500 ppm group at the beginning of week 3. During week 2 (after the reduction in the dose level), another female in the 2500 ppm group died during the second week. Three of the replacement animals in the 2500 ppm group were sacrificed during their first week due to moribundity.

Survival was significantly reduced in the males receiving 4500 ppm.

In addition, there was a significant negative overall trend in survival for males with a significant departure from trend.

Clinical Signs

Clinical signs observed for many of the female animals in the 4500 ppm group prior to their death were: hunched appearance, and rough coat. These observations were also increased relative to the control for the 4500 ppm males and the 2500 females. Males receiving 4500 ppm also exhibited reduced motor activity when compared to the control. Male and female animals receiving 2500 or 4500 ppm had a greater incidence of thin appearance.

2. Body Weight

The absolute weight of both sexes receiving 2500 and males receiving 4500 ppm were consistently lower than their control counterparts. Significant differences were noted by comparison of these groups. Males receiving 300 ppm also exhibited a slight reduction in body weight during the study.

Cumulative body weight gain was significantly reduced in a dose related fashion for the 300 ppm (except for weeks 8 and 76), 2500 ppm (except for week 76), and 4500 ppm males. Females receiving 300 ppm had a significant dose related reduction in cumulative body weight gain during week 13. The 2500 ppm females also exhibited significantly reduced cumulative body weight gain weeks 4, 8, 13, 24, 28, 40, 52, and 76.

The absolute body weight of the 2500 and 4500 ppm recovery males was significantly less than their concurrent controls in a treatment related manner.

3. Food Consumption and Compound Intake

During the first week of the study all animals in the two highest dose groups wasted feed. Statistical analysis of cumulative food consumption weeks 1-52 or weeks 1-76 did not detect significant differences. The animals in the two highest doses tested were omitted from this analysis due to wastage of feed.

The week 56 food consumption values for the recovery animals were similar to the week 52 data (prior to recovery).

Test article intake (mg/kg) was calculated by multiplying the feed consumed daily (g/day) by the projected compound concentration (mg/kg of feed), divided by the average group mid-period body weight (kg).

4. Ophthalmological Examination

The ophthalmology report (p.38) and the individual ophthalmoscopic

findings (p.765) do not address or provide the results of the examination performed prior to treatment. However, the available data do not indicate the presence of treatment related effects. The corneal dystrophy and cataracts which are noted appear to be related to the aging of the animals.

CUMULATIVE GROUP MEAN BODY WEIGHT GAIN (G) FOR MICE ADMINISTERED CGA 169374 IN THE DIET FOR 76 WEEKS

During Week	Males						Females				
	0	10	30	300	2500	4500	0	10	30	300	2500
1	1.2	1.1	1.0	0.3	-2.8	-5.5	0.6	0.6	0.7	0.3	-4.4
2	2.1	2.0	1.9	1.2	-1.2	-3.9	1.5	1.1	1.2	0.8	-1.3
3	2.4	2.5	2.2	1.7	-4	-2.3	1.7	1.8	1.7	1.2	-0.4
4	3.2	3.2	3.1	1.9*	0.9*	-1.3*	2.6	2.7	2.5	2.1	0.9*
5	3.2	3.4	3.4	2.5	1.6	-0.4	3.3	3.1	3.0	2.7	2.0
6	4.2	4.0	4.1	2.9	2.2	0.4	3.8	3.5	3.8	3.6	2.7
7	4.1	4.2	4.6	4.0	3.0	0.1	4.3	3.8	3.9	3.9	2.1
8	4.4	4.6	4.5	4.0	2.9*	-0.1*	4.7	4.2	4.5	4.3	2.9*
9	4.6	[illegible]	4.7	3.8	3.4	0.9	4.5	4.1	4.0	3.8	2.7
10	5.8	[illegible]	6.1	4.4	4.2	1.7	4.8	4.4	4.4	3.8	2.8
11	5.2	[illegible]	5.2	4.5	4.3	2.3	5.0	4.6	5.0	4.2	3.4
12	5.8	[illegible]	6.6	5.2	4.4	2.8	5.5	5.2	5.5	4.9	3.7
13	5.8	5.8	5.6	4.9*	4.7*	2.1*	5.8	5.4	5.8	4.9*	3.9*
14	5.8	5.9	5.8	4.8	4.5	2.4	6.0	5.4	5.7	5.2	3.9
15	6.2	5.8	5.9	4.9	4.8	2.6	6.0	5.6	5.8	5.3	4.2
16	5.8	6.0	6.0	4.9	4.5	2.6	6.3	6.0	6.1	5.5	4.3
20	6.3	6.6	6.5	5.9	5.1	3.5	7.0	6.5	6.8	6.1	5.0
24	7.0	7.2	6.5	5.6*	5.4*	4.0*	7.1	6.5	7.5	7.0	5.7*
28	7.6	7.7	7.1	6.1*	5.7*	4.6*	8.0	7.5	7.6	7.5	5.7*
32	7.6	8.0	7.3	5.9	5.8	4.6	7.9	7.4	7.9	7.6	5.7
36	8.2	8.3	7.7	6.4	6.5	5.3	8.0	7.7	8.1	8.0	6.2
40	8.3	8.8	7.9	6.7*	6.3*	5.5*	8.6	8.1	8.3	8.4	6.5*
44	7.9	8.2	7.9	7.0	6.4	5.6	8.9	8.8	9.2	9.3	7.0
48	8.1	8.2	7.8	7.2	6.6	5.2	8.9	8.5	8.5	8.3	6.9
52	8.5	8.9	8.1	7.2*	6.7*	5.8*	10.0	9.2	9.8	9.2	7.7*
56	9.3	9.8	9.1	7.9	7.3	6.0	9.9	9.5	9.1	9.6	7.9
60	8.8	9.1	9.3	7.8	7.5	7.1	10.3	9.9	10.0	10.5	8.1
64	8.3	9.5	8.8	8.0	7.6	6.6	10.4	9.7	10.2	10.2	8.4
68	8.7	9.1	8.7	7.6	7.9	7.8	10.6	10.6	10.3	9.9	8.0
72	8.2	8.7	8.0	8.1	7.4	6.3	10.6	10.5	10.4	10.4	8.2
76	8.5	9.0	8.0	7.5	7.7	5.6*	11.6	11.0	10.2	10.8	9.0*

Data extracted from Table 48, report HLA 483250 pp. 78-85.
* Significantly different from control ($p \leq 0.05$).

GROUP MEAN FOOD CONSUMPTION (G/WK)

During Week	Males						Females				
	0	10	30	300	2500	4500	0	10	30	300	2500
1	42.3	43.5	43.1	41.7	†	†	42.9	43.5	41.5	42.4	†
2	45.7	44.9	45.4	44.1	50.6	49.9	45.1	45.9	43.6	44.1	47.2
3	42.3	44.5	45.0	43.2	49.1	46.4	43.9	43.4	43.8	44.6	45.9
4	41.8	41.5	43.8	40.4	48.9	45.2	42.0	41.4	44.3	44.5	41.7
5	41.3	42.1	41.9	40.9	44.4	45.0	42.5	42.7	46.3	42.0	45.7
6	41.3	43.4	41.5	40.7	45.1	47.8	44.3	43.4	44.0	42.5	45.2
7	42.9	42.2	43.0	40.9	47.7	48.0	45.5	43.4	44.4	45.1	45.0
8	40.4	40.8	41.9	39.4	46.4	50.1	43.1	44.4	43.9	43.5	46.0
9	43.1	40.7	41.2	40.3	46.4	49.0	42.6	41.2	41.5	41.9	44.8
10	40.0	40.9	41.2	40.9	42.8	49.1	42.9	41.7	40.1	41.2	45.0
11	41.1	39.3	41.6	38.7	41.5	47.7	43.0	41.2	42.9	42.5	43.0
12	42.4	41.9	42.4	40.4	43.7	45.6	42.4	40.6	41.3	40.7	43.6
13	39.8	40.3	41.7	41.1	42.8	46.4	42.3	42.5	43.1	42.6	43.9
14	40.1	40.7	43.0	41.5	42.7	47.4	41.3	42.4	41.2	42.4	43.6
15	39.3	39.9	40.7	40.0	44.3	46.0	41.9	40.7	43.2	43.0	45.6
16	38.5	40.4	40.1	39.2	43.1	45.4	41.6	41.6	42.5	40.8	43.5
20	38.2	38.5	39.9	39.7	40.8	41.9	40.1	39.1	39.9	43.2	40.4
24	37.5	37.2	38.0	36.9	38.7	43.3	37.4	36.9	37.8	38.8	38.4
28	34.5	35.5	36.2	35.1	37.2	41.4	37.7	35.8	36.3	38.0	38.5
32	36.1	36.8	37.1	35.2	36.7	38.3	39.1	38.9	37.2	39.2	39.1
36	35.5	36.4	37.4	36.5	35.6	40.2	36.8	35.4	36.2	37.0	37.3
40	35.1	34.5	35.7	34.3	35.2	36.3	36.3	35.8	35.6	36.7	37.2
44	36.1	36.7	36.6	35.6	35.8	36.9	39.0	38.0	35.9	38.3	35.5
48	36.7	36.5	37.9	37.5	37.2	37.5	38.0	37.5	37.0	38.6	37.4
52	33.0	33.5	34.7	32.2	33.2	34.5	34.2	33.2	34.3	34.9	33.9
56	35.4	35.3	34.0	34.7	35.0	34.6	37.0	37.1	34.9	37.4	35.9
60	37.3	37.6	39.3	40.3	39.6	39.1	38.4	37.3	37.5	39.3	38.5
64	36.7	38.5	38.2	37.7	38.6	40.3	39.5	37.8	36.9	40.4	39.3
68	38.2	39.4	36.9	38.0	37.2	36.3	40.3	39.2	36.8	38.3	37.0
72	38.0	38.7	37.7	37.9	38.1	37.1	37.9	37.7	36.7	38.1	36.7
76	36.1	37.4	35.3	34.9	36.7	38.4	36.0	35.4	34.7	36.2	36.8

Data extracted from Table 5, report HLA 483250 pp. 86-91.
* Significantly different from control ($p \leq 0.05$).
† Data reported as invalid, animals spilled or wasted feed.

GROUP MEAN TEST ARTICLE INTAKE (mg/kg)

During	Males					Females			
Week(s)	10	30	300	2500	4500	10	30	300	2500
1	1.93	5.81	57.23	-	-	2.51	7.07	73.32	-
2	1.93	5.95	58.90	734.02	1187.47	2.60	7.28	74.93	869.56
3	1.89	5.85	56.72	578.42	1038.40	2.38	7.19	74.28	676.59
4	1.73	5.55	52.75	552.14	976.92	2.20	7.06	71.81	583.63
5	1.74	5.25	52.43	491.08	943.32	2.23	7.21	66.29	614.42
6	1.76	5.09	51.57	488.39	978.40	2.24	6.67	64.90	590.71
7	1.71	5.21	50.24	504.66	993.14	2.21	6.71	68.00	602.89
8	1.63	5.09	48.42	493.38	1043.19	2.24	6.50	64.78	598.23
9	1.62	4.97	49.75	486.02	985.95	2.08	6.25	63.49	586.71
10	1.60	4.79	49.71	437.59	965.03	2.09	5.97	62.44	586.39
11	1.53	4	46.89	423.20	921.12	2.05	6.25	63.49	550.09
12	1.62	4.83	47.84	443.33	867.23	1.98	5.92	59.30	551.23
13	1.56	4.90	49.19	430.98	901.72	2.05	6.10	62.12	550.46
14	1.57	5.03	49.80	431.84	912.64	2.05	5.84	61.21	546.67
15	1.54	4.76	47.84	444.72	879.07	1.96	6.12	61.93	565.59
16	1.55	4.67	47.00	435.99	867.89	1.97	5.96	58.24	538.13
20	1.46	4.58	46.21	407.10	779.75	1.82	5.47	60.65	487.42
24	1.39	4.37	43.28	382.35	794.20	1.72	5.06	52.92	452.74
28	1.31	4.08	40.62	363.87	746.40	1.62	4.85	50.87	453.12
32	1.35	4.18	41.04	359.14	692.10	1.76	4.92	52.49	460.93
36	1.32	4.16	41.97	341.75	712.26	1.59	4.76	48.86	432.25
40	1.23	3.95	39.11	340.03	638.72	1.58	4.66	47.87	427.29
44	1.33	4	40.18	343.62	648.13	1.65	4.58	48.57	401.91
48	1.32	4	42.19	356.03	664.58	1.65	4.81	50.46	424.38
52	1.19	3.81	36.20	316.84	601.79	1.43	4.30	44.52	374.41
56	1.22	3.66	38.47	330.49	598.00	1.57	4.48	47.12	397.42
60	1.33	4.21	44.79	371.40	655.24	1.57	4.67	48.21	423.98
64	1.35	4.15	41.64	360.79	684.82	1.59	4.59	50.02	428.38
68	1.39	4.02	42.44	345.35	594.97	1.61	4.55	47.68	408.07
72	1.38	4.20	41.70	358.57	632.04	1.55	4.52	46.75	401.26
76	1.32	3.91	38.99	341.83	661.73	1.44	4.28	43.98	393.44
1-76	1.51	4.65	46.29	423.16	818.87	1.90	5.63	57.79	512.61

Data extracted from Table 6, report HLA 483250 pp. 92-94 and text Table 1 p. 30.

5. Viral Serology

The following screens were performed on 10 randomly selected animals/sex prior to assignment of permanent animals numbers.

Pneumonia Virus of Mice (Enzyme-Linked Immunosorbent Assay)
Reovirus Type 3 (Enzyme-Linked Immunosorbent Assay)
Theiler's Virus (Indirect Fluorescent Antibody)
Sendai Virus (Enzyme-Linked Immunosorbent Assay)
Lymphocytic Choriomeningitis (Indirect Fluorescent Antibody)
Minute Virus of Mice (Enzyme-Linked Immunosorbent Assay)
Mouse Hepatitis (Enzyme-Linked Immunosorbent Assay)
K Virus (Hemagglutination Inhibition)
Mouse Adenovirus (Complement Fixation)
Ectromelia (Indirect Fluorescent Antibody)
Polyoma Virus (Hemagglutination Inhibition)
Mycoplasma Pulmonis (Enzyme-Linked Immunosorbent Assay)

6. Hematology and Clinical Chemistry

A differential leukocyte count was performed before treatment (10 animals/sex from animals not assigned to study). Clinical chemistry was performed on 10 additional animals/sex not assigned to the study. Cell morphology and differential leukocyte counts were performed on 10 animals/sex in the control and high dose groups after 52 and 78 weeks of treatment. These parameters were also evaluated for recovery animals from the 0, 2500 and 4500 (males) ppm groups and the 2500 ppm females after 4 weeks of recovery (at week 57), as well as any animals found in the moribund condition.

Clinical chemistry was performed on 10 animals/sex/group after 52 and 78 weeks of treatment and after 4 weeks of recovery (week 57). Blood smears were obtained from the tail and serum samples were taken from the abdominal aorta of animals that had been fasted overnight (with water available). The following parameters were examined:

sorbitol dehydrogenase (SDH)
gamma glutamyltransferase (GGT)
alanine aminotransferase (ALT)
alkaline phosphatase (ALK P)
total bilirubin (T BILI)

Results

A significant increase in the percent of neutrophils and a decrease in the percent of lymphocytes was observed in females receiving 2500 ppm at week 79. Mean alanine aminotransferase values were elevated in males receiving 2500 or 4500 ppm at week 53 and in females receiving 2500 ppm at week 79. An increase in mean sorbitol dehydrogenase values were observed for males receiving 300 ppm at week 53, 2500 or 4500 ppm at weeks 53 and 79, and females receiving 2500 ppm at week 79. Mean alkaline

phosphatase values were increased for males receiving 4500 ppm at week 79.

Liver enzyme data for the recovery animals (week 57) did not indicate significant differences.

7. Euthanasia and Pathology

Necropsies were performed on all animals that died during the study or were euthanized in a moribund state. An interim sacrifice was performed on 10 animals/sex/dose at week 52. Ten male animals were selected from the 0, 2500, and 4500 ppm groups and ten female animals from the 2500 ppm group were selected as recovery animals that were placed on basal diet for weeks 53-56 prior to euthanasia and necropsy. The remaining surviving animals were exsanguinated under sodium pentobarbital anesthesia. The study pathologist was present at the interim sacrifice and the terminal sacrifice of the first 10 mice/sex/group. The CHECKED (X) tissues were preserved in 10% neutral buffered formalin. The (XX) organs, were weighed (the adrenals, pituitary, and ovaries were weighed postfixation).

X		X		X	
Digestive system		Cardiovasc./Hemat.		Neurologic	
	Tongue	X	Aorta*	XX	Brain*+
X	Salivary glands*	XX	Heart*	X	Periph. nerve*#
X	Esophagus*	X	Bone marrow*	X	Spinal cord (3 levels)*#
X	Stomach*	X	Lymph nodes*	XX	Pituitary*
X	Duodenum*	XX	Spleen	X	Eyes *#
X	Jejunum*	X	Thymus*	Glandular	
X	Ileum*	Urogenital		XX	Adrenal gland*
X	Cecum*	XX	Kidneys*+		Lacrimal gland#
X	Colon*	X	Urinary bladder*+	X	Mammary gland (♀)*#
X	Rectum*	XX	Testes*+	X	Parathyroids*++
XX	Liver *+	XX	Epididymides	X	Thyroids*++
XX	Gall bladder*	X	Prostate	Other	
X	Pancreas*	X	Seminal vesicle		Bone*#
Respiratory		XX	Ovaries*+	X	Skeletal muscle*#
X	Trachea*	X	Uterus*	X	Skin*#
X	Lung*	X	Vagina	X	All gross lesions and masses*
	Nose^				
	Pharynx^				
	Larynx^				

* Required for subchronic and chronic studies.
^ Required for chronic inhalation.
# In subchronic studies, examined only if indicated by signs of toxicity or target organ involvement.
+ Organ weight required in subchronic and chronic studies.
++ Organ weight required for non-rodent studies.

SELECT MEAN CLINICAL HEMATOLOGY & CHEMISTRY DATA

Parameter	Week 0						Week 53						Week 57						Week 79					
	0	10	30	300	2500	4500	0	10	30	300	2500	4500	0	10	30	300	2500	4500	0	10	30	300	2500	4500
% Seg	15	-	-	-	-	-	66	-	-	-	-	69	58	-	-	-	-	59	63	-	-	-	-	73
% Lymph	81	-	-	-	-	-	32	-	-	-	-	29	38	-	-	-	-	37	33	-	-	-	-	26
% Eosin	3	-	-	-	-	-	1	-	-	-	-	1	3	-	-	-	-	2	2	-	-	-	-	0*
ALT U/L	28	-	-	-	-	-	30	32	22	76	104*	114*	34	-	-	-	56	47a	54	52	31	30	89	222*
ALK P U/L	94	-	-	-	-	-	36	41	33	42	39	66	40	-	-	-	31	47a	72	45	34	41	59	392*
GGT U/L	1	-	-	-	-	-	0	1	0	0	0	0	0	-	-	-	0	0a	0	0	0	0	0	5
SDH U/L	53	-	-	-	-	-	45	46	41	89*	179*	215*	49	-	-	-	68	73	51	51	34	43	115*	231*
Females																								
% Seg	23	-	-	-	-	-	66	-	-	-	65	-	-	-	-	-	53	-	61	-	-	-	75*	-
% Lymph	72	-	-	-	-	-	33	-	-	-	33	-	-	-	-	-	44	-	37	-	-	-	23*	-
% Eosin	4	-	-	-	-	-	0	-	-	-	1	-	-	-	-	-	2	-	1	-	-	-	0*	-
ALT U/L	27	-	-	-	-	-	52	25	23	57	45	-	-	-	-	-	30	-	29	28	24	56	182*	-
ALK P U/L	114	-	-	-	-	-	40	39	48	42	39	-	-	-	-	-	52	-	71	57	53	49	88	-
GGT U/L	0	-	-	-	-	-	0	0	0	0	0	-	-	-	-	-	0	-	0	0	0	0	1	-
SDH U/L	33	-	-	-	-	-	72	31*	26*	51	71	-	-	-	-	-	39	-	43	36	27	56	112*	-

Data extracted from Report HLA 483250 tables 7A & 8, pp. 95-98 & 103-104.

a One animal was excluded from calculations due to variation attributed to hepatocellular carcinoma.

* Significantly different from control ($p \leq 0.05$).

Terminal Organ Weight Data

Organ	Absolute Weight										
	Males						Females				
	0	10	30	300	2500	4500	0	10	30	300	2500
Final Bdy Wt (g)	32.5	34.4*	33.0	32.5	32.6	30.6	29.9	29.4	29.3	28.8	27.4*
Brain W/Stem (g)	0.49	0.51	0.50	0.55	0.49	0.48	0.50	0.51	0.51	0.46	0.51
Heart (g)	0.20	0.21	0.20	0.20	0.23	0.22	0.17	0.18	0.16	0.18	0.17
Adrenal (g)	0.006	0.007	0.006	0.006	0.006	0.007	0.011	0.012	0.011	0.010	0.011
Kidney (g)	0.63	0.77*	0.72	0.72	0.70	0.56	0.49	0.51	0.49	0.52	0.45
Liver/Gallbladder (g)	1.64	1.52	1.61	1.65	2.36*	3.48*	1.41	1.27	1.48	1.70	2.56*
Pituitary (g)	0.003	0.002	0.002	0.003	0.002	0.001	0.003	0.003	0.003	0.004	0.002
Spleen (g)	0.09	0.09	0.09	0.09	0.09	0.08	0.14	0.13	0.11	0.12	0.14
Testes/Epidid Ovary (g)	0.36	0.34	0.37	0.34	0.33	0.32	0.128	0.040	0.250	0.048	0.061

Organ	Organ Weight as Percent of BW										
	Males						Females				
	0	10	30	300	2500	4500	0	10	30	300	2500
Brain W/Stem	1.516	1.524	1.481	1.660	1.470	1.550	1.754	1.744	1.750	1.608	1.901
Heart	0.630	0.633	0.595	0.594	0.689	0.715	0.579	0.610	0.552	0.635	0.660
Adrenal	0.0200	0.0201	0.0190	0.0181	0.0182	0.0219	0.0372	0.0401	0.0390	0.0357	0.0421
Kidney	1.972	2.282*	2.127	2.164	2.112	1.808	1.707	1.738	1.684	1.799	1.680
Liver /Gallbladder	5.081	4.523	4.729	4.982	7.036*	11.248*	4.889	4.324	5.056	5.915	9.713*
Pituitary	0.0082	0.0061	0.0047*	0.0077	0.0051	0.0047*	0.0089	0.0100	0.0094	0.0129	0.0090
Spleen	0.305	0.279	0.256	0.287	0.266	0.252	0.483	0.432	0.930	0.420	0.553
Testes/Epidid Ovary	1.107	1.004	1.100	1.030	1.013	1.040	0.4414	0.1391	0.9496	0.1640	0.2218

Data extracted from report HLA 483250 Tables 9C and 9F, pp. 112-113 and 121-122.

* Significantly different from control ($p \leq 0.05$).

Results

Organ Weight

Week 53

There was a significant reduction in the mean absolute weight of the brain (with the brain stem) at the interim sacrifice (week 53) in males receiving 4500 ppm. In addition, there was a significant increase in the mean absolute liver/gallbladder weight of females receiving 300 ppm, males and females receiving 2500, ppm and males receiving 4500 ppm. There was also a significant reduction in mean absolute testicular weight of males receiving 300 ppm at the interim sacrifice.

Statistical analysis of the organ to terminal body weight ratio data at the interim sacrifice revealed significant increases in the liver/gallbladder of females receiving 300 or 2500 ppm, and males receiving 2500 or 4500 ppm in a treatment related manner.

Week 57

Statistical analyses performed on the absolute organ weight of the week 57 recovery animals at the two highest doses tested (2500 and 4500 ppm) did not detect significant differences. However, the absolute terminal body weight of males receiving 2500 and 4500 ppm were significantly reduced.

The heart to body weight ratio of males receiving 4500 ppm was significantly increased at week 57. The liver/gallbladder to body weight ratio of the 4500 ppm males was slightly (nonsignificantly) increased at this time period. (There were no female control values at week 57 to compare with the treated animals.) The adrenal to body weight ratio of males receiving 2500 or 4500 was significantly increased in a treatment related manner at week 57.

Week 79

Absolute body weight was significantly increased in males receiving 10 ppm and was significantly reduced in females receiving 2500 ppm. Males receiving 2500 and 4500 ppm and females receiving 2500 ppm had significant treatment related increases in absolute liver/gallbladder weight.

The kidney to body weight ratio of males receiving 10 ppm was significantly increased. The liver/gallbladder to body weight ratio was significantly increased in males and females receiving 2500 ppm and males receiving 4500 ppm. In addition, the pituitary to body weight ratio of males receiving 30 or 4500 ppm was significantly reduced.

Absolute and Relative Liver Weight Percent Differences From Control[a]

	Males		Females	
	2500	4500	300	2500
Week 53				
Absolute Liver Weights	34	63	20	41
Liver to Body Weight Ratios	38	77	17	46
Week 57				
Absolute Liver Weights	4	9	NDA	NDA
Liver to Body Weight Ratios	6	27	NDA	NDA
Week 79				
Absolute Liver Weights	44	112	21	82
Liver to Body Weight Ratios	38	121	21	99

Data extracted from report HLA 483250 text Table 2 p. 33.
a Calculation: [(absolute difference between control and treated mean/control mean) X 100].
NDA = No Data Available

Gross pathology

Gross observations which were conspicuous among the unscheduled deaths during the first three weeks included liver findings in 7/11 4500 ppm males, 22/70 4500 ppm females, and 2/9 2500 ppm females. In general the livers were observed to be pale in color. Necropsy of animals that died during the first three weeks of the study also revealed stomach findings in 5/11 males and 31/70 females in the 4500 ppm group and 3/9 females in the 2500 ppm group. The report does not indicate that any remarkable gross observations were noted at the 53 or 57 week necropsies. Among the unscheduled deaths weeks 4-87 there was an increased overall incidence of liver findings in both sexes receiving 2500 ppm and in the males receiving 4500 ppm. Enlarged livers and liver masses were observed in about one half of the males of the 4500 ppm group.

Exceptional findings in the liver at the terminal sacrifice included: enlargement, pale areas, and masses. The total number of masses found during necropsy or processing histopathologic tissues for this study were: 3/70, 9/60, 10/60, 8/60, 17/70, and 20/70 for the males (groups 1 - 6 respectively) and 1/70, 1/60, 1/60, 1/60, and 13/70 for the females (groups 1 - 5 respectively).

Microscopic pathology

Histopathologic treatment related lesions were observed in the livers of males and females receiving 300 ppm and above (excluding 4500 ppm for the females). The lesions observed included: necrosis of individual hepatocytes, hypertrophy of

Selected Gross Pathological Findings of the Liver

	Males						Females					
	0	10	30	300	2500	4500	0	10	30	300	2500	4500
Unscheduled Sacrifice (N)	20	18	23	26	16	34	36	15	21	15	21	70
Dark	0	0	0	0	1	1	0	1	0	0	1	7
Enlarged	2	3	6	2	4	14	2	0	2	4	5	3
Pale Area	0	0	1	0	0	13	1	0	0	2	6	13
Mass	0	3	2	4	7	10	0	1	0	0	4	0
Mottled	0	0	1	0	0	4	1	0	0	0	1	0
Interim Sacrifice (N)	10	10	10	10	10	10	10	10	10	10	10	0
Dark	0	0	0	0	0	1	0	0	0	0	0	-
Pale Area	0	0	0	0	1	2	0	0	0	0	1	-
Mass	0	1	2	0	1	2	0	0	0	0	1	-
Recovery Sacrifice (N)	9	0	0	0	10	10	0	0	0	0	10	0
Raised Area	0	-	-	-	0	2	-	-	-	-	0	-
Pale Area	0	-	-	-	0	1	-	-	-	-	0	-
Mass	0	-	-	-	1	3	-	-	-	-	0	-
Terminal Sacrifice (N)	31	32	27	24	34	16	24	35	29	35	29	0
Raised Area	0	0	0	2	0	1	0	0	0	0	1	-
Mottled	0	0	0	0	0	3	0	0	0	0	1	-
Dark	0	0	0	0	3	2	0	0	0	0	1	-
Enlarged	5	0	0	0	8	8	0	3	0	1	13	-
Pale Area	1	1	1	0	12	9	0	3	0	4	12	-
Mass	3	5	5	3	5	7	0	0	1	1	8	-

Data extracted from Report HLA 483250 tables 10A-10D pp. 123-173

Selected Histopathological Findings of the Liver

	Males						Females					
	0	10	30	300	2500	4500	0	10	30	300	2500	4500
# Livers Examined in Unscheduled Deaths	20	17	23	26	16	34	26	14	21	15	21	0
Primary Benign Hepatocellular Adenoma	0	3	1	2	9	6	0	0	0	0	5	-
Primary Malignant Hepatocellular Carcinoma	0	0	1	0	1	4	0	0	0	0	2	-
Necrosis of Individual Cell	0	1	0	3	11	31	0	0	0	1	9	-
Focal/ Multifocal Necrosis	0	0	0	0	12	21	0	0	0	3	6	-
Hypertrophy of Hepatocytes	4	9	7	14	15	34	1	1	1	3	14	-
Bile Stasis	0	0	0	0	12	21	0	0	0	3	6	-
Fatty Change	0	0	0	1	1	15	0	0	0	0	1	-
# Livers Examined at Week 53 Interim Sacrifice	10	10	10	10	10	10	10	10	10	10	10	-
Primary Benign Hepatocellular Adenoma	0	1	2	0	1	2	0	0	0	0	1	-
Primary Malignant Hepatocellular Carcinoma	0	0	0	0	0	2	0	0	0	0	0	-
Necrosis of Individual Cell	0	1	1	3	10	10	0	0	0	3	10	-
Focal/ Multifocal Necrosis	0	0	0	2	1	0	0	0	2	1	3	-
Fatty Change	1	1	0	2	5	9	0	0	2	1	3	-
Hypertrophy of Hepatocytes	0	1	0	1	10	10	0	0	0	0	10	-
Bile Stasis	0	0	0	0	3	10	0	0	0	0	6	-
# Livers Examined in Recovery Sacrifice at Week 57	9	-	-	-	10	10	0	-	-	-	10	-
Primary Benign Hepatocellular Adenoma	0	-	-	-	0	3	-	-	-	-	0	-
Primary Malignant Hepatocellular Carcinoma	0	-	-	-	1	1	-	-	-	-	0	-
Necrosis of Individual Cell	1	-	-	-	6	3	-	-	-	-	0	-
Bile Stasis	0	-	-	-	9	6	-	-	-	-	9	-
Hypertrophy of Hepatocytes	1	-	-	-	4	2	-	-	-	-	1	-
Chronic Inflammation	1	-	-	-	4	4	-	-	-	-	2	-
# Livers Examined in Terminal Sacrifice (Week 79)	31	32	27	24	34	16	24	35	29	35	29	0
Primary Benign Hepatocellular Adenoma	4	6	5	7	3	9	0	0	0	1	10	-

	Males						Females					
	0	10	30	300	2500	4500	0	10	30	300	2500	4500
Primary Malignant Hepatocellular Carcinoma	1	0	0	0	3	6	0	0	1	0	2	-
Necrosis of Individual Cell	4	3	1	7	25	9	3	0	0	2	8	-
Focal/Multifocal Necrosis	3	1	0	1	5	5	3	1	0	6	3	-
Hypertrophy of Hepatocytes	12	6	8	11	32	11	1	6	1	4	28	-
Bile Stasis	1	0	0	3	32	13	0	0	0	0	29	-
Fatty Change	0	0	0	1	7	7	0	0	0	1	4	-
Summary of Hepatic Histopathological Findings - # of Livers Examined	70	59	60	60	70	70	60	59	60	60	70	-
# Examined & Found Unremarkable	26	24	19	12	1	2	21	19	25	13	1	-
Primary Benign Hepatocellular Adenoma	4	10	8	9	13	20	0	0	0	1	16	-
Primary Malignant Hepatocellular Carcinoma	1	0	1	0	5	13	0	0	1	0	4	-

Data extracted from Report HLA 483250 Tables 11A - 11E pp. 174-246.

hepatocytes, bile stasis, focal/multifocal necrosis of hepatocytes and fatty change.

Histopathological examination at the week 53 interim sacrifice and the week 57 recovery sacrifice found lesions consisting of individual hepatocyte necrosis, hypertrophy of hepatocytes, bile stasis and chronic inflammation in both sexes receiving 2500 ppm and males receiving 4500 ppm. Hepatocellular adenoma and/or carcinoma was observed at the interim sacrifice in males receiving 10, 30, 2500, or 4500 ppm and in females receiving 2500 ppm. At the postrecovery sacrifice this observation was only found in the males receiving 2500 or 4500 ppm.

D. DISCUSSION:

The clinical pathology report noted a significant increase in the percentage of neutrophils and decrease in the percentage of lymphocytes for the 2500 ppm females. These findings may be indicative of malignant tumors. In addition, the clinical pathology report indicated a number of enzymes were affected by treatment. Alanine aminotransferase was significantly increased in the 2500 and 4500 ppm males at 52 weeks. An increase in this enzyme may be indicative of liver damage. Sorbitol dehydrogenase levels were significantly increased in the 300, 2500 and 4500 ppm males at 52 weeks. Sorbitol dehydrogenase is a liver enzyme. An increase in this enzyme is thought to be indicative of frank liver damage.

After 79 weeks of treatment alanine aminotransferase was increased in males receiving 4500 ppm and in females receiving 2500 ppm. Alkaline phosphatase was increased in males receiving 4500 ppm. An increase in either of these enzymes may be indicative of hepatocyte necrosis.

Absolute liver organ weight was significantly increased in both sexes receiving 2500 ppm and in males receiving 4500 ppm. Liver weight as a percent of body weight was significantly increased in both sexes receiving 2500 ppm and in males receiving 4500 ppm.

The pathology report indicates the following hepatic lesions were observed:

- hepatocellular adenoma and/or carcinoma observed in males at 2500 or 4500 ppm; in females at 2500 ppm.

- necrosis of individual hepatocytes at 300, 2500, or 4500 ppm in males; in females at 2500 ppm.

- focal/multifocal necrosis of hepatocytes in males receiving 2500 or 4500 ppm.

- hypertrophy of hepatocytes in males at 300, 2500, or 4500 ppm; in females at 2500 ppm.

- bile stasis in males at 2500 or 4500 ppm; in females at 2500 ppm.

- fatty changes of the liver in males receiving 2500 or 4500 ppm.

Based on the available data, it would appear that the target organ in the mouse for CGA 169374 is the liver, when administered in the diet for up to 79 weeks in excess of 30 ppm.

E. Study Deficiencies:

1. The analytical chemistry report does not contain data pertaining to the purity of the test material.

2. The analytical report does not contain the raw data for stability, homogeneity, or concentration analyses.

3. Cageside observations were not performed on eight different days, and were performed twice on one day.

4. Slides for differentials were not performed for a group 2 male, a group 2 female, a group 3 female, a group 4 female, and a group 6 female.

F. Core Classification: **Core Supplementary**

This study does not satisfy the guideline requirements for a oncogenicity study in mice due to deficiencies in the analytical data, namely the raw data pertaining to the purity of the test material, the stability, homogeneity, and concentration analyses. The study may be upgraded upon satisfactory review of the Registrant's response to these deficiencies.

NOEL = 30 ppm
LOEL = 300 ppm

Page_____ is not included in this copy.

Pages 46 through 53 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 confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

Table 11E
Histopathology Incidence Summary
Oncogenicity Study in Mice
Neoplastic Findings

Page_____ is not included in this copy.

Pages 56 through 66 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.

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009689

Primary Review by: K.E. Whitby, Ph.D. 5/8/92
Toxicologist, Review Section II, Tox. Branch (H7509C)
Secondary Review by: K. Clark Swentzel
Section Head, Review Section II, Tox. Branch (H7509C)

DATA EVALUATION REPORT

STUDY TYPE: Combined Chronic Toxicity and Oncogenicity Study in Rats §83-5

HED Project No: 2-0696 MRID NO.: 420900-19

TEST MATERIAL: CGA 169374 SHAUGHNESSY NO: 128847

SYNONYMS: Difenconazole

LABORATORY STUDY NO.: 483-249

SPONSOR: Agricultural Division
CIBA-GEIGY Corporation
P. O. Box 18300
Greensboro, NC 27419-8300

TESTING FACILITY: Hazelton Laboratories America, Inc.
9200 Leesburg Turnpike
Vienna, VA 22182

TITLE OF REPORT: Combined Chronic Toxicity and Oncogenicity Study of CGA 169374 Technical in Rats

AUTHOR(S): Raymond H. Cox, Ph.D.

REPORT ISSUED: March 31, 1989

STUDY DATES: Initiation of Dosing Period: April 16, 1986
Necropsies Completed: April 26, 1988

CONCLUSION:

CGA 169374 was administered in the diet to male and female rats (80/sex) for 104 weeks at 0, 10, 20, 500, and 2500 ppm. The NOEL was 20 ppm. The LOEL was 500 ppm based on reductions in cumulative body weight gains in the 500 and 2500 ppm groups. Mean liver weight was increased at week 53 and at termination in the 2500 ppm group (but not in the recovery group at week 57). Hepatocellular hypertrophy was observed in the 500 and 2500 ppm animals at termination. Additional findings which indicate that the liver is the target organ were observed in the clinical chemistry data. No treatment related increased incidences of neoplastic findings were observed in this study. The study may be upgraded after satisfactory review of the Registrant's response to the deficiencies (raw data for the purity of the test material, the stability, homogeneity, and concentration analyses).

Classification: Core Supplementary

NOEL = 20 ppm
LOEL = 500 ppm

A. MATERIALS:

1. Test Compound:

Test Substance: CGA 169374 technical
Description: dark brown solid
Stability: not stated
Storage Conditions: room temperature

Lot No.	Date Required	Purity	Weeks Used
FL851406	November 22, 1985	94.5%	1-20
FL861408	August 4, 1986	95%	21-46
FL861408	February 6, 1987	95%	47-106

Vehicle: Pesticide grade acetone
Source: Fisher Scientific Company
Description: clear liquid
Storage: nonflammable cabinet

Lot No.	Date Received	Weeks Used
856990	March 4, 1986	1-12
860851	May 20, 1986	13-20
862767	July 28, 1986	21-26
862852	September 24, 1986	27-56
865760	March 16, 1987	57-64
62852	April 29, 1987	65-82
1909	October 7, 1987	83-96
874896	January 13, 1988	97-106

Analytical Chemistry

The report indicates (p. 14) that information on the stability as well as other characteristics of the test material are on file with the Sponsor.

The analytical chemistry report (p. 31) provides the results in the form of text for stability and homogeneity. Purity is not presented. Page 60 of the report provides the analytical chemistry results. The 10 ppm level of CGA 169374 was the only level tested for stability. Duplicate samples found the stability to be 97.23 - 97.97% in the diet at room temperature for 16 days. Homogeneity

analyses means for weeks 1-2 (top, bottom, and middle) ranged from 87.37 to 105.9% of the target concentration. The week 7-8 results of the concentration analyses for the 500 ppm level were 42.86 and 45.32% of the target for the duplicate samples. The 10 ppm group was found to be 74.47 and 79.03% of target value during weeks 47-48. Otherwise, the analytical values ranged from 80.1 to 111.7%.

2. Test animals:

Species: Rats
Strain: Sprague-Dawley
Number Received for Study: 535 ♂
520 ♀
Source: Charles River Laboratories, Inc., Portage MI
Date of Arrival: March 26, 1986
Age: 28 days at receipt
8 weeks at initiation of treatment
Weight: ♂ 213.8 - 334.2 g at initiation of treatment
♀ 137.7 - 230.8 g at initiation of treatment

Not all of the animals received for the purpose of this study were assigned to treatment groups. Animals were selected for participation in the study by the staff veterinarian following examination. Ten animals of each sex were randomly selected for clinical pathology screening.

B. STUDY DESIGN:

1. Animal Husbandry

Purina Certified Rodent Chow® #5002 was used as the basal diet fed ad libitum (during quarantine and study). Tap water was available ad libitum during the study via an automatic watering system. Animals were housed in double in stainless steel hanging wire cages for 8 days. Thereafter the animals were housed singly and assigned permanent identification numbers for the last two weeks of quarantine. Temperature and relative humidity during the study ranged from 66-86° F and 31-79%, respectively. The animals were maintained on a 12 hour light/dark cycle.

Animals were assigned to the following test groups:

Test Group	Dose in diet (ppm)	♂	♀
1 Control	0	90	90
2 Low (LDT)	10	80	80
3 Low Mid (LMDT)	20	80	80
4 High Mid (HMDT)	500	80	80
5 High (HDT)	2500	90	90

2. Diet preparation

The test compound was weighed as 100 % a.i.. Test diets were prepared every 2 weeks. The control group received an acetone feed admixture. Prior to the day of admixing in the diet, the test compound was placed overnight (24 hrs) in a water bath at 70° C to achieve a liquid state. The vehicle was used to dissolve and mix the test compound at a ratio of 5 mL vehicle/kg diet. The beaker containing test compound was set on a 100° F hot plate reach a liquid state. A premix was prepared in a mixer (20 min of mixing). The premix was added to the appropriate volume of feed and mixed for 1 min/kg.

3. Observations

All animals were observed twice daily for mortality, and moribundity. Once daily the animals were observed cage side for toxic effects. Once each week animals were palpated for tissue masses.

Ophthalmoscopic examinations were performed by the staff ophthalmologist for all animals prior to dosing and at 6 month intervals (weeks 28, 52, 78, and 104) using 1% Mydriacyl as a mydriatic and indirect ophthalmoscopy.

Body weight was recorded for all animals prior to dosing. Body weight and food consumption were recorded weekly during weeks 1-16, and then once every 4 weeks thereafter.

4. Statistics

The procedures utilized in analyzing the data are included in Appendix I.

5. Compliance

A signed Statement of No Confidentiality Claim was included which was dated 1/11/91 (p. 2).

A signed Statement of Compliance with EPA GLP's was included which was dated 3/31/89 (p. 5).

A signed Quality Assurance Statement was included and dated 3/31/89 (p. 6).

A signed Flagging Criteria Statement was included which was dated 1/15/91 (p. 4).

C. RESULTS:

1. Observations:

There were no significant treatment/dose related differences in survival among the groups. Evaluation of the clinical data indicated that males exhibited a non-significant increase in the incidence of malocclusion at the HDT relative to the control (8 vs. 29).

Percent Adjusted Survival

% Survival	0	10	20	500	2500
Males	61	50	57	61	64
Females	48	50	44	61	58

2. Body Weight

Prior to the initiation of treatment, there were no significant differences in body weight among the groups. Statistical analyses indicated significantly lower mean weight for the 500 ppm females and 2500 ppm males and females at week 52. By week 76 both sexes receiving 2500 ppm had significantly lower mean body weight. Statistical analyses of the last interval body weight was recorded, day 104, found the 2500 ppm females to be significantly lower in body weight than the concurrent controls. The report indicates that mean growth indices (not defined) for the 500 ppm males and both sexes in the 2500 ppm group were significantly lower than control values.

Cumulative body weight gain was significantly less for both sexes in the 500 ppm group at week 13. Body weight gain of the 500 ppm group remained less than that of controls for males (-6 to -7%) and females (-6 to -11%) through week 52. Significantly lower values for body weight gain were noted throughout the study for males (-8 to -53%) and females (-29 to -71%) receiving 2500 ppm.

GROUP MEAN BODY WEIGHT GAIN (G) FOR RATS ADMINISTERED CGA 169374 IN THE DIET FOR 104 WEEKS

		Males					Females				
WEEKS ON TEST	ppm =	0	10	20	500	2500	0	10	20	500	2500
1.....		52.2	48.7	48.6	46.9	24.5	19.6	18.8	19.7	17.6	5.7
2.....		94.9	89.2	87.1	83.7	60.4	36.3	34.5	35.7	33.5	21.8
3.....		131.1	124.3	122.5	119.4	95.6	52.0	52.3	52.1	46.3	29.5
4.....		159.6	151.7	146.9	143.4	115.9	61.2	60.8	62.0	55.4	37.8
5.....		185.0	174.7	173.1	169.1	139.4	73.0	70.6	71.6	66.0	46.2
6.....		206.7	195.3	193.6	188.0	153.0	80.0	79.0	82.1	72.2	53.5
7.....		227.0	213.7	211.9	207.9	168.7	88.0	87.0	89.1	79.8	59.1
8.....		236.7	224.9	224.7	222.2	182.8	93.2	90.6	91.4	84.2	61.6
9.....		249.2	235.9	236.5	230.3	189.5	101.3	98.1	100.2	91.5	67.4
10.....		259.4	249.8	247.2	243.3	201.2	102.9	101.6	102.0	93.8	69.9
11.....		266.0	257.4	252.9	247.2	205.1	110.7	109.3	110.1	100.6	74.7
12.....		276.7	267.2	262.7	255.9	212.6	115.5	112.9	114.5	103.9	77.6
13.....		286.7	276.3	271.4*	265.3*	222.4*	114.3	111.3	112.4	102.2*	76.7*
14.....		288.3	278.4	277.6	269.8	224.6	116.1	120.3	119.8	109.4	81.1
15.....		295.4	283.3	282.5	276.9	229.9	121.2	124.0	124.4	110.6	83.3
16.....		302.4	290.5	289.7	282.4	234.2	124.6	126.9	126.1	114.6	85.7
20.....		331.4	321.2	317.4	308.2	259.3	134.3	137.6	136.9	124.3	95.1
24.....		350.7	340.7	339.0	325.7*	273.0*	150.6	154.4	151.2	136.0*	101.8*
28.....		358.0	348.0	346.1	332.4	274.6	156.2	163.7	161.0	145.0	105.4
32.....		375.8	363.6	360.1	344.8	289.8	175.2	180.7	176.1	157.4	116.2
36.....		391.0	377.2	374.5	361.7	307.4	183.9	185.2	184.1	169.8	121.4
40.....		398.5	392.6	390.8	374.0	321.3	195.9	201.4	204.8	181.8	127.9
44.....		403.1	398.2	391.2	375.1	323.7	205.3	212.0	210.6	187.4	129.8
48.....		412.0	404.1	400.0	387.4	331.1	218.9	222.9	221.2	195.4	133.9
52.....		423.0	416.5	415.5	397.4*	339.2*	231.5	233.9	237.4	209.1*	138.8*
56.....		428.8	422.1	417.5	403.0	347.7	232.9	234.2	240.3	214.6	144.0
60.....		425.2	417.0	414.7	406.7	349.0	242.0	236.9	238.7	219.4	141.1
64.....		427.0	422.7	416.4	410.8	351.8	238.7	250.4	249.7	223.7	152.3
68.....		422.2	405.4	413.8	406.3	352.9	246.9	245.2	253.3	229.9	155.4
72.....		415.1	404.2	407.0	399.5	351.7	253.4	256.8	258.0	231.7	158.9
76.....		409.7	405.1	398.0	397.2	350.1	251.5	262.4	254.1	237.2	158.6
80.....		402.0	400.3	393.4	392.9	347.7	253.6	267.3	256.9	237.2	162.7
84.....		407.8	395.2	388.6	387.1	345.9	268.3	270.5	264.8	241.0	163.0
88.....		394.5	378.8	376.8	394.2	344.8	256.1	268.9	258.8	238.5	158.3
92.....		372.8	364.8	370.6	396.3	334.0	252.7	267.9	249.0	242.4	162.6
96.....		363.3	355.1	352.7	381.7	323.5	247.6	268.2	264.1	239.4	163.8
100.....		342.1	342.2	350.8	375.1	311.8	251.4	268.5	264.3	240.1	161.7
104.....		331.8	339.5	352.4	358.9	293.8*	250.9	270.3	268.7	238.1	157.6*

Data extracted from Table 4B, report HLA 483249 pp. 70-77.

* Significantly different from control ($p \leq 0.05$).

3.

Food consumption and compound intake

During the first month of the study the authors report a higher incidence of spillage in the high dose females than observed in the controls. In general, the mean food consumption of the animals in the 2500 ppm group was lower than that of the controls. Statistical analyses of feed consumption weeks 52, 76, and 104 found significant reductions in the group receiving 2500 ppm.

GROUP MEAN FOOD CONSUMPTION (G/WK)

	Males					Females				
WEEKS ON TEST ppm =	0	10	20	500	2500	0	10	20	500	2500
1	189.8	188.1	188.8	186.8	166.0	143.0	143.5	142.5	135.7	131.4
2	192.4	191.5	190.0	188.5	182.4	143.7	139.4	143.1	136.3	136.0
3	193.5	193.5	194.1	195.9	184.3	150.1	145.0	148.2	143.6	121.8
4	200.5	199.8	201.9	201.2	190.5	149.7	148.7	153.0	142.5	138.1
5	199.8	196.9	198.6	196.5	184.3	149.3	147.5	145.8	143.6	132.4
6	201.2	196.5	196.6	194.9	180.5	146.9	146.1	146.1	141.6	130.3
7	201.7	198.1	200.5	204.0	188.0	152.6	149.8	151.2	150.8	136.3
8	198.5	198.5	198.5	202.4	186.5	147.5	148.3	150.1	148.0	132.6
9	200.5	200.0	200.7	199.2	186.9	145.2	145.7	147.1	144.5	132.7
10	199.1	204.5	199.0	199.2	181.1	153.4	154.2	150.2	148.6	133.7
11	199.3	198.3	200.1	194.0	182.4	143.9	148.5	147.4	143.3	131.5
12	197.0	195.8	196.9	192.8	182.0	143.4	145.5	145.5	139.7	129.3
13	194.0	190.4	194.2	185.6	174.7	146.9	148.4	144.6	143.7	134.4
14	189.5	192.6	192.7	187.6	179.0	147.6	149.3	143.5	139.2	131.8
15	193.4	190.6	191.0	187.8	182.4	149.6	147.2	144.7	139.6	131.9
16	193.2	192.3	191.0	189.9	182.3	147.1	149.1	144.4	142.1	135.2
20	191.8	186.5	185.8	188.9	182.2	145.9	152.4	149.7	152.6	140.0
24	179.0	184.5	183.7	180.7	167.8	144.1	147.5	147.2	140.1	127.6
28	179.4	182.2	180.6	181.7	172.8	138.2	144.4	138.0	135.6	131.4
32	185.2	186.7	187.2	186.5	173.2	144.3	147.4	138.1	138.9	131.8
36	175.2	180.6	175.2	174.3	162.1	137.1	145.0	148.2	146.6	129.4
40	172.9	176.2	169.1	166.7	154.7	139.3	139.8	142.0	140.2	127.7
44	171.9	170.2	160.7	165.5	157.1	140.2	143.7	143.5	139.0	125.2
48	168.0	173.8	169.6	172.3	161.1	143.4	146.9	145.2	140.5	126.5
52	166.1	163.9	162.8	161.5	154.2*	131.8	133.1	133.4	125.7	114.1*
56	169.3	172.7	174.9	178.0	165.0	146.5	142.6	141.9	142.0	128.0
60	179.7	183.7	180.1	179.0	174.5	150.4	147.2	148.4	142.8	134.2
64	194.7	201.0	198.7	205.3	197.5	171.5	180.7	182.6	169.1	151.5
68	182.8	179.2	177.5	178.1	173.9	150.1	133.0	150.5	148.5	138.7
72	179.7	176.2	179.4	180.4	175.2	156.5	158.2	156.5	149.5	138.5
76	184.2	180.6	180.5	180.1	175.6*	159.0	158.2	156.0	155.3	141.2*
80	176.8	173.9	176.3	179.0	174.1	150.3	154.4	144.8	145.2	140.9
84	177.5	174.6	177.3	168.1	167.4	149.6	142.3	145.3	140.2	129.9
88	178.1	176.6	179.5	178.3	172.2	139.3	152.1	147.2	146.1	130.8
92	183.3	177.0	176.3	178.1	166.1	148.8	153.4	141.1	148.6	139.3
96	184.6	182.1	167.0	175.0	170.3	153.4	152.5	163.3	149.9	139.6
100	174.0	173.2	168.7	173.3	167.2	154.1	153.0	162.5	150.8	137.7
104	165.5	173.9	175.1	168.7	163.4*	155.7	156.3	150.5	148.4	134.3*

Data extracted from Table 5, report HLA 483249 pp. 78-80.
*Significantly different from control ($p \leq 0.05$).

Test article intake (mg/kg) was calculated by multiplying the feed consumed daily (g/day) by the projected compound concentration (mg/kg of feed), divided by the average group mid-period body weight (kg).

GROUP MEAN TEST ARTICLE INTAKE (mg/kg)

	Males					Females				
WEEKS ON TEST |ppm = |	0	10	20	500	2500	0	10	20	500	2500
1.....	0.0	0.84	1.69	42.31	199.42	0.0	1.03	2.01	49.46	252.34
2.....	0.0	0.76	1.52	38.22	197.68	0.0	0.93	1.87	46.17	241.79
3.....	0.0	0.70	1.41	36.00	180.73	0.0	0.89	1.79	45.67	207.93
4.....	0.0	0.68	1.38	34.82	176.98	0.0	0.88	1.80	43.59	227.59
5.....	0.0	0.63	1.28	32.07	161.28	0.0	0.84	1.64	41.97	209.41
6.....	0.0	0.60	1.21	30.53	152.90	0.0	0.81	1.60	40.58	201.07
7.....	0.0	0.59	1.19	30.66	153.34	0.0	0.81	1.59	41.62	204.68
8.....	0.0	0.57	1.15	29.45	147.66	0.0	0.79	1.56	40.27	196.49
9.....	0.0	0.56	1.14	28.51	145.64	0.0	0.75	1.48	38.63	193.43
10.....	0.0	0.56	1.10	27.80	137.97	0.0	0.78	1.51	39.27	191.18
11.....	0.0	0.54	1.10	26.96	137.57	0.0	0.74	1.44	36.77	187.31
12.....	0.0	0.52	1.06	26.31	135.52	0.0	0.72	1.41	35.26	181.25
13.....	0.0	0.50	1.03	24.85	127.27	0.0	0.73	1.41	36.71	188.57
14.....	0.0	0.50	1.01	24.97	129.76	0.0	0.71	1.36	34.68	182.62
15.....	0.0	0.49	0.99	24.70	130.65	0.0	0.70	1.36	34.45	179.65
16.....	0.0	0.49	0.98	24.61	129.71	0.0	0.70	1.34	34.88	183.89
20.....	0.0	0.45	0.91	23.40	123.56	0.0	0.69	1.35	36.21	183.72
24.....	0.0	0.43	0.86	21.77	110.87	0.0	0.63	1.27	31.98	163.41
28.....	0.0	0.42	0.84	21.65	113.93	0.0	0.61	1.16	30.16	166.97
32.....	0.0	0.42	0.85	21.87	111.02	0.0	0.59	1.11	29.71	161.62
36.....	0.0	0.40	0.78	19.81	100.89	0.0	0.57	1.16	30.28	155.91
40.....	0.0	0.38	0.73	18.60	93.81	0.0	0.53	1.06	27.97	151.24
44.....	0.0	0.37	0.70	18.43	94.71	0.0	0.53	1.05	27.39	147.39
48.....	0.0	0.37	0.72	18.86	96.13	0.0	0.53	1.04	27.13	147.11
52.....	0.0	0.34	0.68	17.42	90.80	0.0	0.46	0.91	23.48	130.63
56.....	0.0	0.36	0.73	19.04	95.70	0.0	0.50	0.96	26.17	145.24
60.....	0.0	0.38	0.76	19.05	101.37	0.0	0.51	1.01	25.90	153.94
64.....	0.0	0.42	0.83	21.78	114.12	0.0	0.60	1.21	30.42	168.09
68.....	0.0	0.38	0.74	18.89	100.13	0.0	0.45	0.99	26.20	151.70
72.....	0.0	0.37	0.76	19.31	101.28	0.0	0.52	1.01	26.20	150.73
76.....	0.0	0.38	0.77	19.35	101.77	0.0	0.52	1.02	27.11	154.32
80.....	0.0	0.37	0.76	19.46	101.30	0.0	0.50	0.95	25.26	153.85
84.....	0.0	0.38	0.77	18.45	97.52	0.0	0.46	0.93	24.35	139.73
88.....	0.0	0.39	0.80	19.37	100.64	0.0	0.49	0.96	25.46	143.97
92.....	0.0	0.40	0.79	19.40	98.64	0.0	0.50	0.93	26.17	150.74
96.....	0.0	0.42	0.76	19.29	103.22	0.0	0.49	1.05	26.13	152.03
100.....	0.0	0.41	0.79	19.37	103.34	0.0	0.50	1.06	26.27	148.02
104.....	0.0	0.41	0.82	19.36	104.07	0.0	0.50	0.95	26.03	147.99
Overall Mean	0.0	0.48	0.96	24.12	123.76	0.0	0.64	1.27	32.79	169.67

Data extracted from Table 6, report HLA 483249 pp. 81-84.

4. Ophthalmological examination

The ophthalmology report did not address or provide the results of the examination performed prior to treatment. The data does not indicate the presence of treatment related effects (Appendix 2).

5. Viral Serology

The following screens were performed on 10 randomly selected animals/sex prior to assignment of permanent animals numbers.

Pneumonia Virus of Mice (Enzyme-Linked Immunosorbent Assay)
Reovirus Type 3 (Enzyme-Linked Immunosorbent Assay)
Theiler's Virus (Indirect Fluorescent Antibody)
Sendai Virus (Enzyme-Linked Immunosorbent Assay)
Lymphocytic Choriomeningitis (Indirect Fluorescent Antibody)
Killian's Rat Virus (Enzyme-Linked Immunosorbent Assay)
Toolan's H-1 Virus (Enzyme-Linked Immunosorbent Assay)
Sialodacryoadenitis (Enzyme-Linked Immunosorbent Assay)
Mycoplasma Pulmonis (Enzyme-Linked Immunosorbent Assay)

6. Hematology and Clinical Chemistry

Blood was collected before treatment (10 animals/sex from animals not assigned to study) and 20 animals/sex/group at weeks 27, 52, 78, and 104 of treatment by orbital sinus puncture from animals which had been fasted overnight. The CHECKED (X) parameters were examined. A differential leukocyte count and examination of cell morphology was performed for control and high-dose animals.

a. Hematology

X		X	
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	Corrected Leukocyte Count (COR WBC)		
X	Erythrocyte count (RBC)*	X	Mean corpusc. volume (MCV)
X	Platelet count*		Reticulocyte count
	Blood clotting measurements		
	(Thromboplastin time)		
	(Clotting time)		
	(Prothrombin time)		

* Required for subchronic and chronic studies

NOTE: The differential leukocyte count and examination of cell morphology was performed only for the control and the HDT.

Results

The hematology data revealed significant increases in the MCH of

males at 2500 ppm at weeks 28 and 53. The MCHC was significantly increased in both sexes at 2500 ppm weeks 28, 53, and 79. Platelets were decreased nonsignificantly in females at 2500 ppm and significantly in males at 2500 ppm weeks 28, 53, 79, and 105. Males receiving 500 ppm exhibited significant reductions in platelets weeks 28 and 53. MCV was significantly reduced in the 2500 ppm group of females weeks 28, 53, and 79. In the 2500 ppm males this parameter was significantly reduced weeks 53 and 79. MCV was also significantly reduced in the 10 and 500 ppm males at week 79. Hemoglobin was significantly reduced in the 2500 ppm females weeks 28 and 53. Significant reductions in the hematocrit were observed in 2500 ppm males at week 53, and 2500 ppm females at weeks 28, 53, and 105. Significant reductions in WBC count were observed at 2500 ppm at week 105 for both sexes.

Male Hematology Data

Parameter	Week 28					Week 53					Week 79					Week 105				
	0	10	20	500	2500	0	10	20	500	2500	0	10	20	500	2500	0	10	20	500	2500
MCH - PG	18.5	18.5	18.6	18.7	19.2*	19.9	19.7	19.6	19.8	20.9*	20.1	20.2	20.3	20.4	21.2	18.2	18.5	18.0	18.1	17.8
MCHC - g/dL	34.8	35.0	34.8	35.0	36.0*	36.5	36.2	36.5	37.0	39.7*	35.4	36.9	36.8	37.5	39.5*	34.2	34.5	34.2	34.4	35.1
Lymph. TH/µL	7.1	-	-	-	6.9	6.7	-	-	-	6.5	6.0	-	-	-	5.9	5.8	-	-	-	4.0*
Platelet TH/µL	1144	1089	1160	1040*	943*	1123	1061	1063	994*	855*	1124	1066	1077	1017	872*	1512	1328	1409	1419	1222*
Seg TH/µL	2.2	-	-	-	3.3	2.8	-	-	-	3.2	5.2	-	-	-	4.8	6.5	-	-	-	4.7*
Mono TH/µL	0.2	-	-	-	0.1	0.2	-	-	-	0.2	0.4	-	-	-	0.3	0.3	-	-	-	0.2
Eosin. TH/µL	0.2	-	-	-	0.2	0.2	-	-	-	0.2	0.1	-	-	-	0.1	0.1	-	-	-	0.1
Baso TH/µL	0.0	-	-	-	0.0	0.0	-	-	-	0.0	0.0	-	-	-	0.0	0.0	-	-	-	0.0
MCV - FL	53.2	52.7	53.5	53.4	53.2	54.4	54.3	53.7	53.6	52.6*	56.9	54.6*	55.1	54.2*	53.6*	53.3	53.9	52.8	52.7	50.6
RBC - MI/µL	7.80	8.09	8.33	7.86	7.36	7.44	7.70	7.87	7.63	6.92	6.88	7.51	7.45	7.06	6.79	6.92	7.67	7.46	7.60	7.75
HGB g/dL	14.4	14.9	15.5*	14.7	14.1	14.7	15.1	15.4	15.1	14.3	13.7	15.1	15.1	14.3	14.2	12.5	14.2	13.3	13.6	13.7
HCT %	41.6	42.7	44.7*	42.1	39.1	40.5	41.8	42.3	41.0	36.4*	38.8	41.0	41.1	38.3	36.4	36.5	40.9	38.7	39.6	39.0
WBC TH/µL	9.7	10.1	10.0	10.8	10.5	9.9	9.9	10.1	11.0	10.1	11.8	11.4	11.2	11.9	11.2	12.8	11.9	12.1	10.3	9.0*
Cor WBC - TH/µL	9.7	-	-	-	10.5	9.9	-	-	-	10.1	11.8	-	-	-	11.2	12.8	-	-	-	9.0*

Data extracted from Table 7 report HLA 483249 pp. 85-104.

* Significantly different from control ($p \leq 0.05$).

Female Hematology Data

Parameter	Week 28					Week 53					Week 79					Week 105				
	0	10	20	500	2500	0	10	20	500	2500	0	10	20	500	2500	0	10	20		2500
MCH - PG	19.5	19.3	19.2	19.4	19.7	20.3	20.5	20.6	20.6	21.0	21.1	21.2	21.6	21.4	21.3	19.7	19.6	20.0	19.6	19.1
MCHC - g/dL	34.1	33.6	34.2	34.1	35,9*	35.1	35.4	35.6	35.7	38.0*	34.9	35.0	35.7	35.9	37.6*	34.4	34.6	34.4	34.5	34.6
Lymph. TH/µL	5.6	-	-	-	6.0	4.8	-	-	-	5.1	3.9	-	-	-	4.2	3.7	-	-	-	2.9
Platelet TH/µL	1051	1052	1055	1001	987	944	910	949	878	833	939	869	921	873	841	1113	1105	1038	1043	1024
Seg TH/µL	1.3	-	-	-	1.5	2.0	-	-	-	1.7	4.2	-			2.9	4.7	-	-	-	2.4*
Mono TH/µL	0.1	-	-	-	0.0	0.1	-	-	-	0.1	0.3	-	-	-	0.3	0.2	-	-	-	0.1
Eosin. TH/µL	0.2	-	-	-	0.2	0.2	-	-	-	0.2	0.1	-	-	-	0.1	0.0	-	-	-	0.1
Baso TH/µL	0.0	-	-	-	0.0	0.0	-		-	0.0	0.0	-	-	-	0.0	0.0	-	-	-	0.0
MCV - FL	57.1	57.4	56.2	56.8	54.8*	57.7	57.7	57.8	57.7	55.2*	60.4	60.6	60.6	59.5	56.7*	57.4	56.8	58.3	56.7	55.3
RBC - MI/µL	7.90	7.87	7.63	7.55	7.16*	7.45	7.33	7.33	7.20	6.73*	6.72	7.01	6.66		6.64	7.43	7.16	7.27	7.38	7.07
HGB g/dL	15.3	15.2		14.6*	14.1*	15.1	15.0	15.1	14.8	14.1*	14.0	14.7	14.3	14.7	14.0	14.6	14.0	14.5	14.4	13.4
HCT %	45.2	45.2		42.9	39.3*	43.0	42.4	42.5	41.5	37.2*	40.3	42.1	40.1	41.2	37.6	42.4	40.5	42.2	41.8	38.8*
WBC TH/µL	7.1	7.2	7.9	6.4	7.8	7.1	6.7	7.8	6.0	7.1	8.7	7.5	8.9	6.5	7.6	8.5	7.5	8.6	6.2	5.4*
Cor WBC - TH/µL	7.1	-	-	-	7.8	7.1	-	-	-	7.1	8.7	-	-	-	7.6	8.5	-	-	-	5.4*

Data extracted from Table 7 report HLA 483249 pp. 85-104.

* Significantly different from control ($p \leq 0.05$).

b. Clinical Chemistry

X		X	
	Electrolytes:		Other:
X	Calcium*	X	Albumin*
X	Chloride*		Blood creatinine*
	Magnesium*	X	Blood urea nitrogen*
X	Phosphorous*	X	Total Cholesterol*
X	Potassium*	X	Globulins
X	Sodium*	X	Glucose*
	Enzymes	X	Total bilirubin
	Alkaline phosphatase (ALK)	X	Total serum Protein (TP)*
	Cholinesterase (ChE)#		Triglycerides
X	Creatinine phosphokinase*^		Serum protein electrophoresis
	Lactic acid dehydrogenase (LAD)		
X	Serum alanine aminotransferase (also SGPT)*		
X	Serum aspartate aminotransferase (also SGOT)*		
X	Gamma glutamyl transferase (GGT)		
	Glutamate dehydrogenase		

* Required for subchronic and chronic studies
# Should be required for OP
^ Not required for subchronic studies

RESULTS

No treatment related alterations were observed in the electrolytes. SGPT was significantly increased in males at 500 and 2500 ppm at week 53. Total cholesterol was significantly increased in 2500 ppm males at weeks 28 and 105 and females of the same group at week 28. Glucose was significantly decreased in both sexes receiving 2500 ppm at week 28. Total bilirubin was significantly decreased in 2500 ppm males at week 28 and females at weeks 28, 53, and 79. Albumin was significantly increased at 2500 ppm in females at week 28, and in males at all time periods. Globulins were significantly decreased in males receiving 2500 ppm weeks 53, 79, and 105. The A/G ratio of males receiving 2500 ppm was significantly increased at every interval.

6. Urinalysis

Urine was collected during the overnight fast in individual urine collection cages at the same intervals that hematology was evaluated. The CHECKED (X) parameters were examined.

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

^Not required for subchronic studies
* Required for chronic studies

RESULTS- There were no treatment related changes detected in the urinalysis data.

Male Biochemistry Data

Parameter	Week 28					Week 53					Week 79					Week 105				
	0	10	20	500	2500	0	10	20	500	2500	0	10	20	500	2500	0	10	20	500	2500
T Cholest mg/dL	97	93	102	112	119*	122	117	118	123	129	125	148	137	129	153	119	128	128	117	176*
BUN mg/dL	15	15	15	16	14	14	14	14	14	14	14	13	13	12	13	26	15	26	15	19
Glucose mg/dL	124	119	114	115	109*	114	109	109	108	107	104	105	99	99	97	77	78	79	85	84
Tot Bili mg/dL	0.2	0.1	0.2	0.1	0.1*	0.2	0.2	0.2	0.2	0.1	0.1	0.1	0.1	0.0	0.1	0.1	0.1	0.1	0.1	0.1
Tot Prot g/dL	6.7	6.7	7.0	6.8	6.7	6.8	6.9	7.0	6.9	6.7	6.6	6.9	7.0*	6.7	6.6	6.8	6.9	7.0	6.8	7.1
Albumin g/dL	3.8	3.8	3.9	3.9	4.0*	3.7	3.7	3.7	3.7	3.9*	3.3	3.5	3.4	3.4	3.5*	3.8	4.1	4.0	3.9	4.6*
Globulin g/dL	2.9	2.9	3.1	2.9	2.7	3.1	3.1	3.2	3.1	2.8*	3.4	3.4	3.5	3.3	3.0*	3.0	2.8	3.0	2.9	2.5*
CPK u/L	194	191	210	196	191	241	190	199	221	178	180	175	233	229	172	472	432	436	461	331
SGOT u/L	71	71	72	94	95	78	74	70	93	103	73	79	88	82	114	152	123	121	136	105
SGPT u/L	30	32	29	59	52	33	34	31	47*	71*	30	36	38	39	81	40	43	40	50	42
ALB/GLOB ratio	1.30	1.31	1.29	1.35	1.48*	1.18	1.23	1.17	1.20	1.39*	0.98	1.02	0.97	1.04	1.18*	1.28	1.48	1.43	1.44	1.90*

Data extracted from report HLA 483249 Table 8 pp. 105- 122.

‡ Significantly different from control ($p \leq .05$).

Female Biochemistry Data

Parameter	Week 28					Week 53					Week 79					Week 105				
	0	10	20	500	2500	0	10	20	500	2500	0	10	20	500	2500	0	10	20	500	2500
T Cholest mg/dL	114	109	122	129	146*	127	111	126	121	146	123	117	118	127	147	146	133	134	145	166
BUN mg/dL	15	16	15	15	16	14	15	15	15	17*	14	14	13	13	16	19	15	14	17	15
Glucose mg/dL	111	113	112	109	102*	97	101	104	97	92	89	95	95	97	91	79	81	81	80	84
Tot Bili mg/dL	0.1	0.1	0.1	0.1	0.0*	0.3	0.3	0.3	0.2	0.1*	0.1	0.1	0.1	0.1	0.0*	0.1	0.1	0.1	0.1	0.1
Tot Prot g/dL	7.2	7.3	7.4	7.5	7.6	7.7	7.6	7.8	7.6	7.8	7.2	7.4	7.3	7.4	7.6	7.4	7.5	7.5	7.4	7.7
Albumin g/dL	4.4	4.5	4.5	4.7	4.7*	4.6	4.6	4.7	4.7	4.8	3.9	4.0	4.1	4.1	4.2	5.0	4.8	4.8	5.2	5.3
Globulin g/dL	2.8	2.8	2.8	2.9	2.8	3.1	3.0	3.1	3.0	3.0	3.3	3.3	3.2	3.3	3.4	2.4	2.7	2.7	2.2	2.3
CPK u/L	149	120	127	117	101	153	126	160	145	162	123	154	125	131	118	312	348	335	320	269
SGOT u/L	111	80	88	87	64	82	68	71	68	71	83	82	78	68	84	163	111	116	135	108
SGPT u/L	71	46	51	42*	29*	41	35	33	30	28*	34	39	34	32	33	58	36	35	50	38
ALB/GLOB ratio	1.59	1.60	1.63	1.63	1.68	1.49	1.57	1.52	1.58	1.61	1.25	1.24	1.30	1.27	1.28	2.28	1.87	1.99	2.56	2.59

Data extracted from report HLA 483249 Table 8 pp. 105- 122.

‡ Significantly different from control ($p \leq .05$).

Biochemistry Data

Parameter	Week 28					Week 53					Week 79					Week 105				
	0	10	20	500	2500	0	10	20	500	2500	0	10	20	500	2500	0	10	20	500	2500
Males																				
Ca mg/dL	10.7	10.7	10.9	10.9	10.8	10.7	10.8	10.8	10.8	10.7	10.6	10.9	10.8	10.7	10.6	10.2	10.1	10.2	9.8	10.3
Cl mmol/L	107.0	107.0	107.0	106.9	107.3	105.8	106.4	105.9	106.2	106.9	107.6	108.0	107.3	108.0	108.6	104	104	104	105	104
P mg/dL	7.3	7.2	7.6	7.5	7.4	6.7	6.6	6.7	6.8	6.7	6.5	6.6	6.4	6.5	6.4	7.0	6.3	6.8	6.1	6.5
K mmol/L	5.85	5.83	5.98	5.86	5.71	6.27	6.14	6.24	6.21	5.97	5.91	5.92	6.04	6.07	5.63	6.2	5.9	6.1	6.0	5.7
Na mmol/L	144.1	143.5	144.2	144.2	143.1	145.7	145.3	145.3	145.5	145.0	145.7	145.6	145.5	145.4	145.4	148	147	148	148	148
Females																				
Ca mg/dL	11.0	11.2	11.3	11.2	11.2	11.3	11.4	11.3	11.3	11.1	11.2	11.1	11.1	11.3	11.1	10.5	10.4	10.4	10.6	10.5
Cl mmol/L	106.6	106.7	107.1	107.7	107.7	103.9	103.6	103.7	104.5	105.1	105.5	105.8	105.2	106.1	107.3	101	101	100	101	101
P mg/dL	6.6	6.5	6.6	6.3	6.8	5.9	5.7	5.9	5.9	6.2	6.2	6.3	6.1	6.2	6.8	6.0	5.7	5.8	5.5	5.7
K mmol/L	5.39	5.29	5.37	5.33	5.50	5.22	5.09	5.32	5.21	5.41	5.04	5.10	5.05	5.34	5.45	4.9	5.1	5.0	4.9	5.0
Na mmol/L	142.1	142.7	142.3	142.8	142.7	143.3	142.7	143.1	143.1	143.8	143.2	143.2	143.5	143.2	144.0	147	147	146	147	146

Data extracted from report HLA 483249 Table 8 pp. 105-122.

Terminal Organ Weight Data

Organ	Absolute Weight									
	Males					Females				
	0	10	20	500	2500	0	10	20	500	2500
Final Bdy Wt (g)	576.5	578.4	594.6	599.8	545.1	401.6	415.5	433.3	390.3	313.2*
Brain (g)	2.31	2.27	2.45	2.28	2.24	2.06	2.03	2.05	2.07	2.05
Heart (g)	1.90	1.89	1.93	1.84	1.80	1.36	1.37	1.50	1.33	1.30
Adrenal (g)	.074	.079	.084	.082	.067	.129	.159	.104	.121	.123
Kidney (g)	4.43	4.13	4.16	3.80	4.24	3.09	2.60	2.72	2.69	2.65
Liver (g)	14.83	15.68	14.02	14.78	16.26	11.82	9.75	10.28	10.91	12.53
Pituitary (g)	.060	.025	.025	.029	.045	.060	.131	.134	.114	.071
Spleen (g)	1.09	1.03	0.98	0.96	0.92	0.68	0.81	0.62	0.63	0.66
Testes/Epidid Ovary (g)	4.89	4.98	4.57	5.19	4.88	.100	.123	.101	.185	.189*

Organ	Organ Weight as Percent of BW									
	Males					Females				
	0	10	20	500	2500	0	10	20	500	2500
Brain	.403	.364	.431	.369	.426	.448	.506	.490	.593	.614*
Heart	.333	.300	.345	.298	.343	.294	.339	.360	.362	.384
Adrenal	.0131	.0127	.0157	.0133	.0129	.0275	.0414	.0250	.0348	.0350
Kidney	.815	.661	.761	.614	.818	.678	.652	.652	.749	.778
Liver	2.630	2.499	2.517	2.374	3.091	2.532	2.408	2.436	2.881	3.625*
Pituitary	.0137	.0039	.0045	.0050	.0089	.0141	.0375	.0327	.0418	.0263
Spleen	.185	.163	.174	.155	.175	.148	.204	.147	.166	.188
Testes/Epidid Ovary	.822	.803	.792	.835	.929	.0217	.0311*	.0242	.0463*	.0508*

Data extracted from report HLA 483249 Tables 9C and 9F, pp. 130-131 and 139-140.

* Significantly different from control ($p \leq .05$).

7. Euthanasia and Pathology

Necropsies were performed on all animals that died during the study or were euthanized in a moribund state. An interim sacrifice was performed on 10 animals/sex/dose at week 52. Ten more animals/sex/dose were selected as recovery animals that were placed on basal diet weeks 53-56 prior to sacrifice and necropsy. Animals scheduled for euthanasia were exsanguinated under sodium pentobarbital anesthesia. The CHECKED (X) tissues were collected for histological examination. The (XX) organs, i addition, were weighed (paired organs were weighed as pairs).

X		X		X	
Digestive system		Cardiovasc./Hemat.		Neurologic	
	Tongue	X	Aorta*	XX	Brain*+
X	Salivary glands*	XX	Heart*	X	Periph. nerve*#
X	Esophagus*	X	Bone marrow*	X	Spinal cord (3 levels
X	Stomach*	X	Lymph nodes*	XX	Pituitary*
X	Duodenum*	XX	Spleen	X	Eyes *#
X	Jejunum*	X	Thymus*	Glandular	
X	Ileum*	Urogenital		XX	Adrenal gland*
X	Cecum*	XX	Kidneys*+		Lacrimal gland#
X	Colon*	X	Urinary bladder*	X	Mammary gland (♀)*#
X	Rectum*	XX	Testes*+	X	Parathyroids*++
XX	Liver *+	XX	Epididymides	X	Thyroids*++
	Gall bladder*	X	Prostate	Other	
X	Pancreas*	X	Seminal vesicle		Bone*#
Respiratory		XX	Ovaries*+	X	Skeletal muscle*#
X	Trachea*	X	Uterus*	X	Skin*#
X	Lung*	X	Vagina	X	All gross lesions and masses*
	Nose^				
	Pharynx^				
	Larynx^				

* Required for subchronic and chronic studies.
^ Required for chronic inhalation.
# In subchronic studies, examined only if indicated by signs of toxicity or target organ involvement.
+ Organ weight required in subchronic and chronic studies.
++ Organ weight required for non-rodent studies.

Results

Week 53

The terminal (week 53) body weight of animals of both sexes receiving 2500 ppm was significantly reduced as compared to the control animals. The absolute adrenal weight of male animals receiving 2500 ppm was significantly reduced (23.5%) relative to the control. A non-significant reduction (26.9%) was observed in the females of this group relative to the control. The lack of significance was apparently due to a higher standard deviation in the female control group.

The adrenal organ to terminal body weight ratio (%) was not

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significantly affected in either sex of any of the treatment groups. However, the liver to body weight ratio was significantly increased in both sexes of the HDT. The females receiving 2500 ppm also had significant increases in the relative weights of the heart and kidneys.

Week 57

The recovery animals which were euthanized at week 57 did not have significant reductions in terminal body weight at the 2500 ppm level. However, the absolute weight of the spleen of females at the HDT was significantly reduced (18%). The mean absolute adrenal weight of the males at the HDT was non-significantly increased 96% whereas the mean absolute adrenal weight of the females was non-significantly reduced by 22.8% compared to their respective controls.

No statistically significant alterations were detected in the relative organ weights of the recovery animals.

Week 104

The terminal body weight of the females receiving 2500 ppm was significantly reduced. Mean absolute ovarian weight of this group was significantly increased (89%). No other significant alterations in absolute organ weight were reported.

Organ Weight

Statistical analyses of the organ to terminal (week 104) body weight ratios detected significant increases in brain (37.1%), liver (43.3%), and ovarian (134.1%) weights of females at the HDT which may be due to decreased body weight. Relative ovarian weight was also significantly increased at 10 (43.3%) and 500 (113.4%) ppm.

Gross pathology

Evaluation of the gross pathology data for unscheduled deaths did not reveal any treatment related gross tissue alterations which could be attributed to CGA 169374.

Microscopic pathology

The most outstanding microscopic observation in this study which appeared to be related to treatment was hepatocytic hypertrophy at 500 and 2500 ppm in both sexes. The observation was characterized by the presence of lightly stained enlarged hepatocytes with poorly distinguishable cell borders. This finding varied from centrilobular to diffuse to focal/or multifocal.

Incidence of Hepatocytic Hypertrophy

Observation	Males					Females				
	0	10	20	500	2500	0	10	20	500	2500
Total No. Examined	70	70	70	70	70	70	70	70	70	70
UNSCHEDULED DEATHS	0	0	0	1	0	0	0	0	3	4
WEEK 53 Interim Sacrifice	0	0	0	0	0	0	0	0	0	0
WEEK 57 Recovery Sacrifice	0	-	-	-	0	0	-	-	-	0
TERMINAL SACRIFICE	7	5	8	28	39	4	0	0	14	32
OVERALL No. (%)	7 (10%)	5 (7%)	8 (11%)	29 (41%)	39 (56%)	4 (6%)	0 (0%)	0 (0%)	17 (24%)	36 (51%)
SEVERITY 0 (Finding Absent)	63	65	62	41	31	66	70	70	53	34
1 (Minimal)	7	4	5	25	7	4	0	0	11	19
2 (Slight)	0	1	3	3	27	0	0	0	6	10
3 (Moderate)	0	0	0	1	5	0	0	0	0	7
TOTAL	70	70	70	70	70	70	70	70	70	70
MEAN	0.1	0.1	0.2	0.5	1.1	0.1	0.0	0.0	0.3	0.9

Data extracted from Report HLA 483249 text table 2 p. 38 and Tables 11A-D pp. 207-279.

D. DISCUSSION:

Based on the available data, it would appear that the target organ for chronic toxicity of CGA 169374 in the rat is the liver. The pathology report indicates hepatocytic hypertrophy was observed in groups receiving 500 and 2500 ppm at incidences greater than the concurrent control. Absolute liver organ weight was nonsignificantly increased at 2500 ppm in both sexes. Liver weight as a percent of body weight was increased (significant for females and nonsignificant for males) at 2500 ppm.

Additional support to the theory that the liver is the target organ for CGA 169374 toxicity at 2500 ppm is provided by the observed: increase in total cholesterol (♂ and ♀), decrease in glucose (♂ and ♀), decrease in total bilirubin (♂ and ♀), increase in albumin (♂ and ♀), decrease in globulin (♂), increase in A/G ratio (♂), and increase in SGPT (♂ at 500 and 2500 ppm).

E. Study Deficiencies:

1. The analytical chemistry report does not contain data pertaining to the purity of the test material.

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2. The analytical report does not contain the raw data for stability, homogeneity, or concentration analyses.

3. Bone was not examined histologically. Thyroid/parathyroid was not weighed.

4. Blood creatinine was not measured.

F. Core Classification: **Core Supplementary**

This study does not satisfy the guideline requirements for a chronic rat study due to deficiencies in the analytical data, namely the raw data pertaining to the purity of the test material, the stability, homogeneity, and concentration analyses. The study may be upgraded upon satisfactory review of the Registrant's response to these deficiencies.

NOEL = 20 ppm
LOEL = 500 ppm

Page_____ is not included in this copy.

Pages 88 through 100 are not included.

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_____ 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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X FIFRA registration data.

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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.

Table 11E
Histopathology Incidence Summary
Neoplastic Findings
Combined Chronic Toxicity and Oncogenicity Study of
CGA-169374 Technical in Rats

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Pages 102 through 117 are not included.

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

MAR 30 1994

MEMORANDUM

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

SUBJECT: Dividend (Difenoconazole) Qualitative Risk Assessment Based On Charles River CD-1 Mouse Dietary Study

Caswell No. 955

TO: Jess Rowland, Acting Section Head
Review Section IV
Toxicology Branch II
Health Effects Division (7509C)

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

Lori L. Brunsman 3/30/'94

THROUGH: Hugh M. Pettigrew, Section Head
Statistics Section
Science Analysis Branch
Health Effects Division (7509C)

Hugh M. Pettigrew 3/30/94

Summary

This qualitative risk assessment of Dividend (Difenoconazole) was based upon a chronic carcinogenicity feeding study conducted in Charles River CD-1 mice. The study design specified doses of 0, 10, 30, 300, 3000, or 4500 ppm of Dividend for 78 weeks. The males received actual doses of 0, 1.51, 4.65, 46.29, 423.16, or 818.87 mg/kg/day. All 4500 ppm females died or were sacrificed *in extremis* within the first 2 weeks of the study. The remaining female dose groups received actual doses of 0, 1.90, 5.63, 57.79, or 512.61 mg/kg/day.

The statistical evaluation of mortality indicated a significant increasing trend in mortality with increasing doses of Dividend in male mice. Female mice showed no significant incremental changes in mortality with increasing doses of Dividend.

Male mice had significant dose-related increasing trends in hepatocellular adenomas, carcinomas, and combined adenomas and/or carcinomas. There was a significant difference in the pair-wise comparison of the 46.29 mg/kg/day dose group with the controls for

hepatocellular adenomas. There were significant differences in the pair-wise comparisons of the 423.16 mg/kg/day dose group with the controls for hepatocellular adenomas and combined adenomas and/or carcinomas. There were significant differences in the pair-wise comparisons of the 818.87 mg/kg/day dose group with the controls for hepatocellular adenomas, carcinomas, and combined adenomas and/or carcinomas.

Female mice had significant dose-related increasing trends, and significant differences in the pair-wise comparisons of the 512.61 mg/kg/day dose group with the controls, for hepatocellular adenomas, carcinomas, and combined adenomas and/or carcinomas.

Background

A chronic carcinogenicity feeding study in Charles River CD-1 mice was conducted by Hazleton Laboratories America, Incorporated, Vienna, Virginia, for the Agricultural Division of Ciba-Geigy Corporation, Greensboro, North Carolina, and issued April 3, 1989 (Study No. 483-250; MRID No. 420900-15).

The study design allocated groups of 50 mice per sex to dose levels of 0, 10, 30, 300, 3000, and 4500 ppm of Dividend for 78 weeks. An additional 10 mice per sex per dose were designated for interim sacrifice at week 53. Ten more mice per sex in the control, 3000 and 4500 ppm dose groups were designated as post-recovery animals to be dosed until week 53, then sacrificed at week 57. All 70 of the 4500 ppm females, and 15 of the 70 total 3000 ppm females, died or were sacrificed *in extremis* within the first 2 weeks of the study. The 10 control group animals originally designated as post-recovery animals for sacrifice at week 57 were removed from the control group at week 2 and placed in the 3000 ppm dose group. These 10 replaced 10 of the 15 that died or were sacrificed in extremis within the first 2 weeks of the study in the 3000 ppm dose group and were dosed an additional 2 weeks at the end of the study to allow dosing of all animals on the study a total of 78 weeks. At week 3, the 3000 ppm dose was reduced to 2500 ppm for both males and females for the remainder of the study. The males received actual doses of 0, 1.51, 4.65, 46.29, 423.16, or 818.87 mg/kg/day. The remaining female dose groups received actual doses of 0, 1.90, 5.63, 57.79, or 512.61 mg/kg/day.

Survival Analysis

The statistical evaluation of mortality indicated a significant increasing trend in mortality with increasing doses of Dividend in male mice. Female mice showed no significant incremental changes in mortality with increasing doses of Dividend. See Tables 1 and 2 for mortality test results.

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

Tumor Analysis

Male mice had significant increasing trends in hepatocellular adenomas, carcinomas, and combined adenomas and/or carcinomas, all at $p < 0.01$. There was a significant difference in the pair-wise comparison of the 46.29 mg/kg/day dose group with the controls for hepatocellular adenomas at $p < 0.05$. There were significant differences in the pair-wise comparisons of the 423.16 mg/kg/day dose group with the controls for hepatocellular adenomas and combined adenomas and/or carcinomas, both at $p < 0.05$. There were significant differences in the pair-wise comparisons of the 818.87 mg/kg/day dose group with the controls for hepatocellular adenomas, carcinomas, and combined adenomas and/or carcinomas, all at $p < 0.01$.

These statistical analyses were based upon Peto's prevalence test since there was a statistically significant positive trend for mortality in male mice with increasing doses of Dividend. See Table 3 for male mouse tumor analysis results.

Female mice had significant dose-related increasing trends in hepatocellular adenomas, carcinomas, and combined adenomas and/or carcinomas, all at $p < 0.01$. There were significant differences in the pair-wise comparisons of the 512.61 mg/kg/day dose group with the controls for hepatocellular adenomas ($p < 0.01$), carcinomas ($p < 0.05$), and combined adenomas and/or carcinomas ($p < 0.01$).

These statistical analyses were based upon the Exact trend test since there were small numbers of tumors observed in selected instances. The Fisher's Exact test was used for pair-wise comparisons. See Table 4 for female mouse tumor analysis results.

Table 1. Dividend - Charles River CD-1 Mouse Study

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

Dose (mg/kg/day)	Weeks 1-52	53[i]	53-56	57[i]	57-80[f]	Total
0	2/70	10/68	1/58	9/57	17/48	20/51 (39)**
1.51	2/60	10/58	0/48	0/48	16/48	18/50 (36)
4.65	2/60	10/58	3/48	0/45	18/45	23/50 (46)
46.29	4/60	10/56	0/46	0/46	22/46	26/50 (52)
423.16	0/70	10/70	0/60	10/60	16/50	16/50 (32)
818.87	13[#]/69[a]	10/56	0/46	10/46	20/36	33/49 (67)**

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

[#]All 13 of these animals died within the first 25 weeks of the study; 11 of the 13 died before week 4.

[a]One accidental death at week 39, dose 818.87 mg/kg/day.

[i]Interim sacrifices at weeks 53 and 57.

[f]Final sacrifice at week 79.

( )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 2. Dividend - Charles River CD-1 Mouse Study

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

Dose (mg/kg/day)	Weeks 1-52	53[i]	53-56	57[i]	57-81[f]	Total
0	2/59[a]	10/57	0/47	0/47	23/47	25/49 (51)
1.90	2/59[b]	10/57	1/47	0/46	11/46	14/49 (29)*
5.63	3/59[c]	10/56	2/46	0/44	15/44	20/49 (41)
57.79	4/60	10/56	1/46	0/45	10/45	15/50 (30)
512.61	10#/69[d]	10/59	0/49	10/49	10/39	20/49 (41)

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

#All 10 of these animals died within the first 16 weeks of the study; 9 of the 10 died before week 4.

[a]One accidental death at week 6, dose 0 mg/kg/day.

[b]One accidental death at week 51, dose 1.90 mg/kg/day.

[c]One accidental death at week 19, dose 5.63 mg/kg/day.

[d]One accidental death at week 7, dose 512.61 mg/kg/day.

[i]Interim sacrifices at weeks 53 and 57.

[f]Final sacrifice at week 79 for all animals except the 10 animals in the 512.61 mg/kg/day dose group that were originally in the control group which were sacrificed at week 81.

( )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 3. Dividend - Charles River CD-1 Mouse Study

Male Hepatocellular Tumor Rates[+] and
Peto's Prevalence Test Results (p values)

	Dose (mg/kg/day)					
	0	1.51	4.65	46.29	423.16	818.87
Adenomas (%)	4/68 (6)	10/57 (18)	8/58 (14)	9/56 (16)	13[a]/70 (19)	20/56 (36)
p =	0.000**	0.053	0.078	0.035*	0.036*	0.000**
Carcinomas (%)	1/68 (1)	0/57 (0)	1/58 (2)	0/56 (0)	5/70 (7)	13[b]/56 (23)
p =	0.000**	-	0.546	-	0.093	0.000**
Combined (%)	5/68 (7)	10/57 (18)	9/58 (16)	9/56 (16)	16/70 (23)	28/56 (50)
p =	0.000**	0.114	0.128	0.061	0.023*	0.000**

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

[a]First adenoma observed at week 53, dose 423.16 mg/kg/day.

[b]First carcinoma observed at week 53, dose 818.87 mg/kg/day.

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. Dividend - Charles River CD-1 Mouse Study

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

	Dose (mg/kg/day)				
	0	1.90	5.63	57.79	512.61
Adenomas (%)	0/57 (0)	0/56 (0)	0/56 (0)	1/56 (2)	16[a]/59 (27)
p =	0.000**	1.000	1.000	0.496	0.000**
Carcinomas (%)	0/47 (0)	0/45 (0)	1/44 (2)	0/45 (0)	4[b]/39 (10)
p =	0.002**	1.000	0.484	1.000	0.039*
Combined (%)	0/57 (0)	0/56 (0)	1/56 (2)	1/56 (2)	17/59 (29)
p =	0.000**	1.000	0.496	0.496	0.000**

[+]Number of tumor bearing animals/Number of animals examined, excluding those that died or were sacrificed before week 53 for adenomas and combined, and before week 58 for carcinomas.

[a]First adenoma observed at week 53, dose 512.61 mg/kg/day.

[b]First carcinoma observed at week 72, dose 512.61 mg/kg/day.

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 $p < 0.01$.

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Reviewed by: Dan W. Hanke, Ph. D. Dan W Hanke 14 April 1992
Section III, Tox. Branch II (H7509C)
Secondary Reviewer: James N. Rowe, Ph. D. James N. Rowe 4/22/92
Section III, Tox. Branch II (H7509C)

DATA EVALUATION RECORD

STUDY TYPE: 13-Week Oral Feeding Study (§82-1)

TOX. CHEM. NO. (CASWELL NO.): new chemical

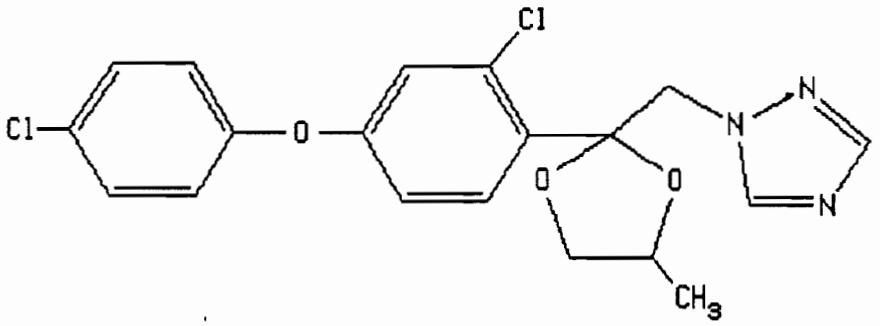
CAS REG. NO.: 119 446-68-3

EPA PESTICIDE CHEMICAL CODE/ACTIVE INGREDIENT CODE (SHANGHNESSY NO.): 128847

HED PROJECT NO.: 2-0696

MRID NO. (ACCESSION NO.): 420900-21

TEST MATERIAL: CGA-169374 Technical; 1-[2-(2-chloro-4-(4-chlorophenoxy)phenyl)-4-methyl-1,3-dioxolan-2-yl)-methyl]-1H-1,2,4-triazole



SYNONYMS: Difenoconazole; Dividend; triazole fungicide

STUDY NUMBER: 483-241

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

TESTING FACILITY: Hazleton Laboratories America, Inc.
9200 Leesburg Turnpike
Vienna, Virginia 22180

TITLE OF REPORT: Subchronic Toxicity/Metabolism Study in Mice

AUTHOR(S): Raymond H. Cox, Ph. D.

REPORT ISSUED: October 20, 1987

SUMMARY AND CONCLUSIONS:
CGA 169374 was offered in feed admixtures to five groups of mice composed of 15 animals/group/sex and 20 mice per sex for controls in dietary concentrations of 20 ppm, 200 ppm, 2500 ppm, 7500 ppm, or 15000 ppm for 13 weeks. Most of the mice fed 7500 ppm or 15,000 ppm test article, groups 5 and 6 respectively, died during the first week on study. There were some CGA 169374-related effects. The statistical analysis of total food consumption and body weight changes over the course of the study showed significantly reduced body weight gain for paired group 4 (2500 ppm) females and a significant negative trend. Compound-related effects from histologic examination were confined to the liver. Hepatotoxicity in mice that DOS was evidenced by hepatocellular enlargement and necrosis of individual hepatocytes. Those mice that survived to the end of the study showed hepatotoxicity that included hepatocellular enlargement in group 4 animals and group 3 males and hepatocytic vacuolization in group 4 animals. Furthermore, coagulative necrosis was observed in the livers of 4/9 group 4 females. This finding, however, was not considered treatment related, because the foci were frequently small and random. The animals in groups 5 and 6, which represent the unscheduled deaths, had a high incidence of changes consistent with stress. The changes included lymphoid depletion or necrosis of the spleen, lymph nodes, and thymus, hypocellularity of the femoral marrow, mucosal erosion/ulceration of the glandular stomach, and in the female mice necrosis of individual cells in the adrenal cortex, specifically in the zona reticularis. Hyperkeratosis of the nonglandular stomach was observed in males especially from group 6. The study director suggests the "stress" effects may be related to inappetence and a failure to eat as opposed to a direct effect of the test article. On the strength of the available data as they relate to the dose levels tested and to the parameters observed, the body weight changes and the liver histopathology form the basis for setting the NOEL at 20 ppm, and the LOEL at 200 ppm. The mortality data indicate the MTD was exceeded and is likely $\leq$ 7500 ppm.

A signed quality assurance statement was present.

Core Classification: Minimum

This study satisfies the guideline requirements (§82-1) for a Subchronic feeding study in mice.

MATERIALS:

1. Test compound. CGA-169374 Technical; difenoconazole; 1-[2-(2-chloro-4-(4-chlorophenoxy)phenyl)-4-methyl-1,3-dioxolan-2-yl)-methyl]-1H-1,2,4-triazole. Description: dark brown solid. Batch #: FL-851406. Purity: 94.5 % run on batch # ACL-5668 (from the rat subchronic/metabolism study); the solvent was dehydrated ethanol. Stability: difenoconazole was stable for at least 24 hrs at room temperature (unspecified) and for 22 days at 6 °C over a concentration range of 0.5 % to 50.0 %. The stability determinations were made on representative samples via gas chromatography equipped with a nitrogen-phosphorus detector. The test article was not detected in the control samples.

2. Test animals.
Species: mouse. Strain: CD-1®(ICR), 144 females and 144 males. Age: At the initiation of treatment mice were approximately seven weeks old. Weight: the range was 17.8 g to 24.3 g for females and 21.3 g to 32.8 g for males. Source: Charles River Laboratories, Inc., Kingston, New York.

METHODS:

1. Animal Dosing/Assignment.
Each test-article dose-group was 15 mice/sex at dose levels of 20 ppm, 200 ppm, 2500 ppm, 7500 ppm, or 15000 ppm. The male and female control groups were 20 mice each. Feed and water were available ad libitum except when fasting for clinical tests and necropsy. The mice were assigned to study groups by first eliminating those with extreme body weights and then by selecting the random assignment which produced homogeneity of both variances and means by Bartlett's test (1937) and one-way ANOVA. The dosing schedule is shown in table 1.

Table 1. Dosing schedule[a]

Group	Number of mice		Dietary level of test article (ppm)
	Male	Female	
1 (Control)	20	20	0[b]
2	15	15	20
3	15	15	200
4	15	15	2,500
5	15	15	7,500
6	15	15	15,000

[a] Data were extracted from p 15 of the study report.

[b] Mice were fed untreated Purina Rodent Diet #5002.

2. Diet Preparation, Dosage Form, and Analytical Chemistry. The test material CGA 169374 was added to Purina Certified Rodent Chow® #5002, which was used as the basal diet, on a weight/weight basis with no adjustment for purity of the test article. The test diets were prepared in two steps. First the test article was melted with the aid of a 70 °C water bath, and then the material was mixed with acetone. The resulting solution was mixed with the basal diet. Dietary administration was used, because the risk of human exposure is via the oral route. Further details of the diet preparation are described in appendix 1 of this DER taken from pp 15-16 of the study report. Routine analyses of the test article were conducted during the study and verified the concentrations of CGA 169374 Technical used on study in the dietary admixtures. Those analyses are summarized in table 2, where the data were taken from p 21 of the study report.

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Table 2. Test concentrations of CGA 169374 Technical

Dose level (ppm)	Percent of Target range Low	High	Mean (SD)
20	91	105	97 (4.3)
200	86	106	98 (6.1)
2500	91	108	101 (5.1)
7500	97	99	98 (1.2)
15000	104	104	104

Data were taken from p 21 of the study report.

3. Statistics.
The cumulative survival data through week 13 were analyzed using the computer software National Cancer Institute Package. Trend analysis of animal survival was evaluated at the 5.0 % one-tailed level of probability. The body weight changes from weeks 0-13, the total food consumption from weeks 1-13, the clinical pathology data with the exception of cell morphology, and organ weight data of the control group were compared statistically to the data from the same sex of the treated groups. The statistical analyses were executed as per the flow chart in appendix 2 of this DER taken from p 20 of the study report. Statistical significance was denoted throughout by the terms "significant" and "trend" where appropriate.

If variances of untransformed data were heterogeneous, then analyses were performed on transformed data to achieve variance homogeneity. When the series of transformations was not successful in achieving variance homogeneity, then analyses were performed on rank-transformed data. Group comparisons were performed routinely at the 5 % two-tailed level of probability unless otherwise specified, or where a trend in the data indicated that a one-tailed test would be more revealing.

RESULTS AND DISCUSSION:

Clinical Signs/Observations.
Clinical signs were generally unremarkable and included hunching, thinness, tremors, languidness, polypnea or hyperpnea (rapid breathing), urine stains, alopecia, squinting, opaque right eye, lacrimation, exophthalmus (bulging eyes), ulcerated right eye, small right eye, and swollen areas. A summary of their incidence may be found in appendix 3 of this DER taken from pp 40-41 of the study report. Additional clinical observations are summarized and discussed in turn below.

Mortality.
With the exception of four mice, all the mice in groups 1 through 4 were alive at the end of the dosing period. Essentially all the mice in groups 5 and 6 were dead within two weeks on study, and no clinical observations were made on those animals. The male mice in groups 5 and 6 that survived until the second week showed thinness, hunched posture, languidness, and tremors. Polypnea was seen in the group 4 females during the first week on study. The clinical observations did not reflect treatment-related effects in group 1, 2, 3, or 4 male mice or in group 1, 2, or 3 female mice. Cumulative mortality (found dead or moribund sacrifice) is shown in table 3 taken from p 23 of the study report.

Table 3. Cumulative Mortality (Found Dead & Moribund Sacrifice)

	Week					
Group/Sex	1	2	3	5	7	10
1 Male	-	-	-	-	1	1
2 Male	-	-	-	-	-	1
3 Female	-	-	-	1	1	1
4 Female	-	-	1	1	1	1
5 Male	3	12	13	13	13	13
5 Female	15	15	15	15	15	15
6 Male	14	15	15	15	15	15
6 Female	15	15	15	15	15	15

The data were taken from p 23 of the study report.

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Body Weight, Food Consumption, and Compound Consumption.
There was a significant reduction in body weight gain for paired group 4 female mice as well as a significant negative trend. Summary data are in appendix 4 of this DER taken from pp 46-48 of the study report. The food consumption data were unremarkable. At the end of the dosing period the mean compound consumption for week 13 in mg/day/kg was 4.37, 41.5, and 558.9 for groups 2, 3, and 4 females or 2.89, 30.76, 383.55 for groups 2, 3, and 4 males. Although there were no significant differences, compound consumption tended to drift down in male mice over the dosing period in groups 2, 3, and 4; whereas in female mice this trend was noticeable only in group 4. There was no discussion of compound consumption in the study report, although the interested reader may read the summary data on compound consumption in table 5 pp 59-62 of the study report.

Ophthalmology.
Ophthalmoscopic findings were unremarkable. There were sporadic incidences of ocular findings noted initially and at week 13, which involved the cornea, lens, and the retina. The findings were not considered to be treatment-related to CGA 169374. Unilateral diffuse retinochoroidal degeneration occurred in one group 4 male and in one group 4 female at the week 13 examination. Again, the low incidence and unilateral occurrence were not considered suggestive of a compound effect. The study report says that there is a detailed report on ophthalmoscopic findings starting on p 20, which is erroneous in that there is no such report on p 20; nor is there a detailed report, by my standards (see p 31), anywhere in the study report on this topic. The interested reader may inspect these data in table 6 p 63 of the study report.

Hematology.
Test article effects on formed elements, etc, in the blood were unremarkable with the sole exception of a significant positive trend for mean platelet counts in males. Those data are in appendix 5 of this DER taken from p 64 of the study report. The parameters examined are shown in table 4 taken from p 17 of the study report.

Table 4. Hematology parameters[a] examined (x).

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

| | (Clotting time)
| | (Prothrombin time)

[a] During week 13 blood samples were drawn via orbital sinus puncture from the first 10 mice/sex/group.

* Required for subchronic and chronic studies

Serum Biochemistry.
This study does not include serum biochemistry determinations. It is important to note, however, that a companion subchronic oral feeding study performed in rats does include the serum biochemistry determinations. The MRID No. for that companion study is 420900-22.

Organ Weight.
Test article effects on absolute and relative organ weights were essentially unremarkable. There were some significant paired differences, however there were no dose-response relationships established. They included absolute heart weight decrease in group 4 females, absolute liver weight increases in both male and female mice of group 4 as well as liver weight increases relative to body weight in both sexes of group 4, and an absolute decrease in ovary weight in group 4. The summary data are in appendix 6 of this DER taken from pp 88-92 of the study report and include the spleen and testes in addition to the organs presented in table 5 of this DER. Findings of trends from statistical analysis of the data are shown in table 5 taken from pp 27-28 of the study report.

Table 5. Treatment effect trends regarding absolute and relative (to body weight) organ weights

Organ.	Finding
Liver.	Absolute and relative weights- positive trend for both sexes; higher-than-control mean values for both sexes of group 4.
	Relative weight only- higher-than-control mean value for group 3 males.
Heart.	Absolute weight- lower-than-control mean value for group 4 females.
Kidney.	Absolute weight- negative trend for females.
Brain	Absolute weight- negative trend for males and females.
Adrenals.	Absolute and relative weights- positive trend for males.
Ovary.	Absolute and relative weights- significant negative trend.
	Absolute weight only- lower-than-control mean value for group 4.

Information was taken from p 27 of the study report.

The spleen and testes were also taken, weighed, and analyzed yielding unremarkable results.

Pathology.

Gross pathology.

The gross pathology findings were generally unremarkable, and the interested reader is referred to tables 8A and 8B of the study report for the summary data of unscheduled and terminal deaths respectively. There were some incidences of dark areas in the stomachs of mice that died early on study in groups 5 and 6. Group 5 males showed 5/15; group 6 males showed 14/15; group 5 females showed 15/15; group 6 females showed 14/15. This is in contrast to the incidence of dark areas in the stomach of less than 1/15 in any other treatment or control group. The gross findings of major interest after 13 weeks on test were in the liver. Liver enlargement was seen in 6/10 group 4 males and 7/9 group 4 females as contrasted to none in the controls. A prominent reticular pattern was noted for the livers of 4/10 group 4 males, again as contrasted to none in the controls. The organs and tissues selected for histology and and/or weight determination are listed in table 6.

Table 6. Tissues selected for histology and organ weights

	Digestive system		Cardiovasc./Hemat.		Neurologic
	Tongue	x	Aorta*	xx	Brain*+
x	Salivary glands* submaxillary	xx	Heart*	x	Periph. nerve, sciatic*#
x	Esophagus*	x	Bone marrow*	x	Spinal cord, cervical lumbar, thoracic (3 levels)*#
x	Stomach*, glandular and nonglandular	x	Lymph nodes* axillary	x	Pituitary*
x	Duodenum*	xx	Spleen	x	Eyes (optic n.)*#
x	Jejunum*	x	Thymic region*		
		x	Lymph nodes, mesenteric & submaxillary		
					Glandular
x	Ileum*		Urogenital	x	Adrenal gland*
x	Cecum*	xx	Kidneys*+	x	Lacrimal gland, exorbital#
x	Colon*	x	Urinary bladder*	x	Mammary gland, female*#
x	Rectum*	xx	Testes*+	x	Parathyroids*++
xx	Liver *+	x	Epididymides	x	Thyroids*++
				x	Thymic region
					Harderian glands
				x	Pancreas
x	Gall bladder*	x	Prostate		Other
x	Pancreas*	x	Seminal vesicle	x	Bone: femur with marrow and joint*#; sternum with marrow
	Respiratory	x	Ovaries*+	x	Skeletal muscle, thigh*#
x	Trachea*	x	Uterus*	x	Skin, abdominal mammary region*#
			Vagina		
x	Lung*	x	Penis	x	All gross lesions and masses*
	Nose^			x	Cranial cavity
	Pharynx^			x	Abdominal cavity
	Larynx^			x	Thoracic cavity

* Required for subchronic and chronic studies.
^ Required for chronic inhalation.
\# In subchronic studies, examined only if indicated by signs of toxicity or target organ involvement.
\+ Organ weight required in subchronic and chronic studies.
++ Organ weight required for non-rodent studies.
x selected for histological examination
xx selected for organ weight determination

Microscopic/histopathology.
Treatment-related microscopic findings were found in the liver, and findings in other organs were unremarkable. The interested reader is directed to tables 10A and 10B of the study report for the histopathology data as they relate to unscheduled and scheduled deaths respectively. Hepatotoxicity in mice that DOS was evidenced by hepatocellular enlargement and necrosis of individual hepatocytes. Those mice that survived to the end of the study showed hepatotoxicity that included hepatocellular enlargement in group 4 animals and group 3 males with hepatocytic vacuolization in group 4 animals. Furthermore, coagulative hepatonecrosis was seen in 4/9 group 4 females. These findings, however, were not considered treatment-related, because the foci were frequently small and random. The animals in groups 5 and 6, which represent the unscheduled deaths, had changes consistent with stress, namely lymphoid depletion or necrosis of the spleen, lymph nodes, and thymus. Hypocellularity of the femoral marrow, mucosal erosion/ulceration of the glandular stomach were also present. In the female mice necrosis of individual cells was observed in the adrenal cortex, specifically in the zona reticularis. The nonglandular portion of the stomach showed hyperkeratosis in group 6 males. The study report author suggested these stress-induced changes may be on account of poor appetite as opposed to a direct effect of the test article. Treatment-related significant findings in the liver for animals' unscheduled and scheduled deaths are summarized in table 7 taken from p 28 of the study report.

Table 7. Microscopic findings in the livers of mice from unscheduled and scheduled deaths

	Males						Females					
Group No.	1	2	3	4	5	6	1	2	3	4	5	6
Number examined	10	10	10	10	15	15	10	10	10	10	10	10
Hepatocellular Enlargement:	0	1	1	10	9	10	0	0	1	10	10	8
Hepatocellular Necrosis:	0	1	0	1	3	3	0	0	0	0	2	0
Hepatocellular Vacuolization:	1	3	1	7	0	0	0	0	2	7	0	0

The data were taken from p 28 of the study report.

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Reviewed by: Dan W. Hanke, Ph. D. Dan W Hanke 19 June 92
Section III, Tox. Branch II (H7509C)
Secondary Reviewer: Whang Phang, Ph. D. W Phang 6/19/92
Section III, Tox. Branch II (H7509C)

DATA EVALUATION RECORD

STUDY TYPE: 13-Week Oral Feeding Study (§82-1)

TOX. CHEM. NO. (CASWELL NO.): new chemical

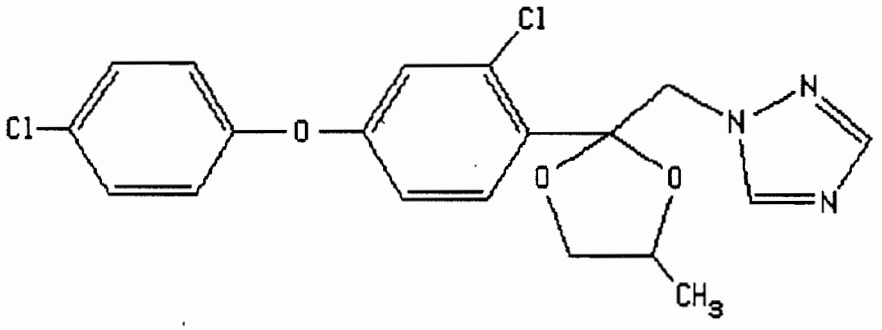
CAS REG. NO.: 119 446-68-3

EPA PESTICIDE CHEMICAL CODE/ACTIVE INGREDIENT CODE (SHANGHNESSY NO.): 128847

HED PROJECT NO.: 2-0696

MRID NO. (ACCESSION NO.): 420900-22

TEST MATERIAL: CGA-169374 Technical; 1-[2-(2-chloro-4-(4-chlorophenoxy)phenyl)-4-methyl-1,3-dioxolan-2-yl)-methyl]-1H-1,2,4-triazole



SYNONYMS: Difenoconazole; Dividend; triazole fungicide

STUDY NUMBER: 483-242

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

TESTING FACILITY: Hazleton Laboratories America, Inc.
9200 Leesburg Turnpike
Vienna, Virginia 22180

TITLE OF REPORT: Subchronic Toxicity/Metabolism Study in Rats

AUTHOR(S): Raymond H. Cox, Ph. D.

REPORT ISSUED: October 28, 1987

SUMMARY AND CONCLUSIONS:
CGA-169374 Technical was administered orally in feed admixtures to six groups of rats of both sexes at 0 ppm, 20 ppm, 200 ppm, 750 ppm, 1500 ppm, and 3000 ppm for 13 weeks. The results of this dietary subchronic evaluation of the toxicity of the test article were generally unremarkable. There was a significant trend for decreased body weights in both sexes, and the 200 ppm female rats showed an approximate 10% decrease in body weight relative to their controls concomitant with decreased food consumption. There was one dose-related effect of the chemical discovered during the histopathology examination, that identified modest diffuse hepatocellular enlargement, vis a vis. increased liver weights, in rats of both sexes at the two highest doses tested. Additionally, although not statistically significant, compared to the other groups there was an increase in the frequency and quantity of ketones in the urine of group 6 males. The presence of elevated ketone levels may be due to gluconeogenesis driven by decreased protein intake from the diet as a result of decreased food intake. The somewhat compromised nutritional status of the rats could possibly and indirectly have promoted the hepatocellular enlargement as well.

It is possible to conclude from this study, that based on approximately 10% decrease in body weight in the 200 ppm females (concomitant with a negative trend for food consumption) and increases in absolute liver weights in both sexes appearing at 750 ppm, the LOEL may be set at 200 ppm. The NOEL of CGA 169374 Technical, therefore, was 20 ppm.

A signed quality assurance statement was present.

Core Classification: Minimum

This study satisfies the guideline requirements (§82-1) for a Subchronic feeding study in rats.

MATERIALS:

1. Test compound. CGA-169374 Technical; difenoconazole; 1-[2-(2-chloro-4-(4-chlorophenoxy)phenyl)-4-methyl-1,3-dioxolan-2-yl)-methyl]-1H-1,2,4-triazole. Description: dark brown solid. Batch #: FL-851406. Purity: 94.5 % run on batch # ACL-5668; the solvent was either dehydrated ethanol or acetone (from the companion mouse subchronic/metabolism study MRID No. 420900.21). Stability: difenoconazole was stable for at least 24 hrs at room temperature (unspecified) and for 22 days at 6 °C over a concentration range of 0.5 % to 50.0 % (from the companion study in mice). The stability determinations were made on representative samples via gas chromatography equipped with a nitrogen-phosphorus detector (see appendix 9, this study, p 426). The test article was not detected in the control samples.

2. Test animals.
Species: rat. Strain: CRL:CD(SD)®, 138 females and 142 males. Age: At the initiation of treatment rats were approximately six weeks old. Weight: the range was 130.1 g to 158.6 g for females and 177.8 g to 209.6 g for males. Source: Charles River Laboratories, Inc., Lakeview, New Jersey.

METHODS:

1. Animal Dosing/Assignment.
Each test-article dose-group was 15 rats/sex at dose levels of 20 ppm, 200 ppm, 750 ppm, 1500 ppm, or 3000 ppm. The male and female control groups were 20 rats each. Feed and water were available ad libitum except when fasting for clinical tests and necropsy. The rats were assigned to study groups by first eliminating those with extreme body weights and then by selecting the random assignment which produced homogeneity of both variances and means by Bartlett's test (1937) and one-way ANOVA. The dosing schedule is shown here in table 1 taken from p 14 of the study report.

Table 1. Dosing schedule[a]

Group	Number of rats		Dietary level of test article (ppm)
	Male	Female	
1 (Control)	20	20	0[b]
2	15	15	20
3	15	15	200
4	15	15	750
5	15	15	1,500
6	15	15	3,000

[a] Data were extracted from p 14 of the study report.

[b] Rats were fed untreated Purina Rodent Diet #5002.

2. Diet Preparation, Dosage Form, and Analytical Chemistry. The test material CGA 169374 was added to Purina Certified Rodent Chow® #5002, which was used as the basal diet, on a weight/weight basis with no adjustment for purity of the test article. The test diets were prepared in two steps. First the test article was melted with the aid of a 70 °C water bath, and then the material was mixed with acetone. The resulting solution was mixed with the basal diet. Dietary administration was used, because the risk of human exposure is via the oral route. Further details of the diet preparation are described in appendix 1 of this DER taken from pp 15-16 of the study report. Routine analyses of the test article were conducted during the study and verified the concentrations of CGA 169374 Technical used on study in the dietary admixtures. Those analyses are summarized here in table 2, where the data were taken from p 22 of the study report.

Table 2. Test concentrations of CGA 169374 Technical

Dose level (ppm)	Percent of Target range Low	High	Mean (SD)
20	92	112	101 (5.5)
200	88	110	100 (5.3)
750	87	110	100 (7.1)
1500	89	112	99 (6.8)
3000	89	106	99 (5.5)

Data were taken from p 22 of the study report.

3. Statistics.
The cumulative survival data through week 13 were analyzed using the computer software National Cancer Institute Package. Trend analysis of animal survival was evaluated at the 5.0 % one-tailed level of probability. The body weight changes from weeks 0-13, the total food consumption from weeks 1-13, the clinical pathology data with the exception of cell morphology, and organ weight data of the control group were compared statistically to the data from the same sex of the treated groups. The statistical analyses were executed as per the flow chart in appendix 2 of this DER taken from p 20 of the study report. Statistical significance was denoted throughout by the terms "significant" and "trend" where appropriate.

If variances of untransformed data were heterogeneous, then analyses were performed on transformed data to achieve variance homogeneity. When the series of transformations was not successful in achieving variance homogeneity, then analyses were performed on rank-transformed data. Group comparisons were performed routinely at the 5 % two-tailed level of probability unless otherwise specified, or where a trend in the data indicated that a one-tailed test would be more revealing.

RESULTS AND DISCUSSION:

Clinical Signs/Observations.
Clinical signs were unremarkable in that no dose-response relationships developed and the rate of incidence was low for all observations that included: hunched appearance, thinness, malocclusion of incisors, soft feces, urine stains, alopecia, sores, lacrimation, chromadacryorrhea, exophthalmus, enlarged and/or swollen ears, and lack of use of the right hind leg. See table 2 of the study report. Additional clinical observations are summarized and discussed in turn below.

Mortality.
Essentially all the rats survived to the end of the dosing period. Notable exceptions during week 10 were one group 5 female found dead and one group 3 male's accidental death.

Body Weight, Food Consumption, and Compound Consumption.
There were some significant differences in body weight changes over the dosing period evidenced by both trend and pair-wise statistical analysis. A negative trend was established for both female and male rat body weight changes. Dose-related test article effects were apparent in paired female groups 3, 4, 5, and 6. Group 6 males also showed decreases in body weight gains. Summary data are in appendix 3 of this DER taken from pp 47-54 of the study report. With the exception of a significant negative trend for female rats, the food consumption data were unremarkable. See table 4 of the study report. The test article consumption data were unremarkable. The decreases in body weight gains, coupled with food consumption essentially comparable to controls, suggests impaired food utilization in the affected groups.

Ophthalmology.
With the exception of a few isolated non-dose-response related effects the ophthalmology was unremarkable. There was one group 6 male that had bilateral diffuse posterior subcapsular cataracts. Also there was one group 2 male, one group 4 female and one group 6 female rat that had unilateral diffuse retinochoroid degeneration (RCD). See table 6 in the study report.

Hematology.
Aside from the exceptions noted below, the hematology findings were unremarkable with regard to biological as well as statistical significance. The parameters examined are shown here in table 3 taken from p 17 of the study report. However, although no dose-response relationships were established, there were some significant paired differences and trends. They are reproduced here in table 4 and were taken from p 30 of the study report. See appendix 3a of this DER taken from pp 69-70 of table 7 of the study report for the numerical values.

Table 3. Hematology parameters[a] examined (x).

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

[a] During week 13 blood samples were drawn via orbital sinus puncture from the first 10 rats/sex/group.

* Required for subchronic and chronic studies

Table 4. Significant treatment effects on hematology parameters

Parameter.	Finding
Red blood cell.	Males- lower-than-control values for groups 4, 5, and 6; and a negative trend. Females- lower-than-control value for groups 5 and 6; and a negative trend.
Hematocrit.	Males- lower-than-control values for groups 4, 5, and 6; and a negative trend Females- lower-than-control values for groups 5 and 6.
Hemoglobin	Females- lower-than-control value for groups 5 and 6; and a negative trend

Information was taken from p 30 of the study report.

Serum Biochemistry.
Aside from the exceptions noted below, the serum biochemistry findings were generally unremarkable with regard to biological as well as statistical significance. The parameters examined are shown here in table 5 taken from p 17 of the study report. However, although no dose-response relationships were established, there were some significant paired differences and trends. They are reproduced here in table 6 and were taken from p 31 of the study report. See the summary data in appendix 3b of this DER taken from pp 77, 79, 80, and 82 of table 8 of the study report for the numerical values.

Table 5. Clinical serum biochemistry parameters[a] examined (x)

	Electrolytes:		Other:
x	Calcium*	x	Albumin*
x	Chloride*	x	Blood creatinine*
	Magnesium#	x	Blood urea nitrogen (BUN)*
x	Phosphorus*	x	Cholesterol*
x	Potassium*	x	Globulins
x	Sodium*	x	Glucose*
	Enzymes	x	Total bilirubin
x	Alkaline phosphatase (ALK)	x	Total serum Protein (TP)*
	Cholinesterase (ChE)#		Triglycerides
x	Creatinine phosphokinase*^		Serum protein electrophoresis
			A/G ratio
			Gamma G-T
		x	LDH[b]
x	Lactic acid dehydrogenase (LAD)		
x	Serum alanine aminotransferase (ALT or serum glutamic pyruvic transaminase, SGPT)*		
x	Serum aspartate aminotransferase (serum glutamic oxaloacetic transaminase, SGOT)*		
x	Gamma glutamyl transferase (or transpeptidase) (GGT)		
	Glutamate dehydrogenase		

[a]During week 13 blood samples were drawn via orbital sinus puncture from the first 10 rats/sex/group.
[b]LDH determinations were not performed during predose.
*Required for subchronic and chronic studies
#Should be required for organophosphorus (OP) pesticides
^Not required for subchronic studies

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Table 6. Significant treatment effects on serum biochemistry parameters

Parameter.	Finding
Globulins.	Males- lower-than-control values for groups 4 and 5.
Total Bilirubin.	Males- lower-than-control value for group 6. Females- lower-than-control values for groups 5 and 6.
Glucose.	Males- negative trend.
Blood Urea Nitrogen.	Males- higher-than-control values for groups 5 and 6; and a positive trend.
Total Cholesterol.	Females- positive trend.
Alanine Aminotransferase.	Females- negative trend.

Information was taken from p 31 of the study report.

Urinalysis.^[a]
Although not statistically significant, compared to the other groups there was an increase in the frequency and quantity of ketones in the urine of group 6 males. The presence of elevated ketone levels may be due to gluconeogenesis driven by decreased protein utilization from the diet as a result of possible decreased food efficiency, since overall food consumption was comparable to controls despite a negative trend for female rats. See appendix 4 of this DER taken from pp 173-175 of the study report. The parameters examined are shown here in Table 7 taken from p 17 of the study report.

Table 7. Urinalysis (x)

X		X	
	Appearance*	x	Glucose*
x	Volume*[b]	x	Ketones*
x	Specific gravity*	x	Bilirubin*
x	pH		Blood*
x	Sediment (microscopic)*		Nitrate
x	Protein*	x	Urobilinogen
x	Occult blood		

[a]Urine was collected by catheterization or the plastic liner method predose and by stainless steel collection pans at termination. When crystals were present microscopically, an identification based upon morphology/pH was entered into the raw data.
[b]Urine volumes were determined at termination only.
^Not required for subchronic studies
*Required for chronic studies

Gross Pathology. Test article effects on select organs at terminal sacrifice, including unscheduled deaths, were unremarkable. See summary data in the study report on pp 84-98. The summary data of the absolute and relative organ weights are displayed in appendix 5 of this DER taken from pp 99-102 of the study report. Appendix 5 also includes the spleen, ovaries, and testes in addition to the list of tissues/organs evaluated, that are presented here in table 8. The tissues/organs selected for histologic evaluation and organ weight comparisons to-terminal body weights are taken from pp 18-19 of the study report.

Table 8. Tissues selected for histology and organ weights

	Digestive system		Cardiovasc./Hemat.		Neurologic
	Tongue	x	Aorta*	xx	Brain*+
x	Salivary glands* submaxillary	xx	Heart*	x	Periph. nerve, sciatic*#
x	Esophagus*	x	Bone marrow*	x	Spinal cord, cervical lumbar, thoracic (3 levels)*#
x	Stomach*, glandular and nonglandular	x	Lymph nodes* axillary	x	Pituitary*
x	Duodenum*	xx	Spleen	x	Eyes (optic n.)*#
x	Jejunum*	x	Thymic region*		
		x	Lymph nodes, mesenteric & submaxillary		
					Glandular
x	Ileum*		Urogenital	xx	Adrenal gland*
x	Cecum*	xx	Kidneys*+		Lacrimal gland, exorbital#
x	Colon*	x	Urinary bladder*	x	Mammary gland, female*#
x	Rectum*	xx	Testes*+	x	Parathyroids*++
xx	Liver *+	x	Epididymides	x	Thyroids*++
				x	Thymic region
					Harderian glands
				x	Pancreas
	Gall bladder*	x	Prostate		Other
x	Pancreas*	x	Seminal vesicle	x	Bone: femur with marrow and joint*#; sternum with marrow
	Respiratory	xx	Ovaries*+	x	Skeletal muscle, thigh*#
x	Trachea*	x	Uterus*	x	Skin, abdominal mammary region*#
x	Lung*		Vagina	x	All gross lesions and masses*
	Nose^		Penis	x	Cranial cavity
	Pharynx^			x	Abdominal cavity
	Larynx^			x	Thoracic cavity

Table 8 continued.

* Required for subchronic and chronic studies.
^ Required for chronic inhalation.
# In subchronic studies, examined only if indicated by signs of toxicity or target organ involvement.
\+ Organ weight required in subchronic and chronic studies.
++ Organ weight required for non-rodent studies.
x selected for histological examination
xx selected for organ weight determination and histologic examination

Organ weight.
Aside from the exceptions noted below, test article effects with regard to absolute and relative organ weights were for the most part unremarkable. However, although no dose-response relationships were established, there were some significant paired differences and trends. They are reproduced here in table 9 and were taken from pp 26-27 of the study report. The significant relative-to-body-weight positive trends for liver, heart, kidney, and brain may be explained by the negative trends and pairwise decrements in body weights among the affected groups. These could be valid comparisons, since food consumption in the treated groups was essentially comparable to the controls.

Table 9. Significant treatment effects regarding absolute and relative (to body weight) organ weights

Organ.	Finding
Liver.	Absolute weights- significantly higher-than-control mean weights for groups 4, 5, and 6 in both sexes; and a significant positive trend for both sexes. Relative weights- significantly higher-than-control mean weights for groups 4, 5, and 6 males, and groups 3, 4, 5, and 6 females; and a significant positive trend for both sexes.
Heart.	Relative weights- significant positive trend for males.
Kidney.	Relative weights- significant positive trend for females
Brain.	Relative weights- significantly higher-than-control mean weight for group 6 females; and a significant positive trend for females.
Adrenals.	Absolute weights- significantly lower-than-control mean weights for groups 4 and 6 males. Relative weights- significantly lower-than-control mean weights for groups 3 and 4 males.

Table 9 continued.

Information was taken from pp 26-27 of the study report. The spleen, testes, and ovaries were also taken, weighed, and analyzed yielding unremarkable results.

Histopathology.
Test article effects with regard to microscopic evaluation of the select tissues/organs listed in table 9 of this DER were for the most part unremarkable. There was, however, one dose-response relationship established identifying modest diffuse hepatocellular enlargement in rats of both sexes (more demonstrative in the females) at the two highest doses (1500 ppm and 3000 ppm). The hepatocellular enlargement is mirrored by the significant increases in absolute and relative liver weights (see table 9). Although there is no dose-response relationship sustained by both sets of data, these data do suggest the liver may be a target organ for the test article which is consistent with the toxicity of other compounds containing a conazole moiety. Select histopathology summary data on microscopic evaluation of hepatocytes are in appendix 6 of this DER taken from pp 114 of the study report. It is important to note that a companion study in mice (MRID NO. 420900-21) also revealed test-article related hepatocellular enlargement in mice that DOS as well as in those animals that survived until termination of the study.

It is possible to conclude from this study, that based on approximately 10% decrease in body weight in the 200 ppm females (concomitant with a negative trend for food consumption) and increases in absolute liver weights in both sexes appearing at 750 ppm (hepatocellular enlargement showed up in the two highest doses tested), the LOEL may be set at 200 ppm. The NOEL of CGA 169374 Technical, therefore, was 20 ppm.

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Toxicologist, Review Section II, Tox. Branch (H7509C)
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Section Head, Review Section II, Tox. Branch (H7509C)

DATA EVALUATION REPORT

STUDY TYPE: Chronic Toxicity in Dogs §83-1 SHAUGHNESSY NO: 128847

HED Project No: 2-0696 MRID NO.: 420900-14

TEST MATERIAL: CGA 169374

SYNONYMS: Difenconazole

TOXICOLOGY/PATHOLOGY REPORT NO.: 88058 MIN NO.: 862010

SPONSOR: Agricultural Division
CIBA-GEIGY Corporation
P. O. Box 18300
Greensboro, NC 27419

TESTING FACILITY: Research Department
Pharmaceuticals Division
CIBA-GEIGY Corporation
556 Morris Ave.
Summit, New Jersey 07901

TITLE OF REPORT: CGA 169374 Technical 52-Week Oral Toxicity Study in Dogs

AUTHOR(S): M.W. Rudzki, G.C. McCormick, and A.T. Arthur

REPORT ISSUED: August 22, 1988

STUDY DATES: Dosing Period: August 19, 1986 - August 20, 1987
Necropsies Performed: August 19 - 21, 1987

CONCLUSION:

CGA 169347 was administered in the diet to male and female dogs at 0, 20, 100, 500, or 1500 ppm. The NOEL was 100 ppm and the LEL was 500 ppm based on the following. Females receiving 1500 ppm in the diet had a significant reduction in body weight gain on day 7. Females in the 500 and 1500 ppm groups, although not statistically significant, had inhibited body weight gain throughout the study. These animals also had significant reductions in food consumption on days 7, 35, 70, and 357. The reduction in mean percent reticulocytes at the highest dose tested on day 359 may have been related to treatment. Significant increases (treatment related at day 85; dose-related at days 175 and 359) were observed in alkaline phosphatase in males receiving 1500 ppm. This study may be upgraded upon satisfactory review of the registrants response to

the deficiencies (submission of the purity and raw daily observation data).

Classification: core-supplementary

A. MATERIALS:

A copy of the "materials and methods" section from the investigators report is appended (Appendix I).

1. Test compound:

Test Substance:	CGA 169374
Purity:	96.1% (by the Agricultural Division)
Description:	not provided
Batch No.:	FL 851406
Stability:	see below
Storage Conditions:	see below

Page 10 of the report indicates that "the Ag Division accepts all responsibilities relating to the stability of the test substance for the duration of the study as long as the test substance was stored at room temperature...additionally, the Sponsor accepts responsibility for the thermal stability of the test substance following heating to 70° C when necessary for diet preparation." Samples (5 g) were taken for confirmatory analysis prior to the start of the study, at 1 and 6 months of the study, and at termination. Analytical results were retained by the Sponsor. The dietary admixtures were determined to be stable for at least 46 days at room temperature, by the TPATO.

2. Test animals:

Species:	dog
Strain:	purebred beagles
Source:	Marshall Farms, North Rose, New York
Date:	April 2, 1986
Age:	approx. 5 mo.
Weight:	6.7-9.0 kg (♂ at beginning of treatment)
	4.8-8.1 kg (♀ at beginning of treatment)

Test animals were acclimated to the test facility for 6 weeks prior to treatment. Prior to their assignment to the study, animals were assessed for suitability based on general observations, litter mates, body weights, physical and ophthalmoscopic examinations, and clinical lab tests. Those which were not found to be suitable were returned to stock. The animals were randomly assigned to treatment groups by use of a computer-generated randomization table that was supplied by the Research Statistics Department.

B. STUDY DESIGN:

1. Animal Husbandry

Animals were housed in animal rooms at the Summit facilities; with a humidity range of 50±20% and a temperature range of 69±5° F. The rooms were maintained on a 12 hour light/dark cycle. During the acclimation period, the animals were provided a daily ration of Certified Purina Lab Canine Diet (#5007) and tap water ad lib via an automatic watering system. During the experiment the dogs were fed the same diet in the powered form with or without the test substance.

Animals were assigned to the following test groups:

Test Group	Dose in diet (ppm)	Number of Dogs Assigned ♂	♀	Least Number of Weeks Dosed
1 Control	0	4	4	52
2 Low (LDT)	20	4	4	52
3 Low Mid (LMDT)	100	4	4	52
4 High Mid (HMDT)	500	4	4	52
5 High (HDT)	1500	4	4	52

The rationale for the selection of doses in this investigation was a six month dog study, evaluating 0, 100, 1,000, 3,000 and 6,000 ppm of the test article in the diet. The NOEL in the six month study was 100 ppm. EPA was consulted for the selection of the top dose in the current 1-year study; it was agreed that 1500 ppm was appropriate as the HDT.

The dietary concentration was not adjusted for purity. Diets (both test and control) were offered to the animals for approximately 3 hours/day.

2. Diet preparation

The test substance was prepared weekly as a premix by admixing the test article with acetone in powdered diet. The acetone was evaporated overnight and the premix was used to prepare the test substance feed admixture. Samples of each batch were submitted for possible analyses for concentration. Prior to initiation of the study, a statistical sampling approach determined that 13 of the 52 possible analyses would be sufficient to confirm the integrity of the feed mixing. The preselected weeks were 1, 4, 5, 7, 11, 14, 15, 20, 21, 31, 32, 34, 35, and 48. When performed, analyses were conducted prior to the use of the admixture. The admixture had to be ±15% of the theoretical to be used. Homogeneity was confirmed week 1 of the investigation. The analytical data for the concentration analyses were within ±15% of the theoretical values. The stability data indicate that the test material was stable in the diet stored in closed containers at room temperature for 46

days. The analytical report does not include information pertaining to purity.

3. Observations

All animals were observed twice daily (once a day on weekends and holidays) for appearance, mortality, and clinical observations.

Physical/auditory examinations were performed on all animals prior to dosing at week -3, and during weeks 14, 25, 39, and 52 of test. The exam which was performed by a veterinarian, included measurement of the heart rate and rectal temperature.

Ophthalmoscopic examinations were performed by the staff ophthalmologist for all animals two weeks prior to dosing and at weeks 11, 27 39, and 51 of test.

Body weight was measured for all animals prior to dosing during weeks -3 and -2. Body weight was also measured prior to dosing on test day 1, weekly during the first 13 weeks, and monthly thereafter starting week 15.

Food consumption was measured for all animals prior to dosing at week -2, weekly during the first 13 weeks, and monthly thereafter starting on week 15.

4. Statistics

The procedures utilized in analyzing the data are included in the appended materials and methods section (Appendix I).

5. Compliance

A signed Statement of No Confidentiality Claim was included which was dated 6/13/90 (p. 2).

A signed Statement of Compliance with EPA GLP's was included which was dated 8/24/88 (p. 5).

A signed Quality Assurance Statement was included and dated 8/22/88 (p. 469).

A signed Flagging Criteria Statement was included which was dated 6/14/90 (p. 4).

C. RESULTS:

1. Observations:

All animals survived until terminal sacrifice for this investigation. Evaluation of the summary data for the clinical signs did not indicate any treatment related differences. However,

the individual data which coincide with table 8.2 of the report are not presented.

The results of the physical/auditory examinations and the ophthalmology report did not indicate any treatment related findings.

2. Body Weight

The collection intervals were standardized in the body weight table. The mean body weight of males and females were not significantly altered by treatment. The mean body weight gain of females in the 100, 500, and 1500 ppm groups was decreased. This decrement was significant for the 100 and 1500 ppm females on day 7 of treatment. Throughout the study the females receiving 500 or 1500 ppm exhibited decreased mean body weight gain. Females receiving 100 ppm appeared to recover from these effects by day 56.

GROUP MEAN PERCENT BODY WEIGHT GAIN (KG) FOR DOGS ADMINISTERED CGA 169374 IN THE DIET FOR 52 WEEKS

	Males					Females				
DAYS ON TEST / ppm =	0	20	100	500	1500	0	20	100	500	1500
7	2.84	3.10	3.78	2.93	1.25	4.58	1.49	1.16*	2.31	0.40*
14	3.48	4.05	4.20	4.51	2.60	4.81	2.53	2.02	2.77	2.48
21	8.39	7.45	8.54	7.80	6.98	8.47	9.78	7.28	7.47	6.98
28	11.15	9.35	12.83	10.07	9.86	14.09	13.45	8.86	10.49	10.24
35	15.32	10.58	16.33	13.52	12.50	16.29	14.24	11.31	12.43	13.24
42	17.18	12.83	19.21	17.11	15.15	20.57	17.44		14.64	16.09
49	19.45	14.37	22.54	19.76	19.29	22.30	19.97	16.28	16.09	18.82
56	21.54	17.50	24.19	20.70	19.65	23.91	23.23	17.80	17.77	21.34
63	24.10	19.40	27.86	23.84	23.12	26.53	25.01	20.32	20.08	22.56
70	25.63	20.73	30.55	26.48	24.02	29.43	27.60	22.00	22.05	24.67
77	26.32	22.23	31.17	27.13	24.39	28.62	27.30	24.49	21.59	24.27
84	28.53	23.46	33.23	28.53	27.02	32.49	28.54	25.57	23.73	25.93
91	28.94	25.06	34.88	30.59	27.91	33.30	29.21	29.45	25.24	27.23
105	27.64	27.23	36.29	31.97	28.43	32.94	32.73	27.87	22.38	25.80
133	28.79	33.19	41.83	38.41	32.94	39.54	39.84	35.65	25.20	31.05
161	28.13	35.66	43.11	39.63	33.62	40.88	41.31	39.68	24.54	29.73
189	30.27	38.43	45.42	40.77	36.30	42.35	40.66	41.47	27.58	32.36
217	27.87	38.43	46.02	39.71	38.55	40.77	37.45	37.64	26.08	29.76
245	34.35	44.13	49.98	43.38	41.70	46.01	42.25	44.27	32.50	33.34
273	35.86	46.08	51.68	45.44	43.34	44.21	45.55	47.21	30.17	34.55
301	35.57	45.76	51.26	45.46	41.30	40.91	44.53	47.51	25.95	31.28
329	36.22	47.66	53.66	43.89	41.60	41.87	46.65	45.22	25.36	31.28
357	35.14	48.63	54.30	42.56	40.94	41.68	44.93	43.49	24.87	28.94

Data extracted from report MIN 862010 pp. 66-70 and 89-93.

3. Food consumption and compound intake

Consumption was determined and mean daily diet consumption was calculated. The collection intervals were standardized in the food consumption table. Statistically significant reductions in food consumption were observed only in the 1500 ppm females after 7, 35, 70, and 357 days on test. These findings appear to correlate with the reduced mean body weight gain observed in this group. Compound intake was calculated from the consumption and body weight gain data.

GROUP MEAN FOOD CONSUMPTION (KG/WK)

	Males					Females				
DAYS ON TEST ppm =	0	20	100	500	1500	0	20	100	500	1500
7	2.48	2.64	2.22	2.07	1.98	1.94	1.76	1.69	1.79	1.50*
14	2.42	2.65	2.30	2.17	2.08	1.89	1.88	1.69	1.82	1.59
21	2.56	2.68	2.41	2.18	2.27	1.89	2.03	1.91	1.95	1.68
28	2.46	2.60	2.46	2.21	2.33	2.06	2.20	1.95	1.89	1.75
35	2.65	2.64	2.46	2.32	2.43	2.19	1.99	2.16	2.02	1.77*
42	2.47	2.53	2.44	2.46	2.44	2.12	2.16	2.14	1.90	1.78
49	2.32	2.65	2.43	2.41	2.47	1.89	2.05	2.09	1.90	1.70
56	2.50	2.72	2.51	2.44	2.45	2.07	2.19	2.05	2.01	1.79
63	2.70	2.77	2.56	2.37	2.45	2.06	2.22	2.16	2.03	1.69
70	2.52	2.71	2.56	2.40	2.49	2.22	2.08	2.25	2.16	1.69*
77	2.42	2.74	2.42	2.36	2.51	2.03	2.02	2.18	2.01	1.65
84	2.58	2.61	2.45	2.39	2.47	2.13	2.11	2.25	2.24	1.71
91	2.59	2.76	2.59	2.54	2.59	2.23	2.04	2.32	2.26	1.75
105	1.87	2.24	1.92	1.78	1.89	1.55	1.65	1.51	1.55	1.37
133	2.57	2.58	2.48	2.41	2.53	2.01	2.31	2.32	2.45	1.70
161	2.47	2.64	2.51	2.41	2.55	2.14	2.18	2.21	2.43	1.63
189	2.54	2.59	2.32	2.34	2.46	1.97	1.89	2.08	2.37	1.67
217	2.40	2.39	2.22	2.19	2.40	1.91	1.84	2.09	2.06	1.29
245	2.57	2.53	2.47	2.30	2.52	2.06	2.05	2.31	2.36	1.61
273	2.57	2.65	2.40	2.41	2.60	1.98	2.21	2.21	2.08	1.63
301	2.54	2.70	2.60	2.34	2.58	2.04	2.17	2.27	2.22	1.63
329	2.49	2.65	2.54	2.25	2.59	2.13	2.04	1.72	2.20	1.66
357	2.33	2.61	2.38	2.14	2.44	2.12	1.82	1.86	2.08	1.51*

Data extracted from report MIN 862010 pp. 74-78 and 97-101.
* .01 < p < = 0.05, two tailed Dunnett t on raw data.

Test article intake (mg/kg) was calculated by multiplying the feed consumed daily (g/day) by the projected compound concentration (mg/kg of feed), divided by the average group mid-period body weight (kg).

GROUP MEAN TEST ARTICLE INTAKE (mg/kg)

	Males				Females			
WEEKS ON TEST ppm =	20	100	500	1500	20	100	500	1500
1	0.94	4.2	19.5	53.4	0.71	4.0	19.4	51.4
2	0.92	4.2	19.9	55.4	0.75	3.9	19.5	53.8
3	0.91	4.3	19.5	58.8	0.78	4.3	20.4	55.3
4	0.86	4.2	19.3	58.3	0.80	4.2	19.1	55.7
5	0.87	4.1	19.7	59.4	0.72	4.6	20.0	54.8
6	0.82	3.9	20.3	58.2	0.76	4.4	18.4	53.5
7	0.84	3.8	19.4	57.4	0.71	4.2	18.2	50.0
8	0.84	3.9	19.3	55.9	0.74	4.1	19.0	51.5
9	0.84	3.9	18.4	54.9	0.73	4.3	18.8	48.0
10	0.81	3.8	18.2	54.7	0.68	4.4	19.7	47.3
11	0.81	3.5	17.7	55.0	0.65	4.2	18.2	45.8
12	0.77	3.5	17.8	53.4	0.68	4.2	20.1	47.4
13	0.80	3.7	18.7	55.3	0.65	4.3	20.0	47.7
15	0.75	3.2	15.0	46.8	0.60	3.2	16.1	43.6
19	0.71	3.4	17.0	52.9	0.69	4.1	21.9	45.7
23	0.71	3.4	16.5	52.2	0.64	3.8	21.5	43.2
27	0.68	3.1	15.9	49.7	0.55	3.5	20.7	44.0
31	0.62	2.9	14.9	47.5	0.54	3.5	17.9	33.9
35	0.64	3.2	15.5	49.0	0.60	3.9	20.1	42.2
39	0.66	3.0	15.9	49.6	0.63	3.6	17.4	42.1
43	0.67	3.3	15.3	49.4	0.61	3.6	19.1	42.5
47	0.65	3.2	14.8	49.8	0.57	2.8	19.3	43.7
51	0.63	2.9	14.3	47.0	0.51	3.0	18.3	40.1
Mean =	0.71	3.4	16.4	51.2	0.63	3.7	19.4	44.3

Data extracted from report MIN 862010 table 8.5 pp. 51-54.

4. Ophthalmological examination

The ophthalmological examinations did not detect any treatment related findings in this study. In a previous 6 month dog study cataracts were observed at ≥ 3000 ppm with CGA 163974 technical. These findings were not observed in the current study.

5. Blood was collected before treatment (week -2) and at weeks 13, 26, and 52-53 for hematology and clinical analysis from all animals. The CHECKED (X) parameters were examined.

a. Hematology

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

* Required for subchronic and chronic studies

The hematology data did not indicate treatment related alterations. Significant reductions were observed at day 85 in the mean percent eosinophils in females receiving 100 ppm; and the mean percent reticulocytes in females receiving 1500 ppm at 359 days. The effect on mean percent eosinophils did not appear to be treatment related. The reduction in mean percent reticulocytes at the highest dose tested on day 359 may have been related to treatment. However, due to the fact that this parameter was not evaluated for the three lower doses does not permit the observation of a dose-response relationship.

Male Hematology Data

Parameter	Day -13					Day 85					Day 175					Day 359				
	0	20	100	500	1500	0	20	100	500	1500	0	20	100	500	1500	0	20	100	500	1500
Neutro. %	63.5	64.25	57.00	60.00	53.00	66.00	63.75	69.50	62.50	63.00	66.50	60.75	65.00	64.25	62.50	65.25	63.25	70.50	63.00	68.25
Lymph. %	26.75	25.75	32.75	24.5	32.75	20.25	22.75	21.00	30.50	27.25	18.50	27.25	24.75	25.50	23.00	21.50	24.25	20.00	25.75	21.75
Platelet 10^3/mm^3	372.8	344.0	394.5	368.2	440.8	301.2	356.5	320.5	293.0	346.5	275.2	278.8	265.8	268.8	342.5	286.2	276.2	284.2	250.5	316.8
Heinz %	0	0	0	0	0	0	-	-	-	0	0	-	-	-	0	0	-	-	-	0
Reticu. %	1.88	1.90	2.25	2.25	2.05	0.55	-	-	-	0.95	1.0	-	-	-	1.3	0.8	-	-	-	0.72
NSeg %	0.50	0.25	0.25	1.5	2.0	1.50	0.50	1.50	0.75	0.50	1.75	0.75	0.5	0.5	0.25	0	0.25	0.5	0.25	0
Mono %	7.25	7.00	6.75	10.0	9.25	8.5	7.0	4.0	3.75	6.25	8.5	8.25	5.75	7.25	8.5	9.0	8.0	4.5	6.0	6.75
Eosin. %	2.00	2.75	3.25	4.00	3.00	3.75	6.0	4.0	2.5	3.0	4.75	3.00	4.00	2.50	5.75	4.25	4.25	4.50	5.00	3.25
Baso %	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Prothrom Sec.	6.55	6.60	6.55	6.52	6.50	6.68	6.32	6.40	6.48	6.58	6.58	6.40	6.50	6.42	6.50	6.18	5.90	5.98	6.15	6.08
RBC 10^6/mm^3	5.68	5.46	5.67	5.48	5.59	5.90	6.23	6.39	6.30	6.26	6.31	6.38	7.12	6.90	6.74	6.52	6.62	7.07	6.78	6.76
HGB g/dL	14.07	13.25	13.57	13.70	13.95	13.92	14.67	14.80	15.12	15.20	15.42	15.50	17.15	17.12	16.62	16.50	16.55	17.55	17.80	17.50
HCT %	41.75	39.75	40.25	41.00	41.25	40.50	42.75	42.75	43.50	43.50	44.25	45.00	49.00	49.00	47.00	48.50	48.50	50.50	50.00	50.00
WBC 10^3/mm^3	10.67	11.60	11.20	9.77	11.22	12.95	10.80	11.22	10.55	11.90	13.05	9.77	11.02	11.60	12.27	12.37	9.80	11.00	10.52	12.77

Data extracted from report MIN 862010 table 8.7 pp. 103-142.

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Female Hematology Data

Parameter	Day -13					Day 85					Day 175					Day 359				
	0	20	100	500	1500	0	20	100	500	1500	0	20	100	500	1500	0	20	100	500	1500
Neutro. %	58.50	57.25	67.00	58.50	59.50	69.25	67.00	73.50	70.00	68.00	63.50	62.75	63.00	67.75	68.75	60.25	62.75	62.25	64.50	61.00
Lymph. %	32.50	30.25	23.50	29.00	30.25	20.00	25.00	19.75	18.00	26.25	23.50	27.25	26.00	20.75	21.50	30.75	28.75	29.75	23.25	27.25
Platelet $10^3/mm^3$	381.25	306.50	247.75	340.75	377.50	325.25	246.00	384.25	301.75	330.50	322.00	265.50	329.00	320.00	280.75	329.50	247.25	361.75	329.50	284.00
Histx %	0	0	0	0	0	0	-	-	-	0	0	-	-	-	0	0	-	-	-	0
Reticu. %	1.68	1.30	1.52	1.45	1.02	0.65	-	-	-	0.62	0.48	-	-	-	0.80	0.72	-	-	-	0.30***
NSeg %	0	0.75	0.25	0	0.75	0.75	0.50	0.50	2.25	1.75	1.25	0	0	0.25	1.0	0.25	0	0.50	0	0.25
Mono %	6.75	9.00	8.00	8.00	6.25	5.25	4.25	4.50	4.25	1.50	6.25	8.25	7.00	7.75	7.00	4.75	5.00	4.75	5.75	7.25
Eosin. %	2.00	2.75	1.25	4.50	3.25	4.75	3.25	1.75**	5.50	2.50	5.50	1.75	4.00	3.50	1.75	4.00	3.50	2.75	6.50	4.25
Baso %	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Prothrom Sec.	6.70	6.55	6.85	6.75	6.62	6.15	6.08	6.18	6.28	6.58	6.50	6.32	6.50	6.48	6.38	6.10	5.82	6.12	6.12	6.20
RBC $10^6/mm^3$	5.75	5.60	5.50	5.54	5.85	6.62	6.31	6.28	6.15	6.64	6.70	6.29	6.45	6.91	7.19	6.56	6.85	6.72	6.95	7.17
HGB g/dL	14.02	13.77	13.67	13.75	14.17	15.67	15.40	14.97	14.70	15.75	16.17	15.75	16.22	16.87	17.32	16.37	16.85	16.82	17.17	18.00
HCT %	41.50	42.00	41.00	41.00	41.75	45.75	44.75	43.75	43.00	45.75	46.00	44.25	45.25	47.75	49.50	47.75	49.25	49.50	50.25	52.50
WBC $10^3/mm^3$	10.35	8.35	9.77	9.25	9.05	10.55	10.22	12.32	10.10	9.87	10.57	11.42	12.12	13.02	9.47	9.80	7.62	9.47	9.47	9.40

Data extracted from report MIN 862010 table 8.7 pp. 143-182.

b. Clinical Chemistry

X	Electrolytes:	X	Other:
X	Calcium*	X	Albumin*
X	Chloride*	X	Blood creatinine*
	Magnesium*	X	Blood urea nitrogen*
X	Phosphorous*	X	Cholesterol*
X	Potassium*	X	Globulins
X	Sodium*	X	Glucose*
	Enzymes	X	Total bilirubin
X	Alkaline phosphatase (ALK)	X	Total serum Protein (TP)*
	Cholinesterase (ChE)#		Triglycerides
X	Creatinine phosphokinase*^		Serum protein electrophorese
	Lactic acid dehydrogenase (LAD)		
X	Serum alanine aminotransferase (also SGPT)*		
X	Serum aspartate aminotransferase (also SGOT)*		
	Gamma glutamyl transferase (GGT)		
	Glutamate dehydrogenase		

* Required for subchronic and chronic studies
# Should be required for OP
^ Not required for subchronic studies

Significant treatment related increases in mean alkaline phosphatase were noted throughout the study in the males receiving 1500 ppm and on day 359 in the males receiving 500 ppm. In the 6 month dog study previously conducted, at ≥ 3000 ppm increases in mean alkaline phosphatase were also observed. Females receiving 1500 ppm exhibited a dose related increase in mean sodium at day 359 only. Other statistically significant alterations in clinical chemistries were considered to be unrelated to treatment.

6. Urinalysis

Urine was collected from all animals at 2 and 3 weeks prior to dosing and during weeks 13, 26, and 53 of dosing. The CHECKED (X) parameters were examined.

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

* Required for chronic studies

There were no treatment related changes detected in the urinalysis in males or females exposed to the test substance.

Male Biochemistry Data

Parameter	Day -13					Day 85					Day 175					Day 359				
	0	20	100	500	1500	0	20	100	500	1500	0	20	100	500	1500	0	20	100	500	1500
Cholest mg/dL	187.0	171.5	164.8	177.5	176.0	178.5	171.5	134.2	192.2	167.0	169.5	160.5	153.8	194.8	170.2	187.0	174.8	153.5	200.2	186.8
Creat mg/dL	0.68	0.68	0.68	0.64	0.66	0.78	0.80	0.76	0.75	0.70	0.80	0.81	0.70	0.73	0.67	0.78	0.80	0.70	0.75	0.74
BUN mg/dL	11.25	12.25	11.25	11.00	11.25	13.25	14.00	12.25	13.25	14.50	15.25	15.50	11.75	14.00	12.25	15.50	13.75	11.00**	14.25	11.25**
Glucose mg/dL	98.50	101.75	100.75	99.25	103.25	92.50	96.50	89.75	90.00	90.75	102.25	100.50	93.50	101.00	96.75	91.75	92.00	88.75	83.50	92.00
Tot Bili mg/dL	0.18	0.19	0.19	0.18	0.20	0.18	0.19	0.26	0.15	0.14	0.28	0.36	0.35	0.37	0.34	0.10	0.12	0.22***	0.12	0.10
Tot Prot g/dL	5.02	5.10	5.08	4.80	4.88	5.58	5.85	5.70	5.32	5.45	5.65	5.82	5.85	5.58	5.72	6.10	6.35	6.22	5.98	6.20
Albumin g/dL	3.20	3.18	3.30	3.10	3.10	3.48	3.55	3.48	3.42	3.35	3.50	3.58	3.52	3.55	3.38	3.65	3.60	3.58	3.60	3.52
Globulin g/dL	1.82	1.92	1.78	1.70	1.78	2.10	2.30	2.22	1.90	2.10	2.15	2.25	2.32	2.02	2.35	2.45	2.75	2.65	2.38	2.68
CPK u/L	130.50	173.50	200.50	130.25	139.25	222.75	239.00	251.75	234.50	184.25	112.75	195.50	163.50	155.00	125.75	59.50	80.00	85.25	68.50	90.00
ALKPH u/L	110.75	129.25	131.25	93.00	90.75	88.00	108.00	107.75	94.00	125.00‡	62.50	70.00	79.50	89.25	123.50‡‡	41.50	55.25	57.75	64.25‡	100.75‡‡
SGOT u/L	18.50	27.75	26.50	21.00	26.00	28.50	34.00	34.25	31.25	31.75	23.75	31.25	27.25	28.50	27.50	21.00	27.75	26.25	24.75	27.75
SGPT u/L	19.25	23.00	21.25	18.75	19.25	21.75	25.50	25.50	21.75	23.50	24.25	25.25	26.50	24.75	27.50	20.50	24.25	25.00	27.50	23.50
ALB/GLOB ratio	1.75	1.67	1.85	1.87	1.75	1.70	1.57	1.60	1.80	1.60	1.62	1.60	1.57	1.77	1.45	1.47	1.30	1.40	1.55	1.32

Data extracted from report MIN 862010 table 8.8 pp. 183-218.

‡ $.01 < p \leq .05$, two tailed Dunnett t on differenced data.

‡‡ $p \leq 0.01$, two tailed Dunnett t on differenced data.

Female Biochemistry Data

Parameter	Day -13					Day 85					Day 175					Day 359				
	0	20	100	500	1500	0	20	100	500	1500	0	20	100	500	1500	0	20	100	500	1500
Cholest mg/dL	152.5	163.8	163.2	158.8	166.0	148.0	157.5	160.8	158.5	170.0	168.2	206.2	179.2	166.8	185.8	187.8	238.2	175.2	178.0	176.2
Creat mg/dL	0.62	0.66	0.65	0.67	0.67	0.72	0.74	0.74	0.67	0.79	0.74	0.81	0.74	0.72	0.83	0.70	0.75	0.73	0.68	0.75
BUN mg/dL	13.25	13.00	12.75	13.50	14.25	14.75	14.00	13.75	13.50	13.50	16.00	16.00	13.50	14.75	14.75	12.00	13.50	12.00	13.00	12.75
Glucose mg/dL	80.75	92.25	91.25	100.5	97.00	83.25	90.75	88.25	96.25	95.50	90.50	104.00	99.00	107.5	104.5	81.25	85.25	93.50	95.75	89.00
Tot Bili mg/dL	0.19	0.23	0.20	0.24	0.24	0.30	0.30	0.28	0.30	0.24	0.35	0.34	0.31	0.30	0.35	0.10	0.09	0.11	0.14	0.10
Tot Prot g/dL	5.05	5.12	5.20	4.88	5.12	5.85	5.70	5.78	5.42	5.60	5.78	5.70	5.78	5.62	5.62	6.15	6.25	6.12	6.15	6.05
Albumin g/dL	3.20	3.20	3.30	3.20	3.35	3.68	3.70	3.65	3.58	3.70	3.52	3.60	3.65	3.62	3.72	3.62	3.75	3.70	3.78	3.82
Globulin g/dL	1.85	1.92	1.90	1.68	1.78	2.18	2.00	2.12	1.85	1.90	2.25	2.10	2.12	2.00	1.90	2.52	2.50	2.42	2.38	2.22
CPK u/L	145.25	129.75	153.50	101.00	136.50	185.75	169.50	202.00	170.75	120.25	108.00	115.50	108.25	336.75	265.00	70.50	71.00	92.50	76.00	66.50
ALKPH u/L	122.50	80.25	103.25	112.25	122.50	105.00	72.50	98.75	92.75	94.75	71.00	68.00	76.75	104.25	79.25	52.25	51.75	55.50	77.00	66.75
SGOT u/L	23.25	19.75	21.25	15.00	21.50	31.00	26.50	32.75	26.00	26.00	26.50	25.25	25.00	33.75	26.00	24.25	17.75	26.25	24.50	21.75
SGPT u/L	20.25	18.25	19.75	16.50	16.50	24.25	22.25	23.50	20.25	20.00	22.25	23.25	23.25	26.50	21.75	18.25	17.25	22.50	21.50	21.25
ALB/GLOB ratio	1.70	1.67	1.75	1.90	1.92	1.70	1.85	1.75	1.92	1.97	1.60	1.75	1.72	1.82	1.97	1.45	1.50	1.52	1.57	1.75

Data extracted from report MIN 862010 table 8.8 pp. 219-253.

‡ $.01 < p \leq .05$, two tailed Dunnett t on differenced data.

‡‡ $p \leq 0.01$, two tailed Dunnett t on differenced data.

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Biochemistry Data

Parameter	Males																			
	Day -13					Day 85					Day 175					Day 359				
	0	20	100	500	1500	0	20	100	500	1500	0	20	100	500	1500	0	20	100	500	1500
Ca mg/dL	10.92	10.72	11.05	10.77	10.77	10.85	10.87	10.65	10.40	10.32	10.95	10.30	10.05*	10.67	10.42	10.45	10.35	10.17	10.00	10.12
Cl meq/L	106.75	106.25	105.50	106.00	106.50	100.50	107.75	108.50	107.25	108.25	106.00	107.00	103.00	106.00	105.25	110.25	111.75	108.50	110.75	111.00
P mg/dL	6.85	5.85	6.90	6.65	6.17	4.47	4.50	4.77	4.20	4.32	3.90	3.47	4.00	3.82	3.80	3.57	3.30	3.55	3.25	3.02
K meq/L	5.05	4.97	5.07	4.97	4.97	4.40	5.17*	4.67	4.55	4.52	4.52	4.55	4.62	4.65	4.80	4.65	4.55	4.65	4.55	4.52
Na meq/L	148.25	148.25	146.75	150.00	148.50	147.25	148.25	146.75	146.75	147.25	149.75	150.00	149.00	150.75	150.50	149.25	149.50	146.50*	148.50	149.50
	Females																			
Ca mg/dL	10.77	11.20	10.95	10.95	11.10	10.65	11.05	10.65	10.80	10.70	10.52	10.62	10.72	10.82	10.90	10.30	10.80	9.97	10.15	10.25
Cl meq/L	105.75	106.00	106.50	106.25	106.00	107.25	107.25	107.25	107.00	108.00	105.25	105.25	106.75	107.00	106.25	110.25	109.75	110.50	110.50	110.25
P mg/dL	6.52	6.77	6.97	6.00	6.87	4.47	4.60	4.72	4.55	4.32	3.87	3.97	3.90	3.45	3.45	3.30	3.65	3.40	2.82	3.35
K meq/L	4.95	4.72	5.17	4.77	4.80	4.50	4.52	4.72	4.47	4.32	4.37	4.50	4.67	4.47	4.12	4.60	4.57	4.77	4.52	4.45
Na meq/L	144.50	146.00	144.25	143.75	143.75	149.25	150.25	148.75	149.00	150.50	150.00	149.75	148.50	149.00	150.25	147.50	149.50	149.75	151.00	151.50*

Data extracted from report MIN 862010 table 8.8 pp. 254-273.

* $.01 < p \leq 0.05$, two tailed Dunnett t on raw data.

Urinalysis Data

Parameter	Males																			
	Day -14					Day 87					Day 177					Day 365				
	0	20	100	500	1500	0	20	100	500	1500	0	20	100	500	1500	0	20	100	500	1500
Specific Gravity	1.02	1.02	1.03	1.04	1.02	1.02	1.02	1.02	1.03	1.02	1.02	1.02	1.02	1.01	1.02	1.02	1.02	1.02	1.02	1.02
	Females																			
Specific Gravity	1.03	1.03	1.02	1.03	1.02	1.02	1.02	1.02	1.02	1.02	1.02	1.02	1.02	1.02	1.02	1.02	1.03	1.02	1.01	1.02

Data extracted from report no. MIN 862010 table 8.9 pp. 274-286.

Organ Weight Data

Organ	Absolute Weight									
	Males					Females				
	0	20	100	500	1500	0	20	100	500	1500
Final Bdy Wt (kg)	10.94	11.91	11.33	10.83	10.88	8.16	9.85	8.61	8.04	7.90
Brain (g)	78.30	83.11	80.85	79.79	86.44	75.31	76.54	72.50	78.24	77.47
Heart (g)	107.17	116.81	101.69	100.38	108.78	79.37	74.83	76.35	76.72	79.64
Adrenal (g)	1.39	1.05*	1.26	1.10	1.22	1.12	1.08	1.13	1.32	1.13
Kidney (g)	52.20	54.23	58.27	50.34	63.62	36.57	37.95	38.05	39.42	36.99
Liver (g)	355.93	368.86	343.35	342.50	382.32	259.34	286.68	285.09	289.90	241.70
Pituitary (g)	0.070	0.090	0.092	0.080	0.080	0.062	0.065	0.065	0.075	0.058
Spleen (g)	37.03	37.59	42.81	48.67	34.32	33.90	48.76	43.00	34.38	42.30
Testes/Epidid (g)	21.73	20.55	19.06	19.30	22.08	1.06	1.27	1.15	1.06	0.74
Thymus (g)	8.52	7.52	8.18	7.60	7.55	7.74	6.44	6.71	5.36	6.32
Thyroid/Para (g)	0.86	0.97	0.82	0.84	0.91	0.63	0.91*	0.66	0.75	0.68

Organ	Organ Weight as Percent of BW									
	Males					Females				
	0	20	100	500	1500	0	20	100	500	1500
Brain	0.722	0.705	0.718	0.738	0.802	0.936	0.780	0.859	0.986	0.980
Heart	0.982	0.983	0.901	0.925	0.997	0.989	0.770	0.891	0.964	1.00
Adrenal	0.013	0.009**	0.011	0.010*	0.011	0.014	0.011	0.013	0.016	0.014
Kidney	0.480	0.458	0.501	0.465	0.584	0.0455	0.390	0.452	0.492	0.467
Liver	3.28	3.13	3.02	3.16	3.53	3.21	2.95	3.36	3.65	3.06
Pituitary	0.0006	0.0008	0.0008	0.0007	0.0007	0.0008	0.0007	0.0008	0.0009	0.0007
Spleen	0.345	0.316	0.386	0.452	0.314	0.407	0.514	0.479	0.428	0.540
Testes/Epidid Ovary	0.201	0.173	0.171	0.178	0.203	0.013	0.013	0.013	0.014	0.009
Thymus	0.077	0.063	0.074	0.070	0.071	0.099	0.063	0.082	0.068	0.079
Thyroid/Para	0.008	0.008	0.007	0.008	0.008	0.008	0.009	0.008	0.010	0.009

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Data extracted from report MIN 862010 table 8.10, pp. 288-307.

* $.01 < p \leq .05$, two tailed Dunnett t on raw data.

** $p \leq 0.01$, two tailed Dunnett t on raw data.

7. Sacrifice and Pathology

All animals scheduled for sacrifice were fasted for at least 12 hours prior to necropsy. All animals that died and that were sacrificed on schedule were subject to gross pathological examination and the CHECKED (X) tissues were collected for histological examination. The (XX) organs, in addition, were weighed (paired organs were weighed as pairs).

X		X		X	
Digestive system		Cardiovasc./Hemat.		Neurologic	
	Tongue	X	Aorta*	XX	Brain*+
X	Salivary glands*	XX	Heart*	X	Periph. nerve*#
X	Esophagus*	X	Bone marrow*	X	Spinal cord (3 levels)*#
X	Stomach*	X	Lymph nodes*	XX	Pituitary*
X	Duodenum*	XX	Spleen	X	Eyes (optic n.)*#
X	Jejunum*	XX	Thymus*	Glandular	
X	Ileum*	Urogenital		XX	Adrenal gland*
X	Cecum*	XX	Kidneys*+		Lacrimal gland#
X	Colon*	X	Urinary bladder*+	X	Mammary gland (♀)*#
X	Rectum*	XX	Testes*+	XX	Parathyroids*++
XX	Liver *+	XX	Epididymides	XX	Thyroids*++
X	Gall bladder*	X	Prostate	Other	
X	Pancreas*		Seminal vesicle	X	Bone*#
Respiratory		XX	Ovaries*+	X	Skeletal muscle*#
X	Trachea*	X	Uterus*	X	Skin*#
X	Lung*	X	Vagina	X	All gross lesions and masses*
	Nose^				
	Pharynx^				
	Larynx^				

* Required for subchronic and chronic studies.
^ Required for chronic inhalation.
# In subchronic studies, examined only if indicated by signs of toxicity or target organ involvement.
+ Organ weight required in subchronic and chronic studies.
++ Organ weight required for non-rodent studies.

a. Organ weight -

The thymus organ weight data in table 8.10 is presented transposed (the organ weight relative to body weight appears under the relative to brain weight heading and vice versa) for males and females. Significant reductions in adrenal to body weight ratios were observed in males receiving 500 ppm. Significant reductions in adrenal weight (absolute and relative to body or brain weight) were observed in the males receiving 20 ppm. Females receiving 20 ppm exhibited significant increases in absolute thyroid/parathyroid weight as well as thyroid/parathyroid to brain weight, however, a dose-related change in any organ weight was not apparent.

b. Gross pathology -

Evaluation of the gross pathology data did not reveal any treatment related gross tissue alterations which could be attributed to CGA 169374.

c. Microscopic pathology -

The microscopic pathology data did not indicate the presence of treatment related lesions.

D. DISCUSSION:

Females receiving 1500 ppm in the diet had a significant reduction in body weight gain on day 7. Females in the 500 and 1500 ppm groups, although not statistically significant, had inhibited body weight gain throughout the study. These animals also had significant reductions in food consumption on days 7, 35, 70, and 357. The reduction in mean percent reticulocytes at the highest dose tested on day 359 may have been related to treatment. Significant increases (treatment related at day 85; dose-related at days 175 and 359) were observed in alkaline phosphatase in males receiving 1500 ppm.

E. Study Deficiencies:

1. The analytical report does not contain data pertaining to the purity of the test material.

2. The individual (raw) data for the clinical observations which coincide with table 8.2 of the report was not included.

F. Core Classification: **Core Supplementary Data.**

NOEL = 100 ppm
LOEL = 500 ppm

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FINAL

DATA EVALUATION REPORT

CGA 169374

Study Type: Metabolism

Prepared for:

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

Prepared by:

Clement International Corporation
9300 Lee Highway
Fairfax, VA 22031-1207

Principal Reviewer Pia Lindström Date 7/13/93
Pia Lindström, DPH

Independent Reviewer Karen N. Gan Date 7/13/93
Karen Gan, M.S.

QA/QC Manager Sharon E. Segal Date 7/13/93
Sharon Segal, Ph.D.

Contract Number: 68D10075
Work Assignment Number: 2-105
Clement Numbers: 324 and 325
Project Officer: Caroline Gordon

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GUIDELINE SERIES 85-1: Metabolism

EPA Reviewer: Jess Rowland, M.S. Signature: Jess Rowland
Review Section II, Toxicology Branch II/HED Date: 9/15/93

EPA Section Head: Clark Swentzel Signature: Susan Lewis for
Review Section II, Toxicology Branch II/HED Date: 9/16/93

DATA EVALUATION REPORT

STUDY TYPE: Metabolism in rats; Guideline Series 85-1

EPA IDENTIFICATION NUMBERS

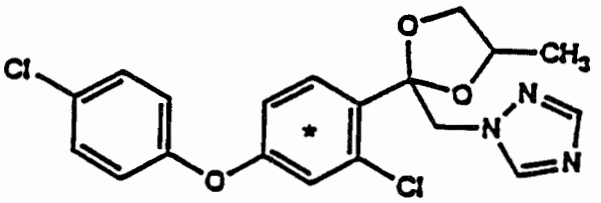
Tox. Chem. Number:

P.C. Code:

MRID Numbers: 427100-14; 427100-13

TEST MATERIAL: CGA 169374

CHEMICAL STRUCTURE:



*[^{14}C] labelling position

SYNONYM: 1-[2-[4-(-chlorophenoxy)-2-chlorophenyl-(4-methyl-1,3 -dioxolan-2-yl)-methyl]]-1H-1,2,4-triazole

SPONSOR: Ciba-Geigy Corporation, Greensboro, NC

TESTING FACILITIES: Tokai Research Laboratories, Ibaraki-ken, Japan (MRID No. 427100-14); Ciba-Geigy Corporation, Greensboro, NC (MRID No. 427100-13)

AUTHORS: Y. Esumi (MRID No. 427100-14)
T. M. Capps (MRID No. 427100-13)

TITLE OF REPORTS: 1) Absorption, Distribution and Excretion of CGA-169374 in Rats (MRID No. 427100-14); 2) Amendment to Characterization and Identification of Major Triazole-^{14}C and Phenyl-^{14}C CGA-169374 Metabolites in Rats (MRID No. 427100-13)

STUDY NUMBERS: AE-1488 (Report 1); ABR-90019 (Report 2)

REPORTS ISSUED: December 9, 1992 (Report 1); September 13, 1990 (Report 2)

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CONCLUSIONS: These studies were submitted because EPA requested additional information not provided in the Sponsor's previously submitted metabolism studies (MRID Nos. 420900-28/29/30/31). The present studies describe the absorption, distribution, and excretion, as well as pharmacokinetics, of ^{14}C-CGA 169374 after a single oral gavage dose of 0.5 or 300 mg/kg in rats (Report 1) and isolated and identified urinary metabolites in three females after a single oral gavage dose of 300 mg/kg (Report 2).

Following oral administration of 0.5 or 300 mg/kg ^{14}C-CGA 169374 in rats, the test compound was adequately absorbed and mainly eliminated via the bile; no evidence of bioaccumulation in any tissue was noted. After 48 hours, total recovery (independent of dose and sex) was ≈96% of the administered dose. Biliary excretion constituted the main route of elimination with some dose- and sex-dependency (≈75% at the low dose for both sexes; 56% for males and 39% for females at the high dose). Urinary and fecal eliminations exhibited a dose-related pattern at 48 hours. In the urine, ≈9%-14% was eliminated at the low dose versus 1% in the high-dose rats. In the feces, ≈2%-4% was eliminated at the low dose versus ≈17%-22% at the high dose. In cannulated males after 48 hours, ≈80% was eliminated via the bile, while ≈4% and ≈14% were eliminated via urine and feces, respectively. Therefore, this study indicates that most of the dose following oral administration is absorbed as indicated by the biliary excretion data. The dose-related difference in elimination suggests that saturation is reached at the higher dose level resulting in an increase of unabsorbed test material.

Maximum concentration in blood was reached within 2 hours at the low dose and 4 hours at the high dose. By 24 hours, <0.05 ppm equivalent was detected in the blood. Total recovery ranged from 95% to 97% after 48 hours, irrespective of dose and sex. During the first 12 hours, slight differences were evident between males and females with regard to Tmax, Cmax, and rate of elimination. The concentration in females was approximately half of that in males and was eliminated faster than in males. Mean half-lives in males and females from Tmax to 12 hours, were 6.2 and 4.4 hours, respectively; from 24 to 168 hours, they were 2.8 and 3.7 days, respectively.

Following administration of 300 mg/kg of [^{14}C-phenyl] CGA 169374, 3 major urinary metabolites were identified: sulfate conjugates (and their isomers) of HO-CGA 205375, isomers of HO-CGA 205375, and the hydroxyacetic metabolite of HO-CGA 205375. The major urinary metabolites of CGA 169374 have been identified and no single unknown metabolite accounted for >1.1% of the dose.

CORE CLASSIFICATION: These studies alone do not meet the minimum requirements for Guidelines 85-1. However, these studies combined with previously submitted studies (MRID Nos. 420900-28/29/30/31) are considered to be acceptable.

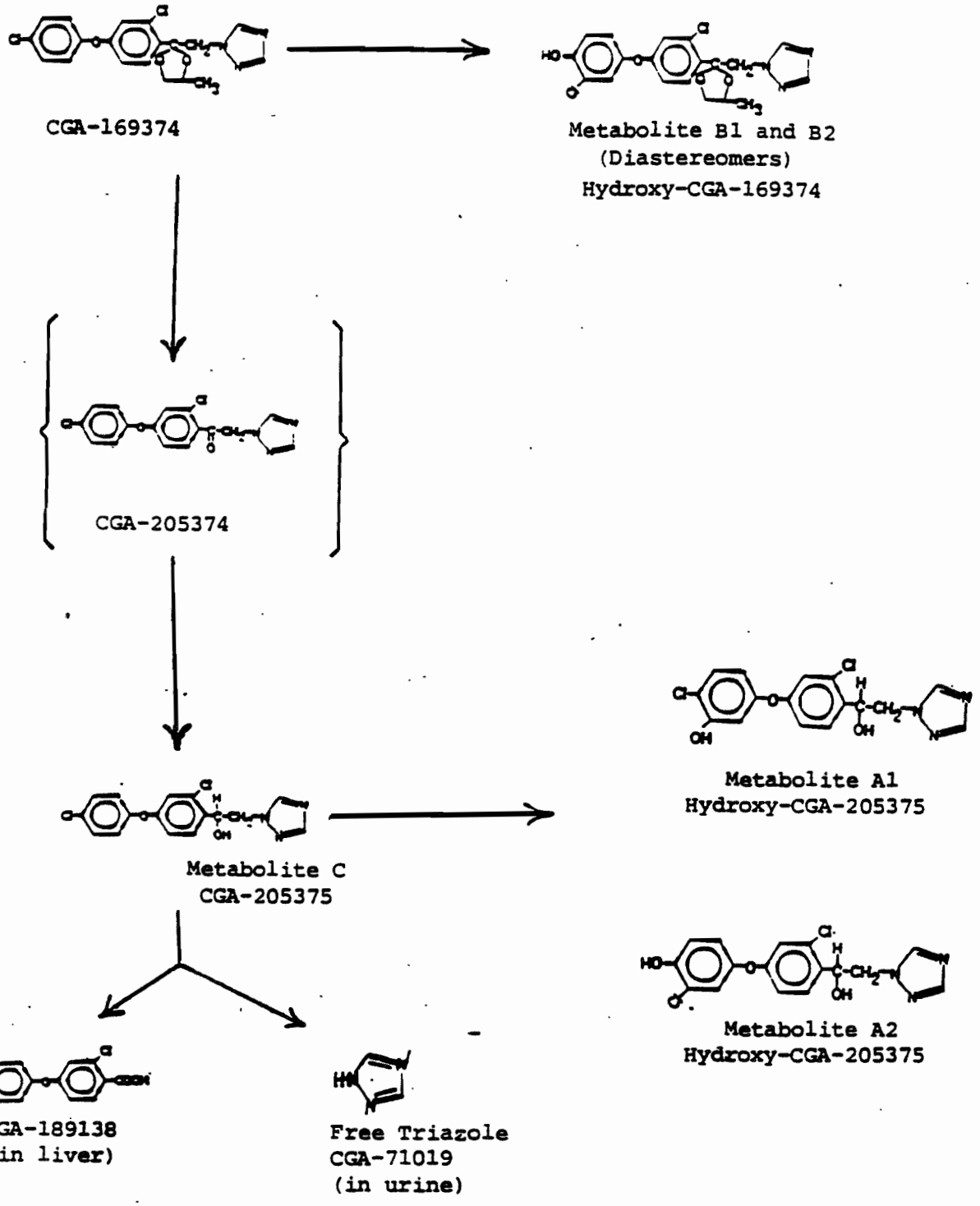

Figure 1. The Proposed Metabolic Pathway of CGA-169374
(metabolites were detected in feces except where indicated)

Source: CBI Report 1, Figure 22, p. 57

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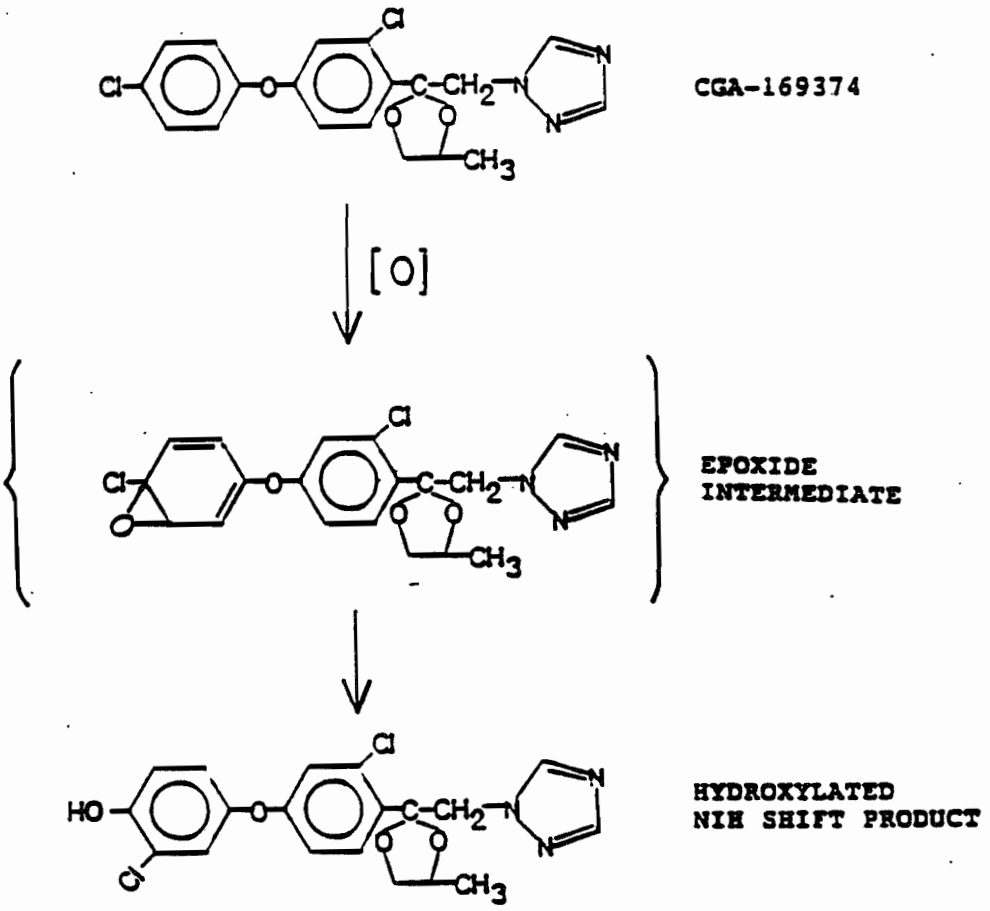

Figure 2. The Proposed Mechanism for Formation of Metabolites A and B by the NIH Shift

Source: CBI Report 1, Figure 21, p. 56

DOC930092

FINAL

009689

DATA EVALUATION REPORT

CGA-169374

Study Type: Metabolism

Prepared for:

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

Prepared by:

Clement International Corporation
9300 Lee Highway
Fairfax, VA 22031-1207

Principal Author Karen N. Gan Date 7/23/92
Karen Gan, M.S.

Reviewer Sanju Diwan Date 7/23/92
Sanju Diwan, Ph.D.

QA/QC Manager Sharon A. Segal Date 7/23/92
Sharon Segal, Ph.D.

Contract Number: 68D10075
Work Assignment Number: 1-79
Clement Number: 91-269, 91-270, 91-271, 91-272
Project Officer: James Scott

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Approved by:
EPA Reviewer: Karen E. Whitby, Ph.D.
Review Section II, Toxicology Branch II,
Health Effects ~~Division~~

Signature: [signature]
Date: 7/28/92

EPA Section Head: Clark Swentzel
Review Section II, Toxicology Branch II,
Health Effects Division

Signature: [signature]
Date: 8/3/92

DATA EVALUATION REPORT

STUDY TYPE: Metabolism

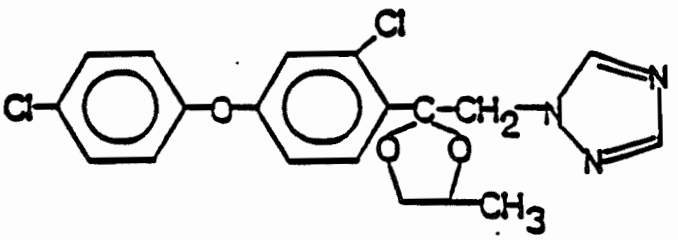
EPA IDENTIFICATION NUMBER:

Tox. Chem. Number:

MRID Numbers: 420900-28; 420900-29; 420900-30; 420900-31

TEST MATERIAL: CGA-169374

SYNONYM: 1-[[2-[2-Chloro-4-(4-chlorophenoxy)phenyl]-4-methyl-1,3-dioxolan-2-yl]-1H-1,2,4-triazole; difenoconazole.



The [^{14}C]-label was positioned at the phenyl or the triazole ring.

SPONSOR: Agricultural Division, CIBA-GEIGY Corporation, P.O. Box 18300, Greensboro, NC 27419

TESTING FACILITIES: Metabolism Department, CIBA-GEIGY Corporation, 410 Swing Road, P.O. Box 18300, Greensboro, NC 27419 and WIL Research Laboratories, Inc., Ashland, OH 44805-9281.

AUTHORS: Thomas Capps (Reports 1 and 4) and Elliott Raine (Reports 2 and 3)

REPORTS: 1. Characterization and Identification of Major Triazole-14C and Phenyl-14C-CGA-169374 Metabolites in Rats. Study Number ABR-90019. 109 pp. [MRID 420900-28]

2. Metabolism of Triazole-14C-CGA-169374 in Rats. Study Number WIL-82014. 84 pp. [MRID 420900-29]

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3. Metabolism of Phenyl-14C-CGA-169374 in Rats. Study Number WIL-82013. 85 pp. [MRID 420900-30]

4. Metabolism of Triazole-14C and Phenyl-14C-CGA-169374 in Rats - Distribution of Radioactivity. Study Number ABR-88043. 48 pp. [MRID-420900-31]

DATES OF COMPLETION: September 13, 1990 (Report 1); July 20, 1987 (Report 2 and 3); and April 25, 1988 (Report 4)

CONCLUSIONS: The absorption, distribution, metabolism, and excretion of CGA-169374 were studied in groups of male and female Sprague-Dawley rats. Animals were administered a single oral gavage dose of 0.5 or 300 mg/kg [^{14}C]CGA-169374, or 0.5 mg/kg unlabeled CGA-169374 by gavage for 14 days followed by a single gavage dose of 0.5 mg/kg [^{14}C]CGA-169374 on day 15. The test compound was labeled with [^{14}C] at either the phenyl or triazole ring.

[^{14}C]CGA-169374 was rapidly and extensively distributed, metabolized, and excreted in rats for all dosing regimens. The extent of absorption is undetermined pending determination of the extent of biliary excretion. The 4-day recoveries were 97.94-107.75% of the administered dose for all dosing groups. The elimination of radioactivity in the feces (78.06-94.61% of administered dose) and urine (8.48-21.86%) were almost comparable for all oral dose groups, with slightly higher radioactivity found in the feces of the high-dose group than the low-dose groups. This was probably due to biliary excretion, poor absorption or saturation of the metabolic pathway. The radioactivity in the blood peaked at about 24-48 hours for all dosing group. Half-lives of elimination appear to be approximately 20 hours for the low-dose groups and 33-48 hours for the high-dose group. The study results also indicate that CGA-169374 and/or its metabolites do not bioaccumulate to an appreciable extent following oral exposure since all the tissues contained negligible levels (<1%) of radioactivity 7 days postexposure.

The metabolism of CGA-169374 appears to be extensive because the metabolites accounted for most of the recovered radioactivity in the excreta. Three major metabolites were identified in the feces (i.e. metabolites A, B, and C). Two of the metabolites were separated into isomers (i.e., A1, A2, B1, and B2). Metabolite C was detected only in the high-dose groups, indicating that metabolism of CGA-169374 is dose-related and involves saturation of the metabolic pathway. Free triazole metabolite was detected in the urine of triazole-labeled groups and its byproduct was detected in the liver of phenyl-labeled groups only. Other urinary metabolites were not characterized.

These study results indicate that distribution, metabolism, and elimination of CGA-169374 were not sex related. There was a slight dose-related difference in the metabolism and elimination of CGA-169374. In phenyl- and triazole-labeling studies, fecal excretion of radioactivity was higher in the high-dose animals compared to the low-dose animals, and an additional metabolite was found in the feces of the high-dose animals compared to the low-dose animals. There were no major differences in the distribution and excretion of radioactivity with labeling at the phenyl and triazole ring positions, however, there were some different metabolites identified. The studies also showed that administration of 0.5 and 300 mg/kg CGA-169374 did not induce any apparent treatment-related clinical effects.

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STUDY CLASSIFICATION: The study is classified as Supplementary. This study may be upgraded if the following additional information is provided and is judged to be acceptable:

1. Determination of the fraction of dose excreted in bile after oral dosing (e.g., by cannulation). This determination appears to be the simplest way to assess absorption after oral dosing, given the plausibility of appreciable biliary excretion after dosing. Estimation of absorption after oral dosing is one of the primary purposes of the metabolism study; the present data allow only speculation as to the extent of this absorption.

2. Determination of metabolites present in the excreted bile of low and high dose animals (since different metabolites might be formed at the high dose). Fecal metabolites A-C may not necessarily reflect the results of biotransformation in the rat and may reflect the results of the action of the gut flora. With the available data, it is only possible to speculate as to the nature of the metabolites.

3. Identification of major peaks in urine or evidence that the metabolite identification are impractical. No specific attempts at identification were indicated in the study, in spite of the presence of at least two peaks in high-dose female urines in which each peak contained 4.0-5.0% of the dose.

SECTION HEAD

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

010583

SEPTEMBER 15, 1993

MEMORANDUM

OFFICE OF PESTICIDES AND TOXIC SUBSTANCES

SUBJECT **DIFENOCONAZOLE:** Registrant's Response to Deficiencies Cited in Toxicology Review.

FROM: Jess Rowland, M.S, Acting Section Head Jess Rowland 9/15/93
Section IV, Toxicology Branch II, Health Effects Division (H7509C)

TO: Cynthia Giles-Parker/Jim Stone
Product Manger 22, Registration Division

THRU: Marcia van Gemert, Ph.D., Chief Marcia van Gemert 9/16/93
Toxicology Branch II, Health Effects Division (H7509C)

TASK IDENTIFICATIONS: Submission: S438223 DP Barcode: D189836 PC Code: 128847

ACTION REQUESTED: Review Registrant's response to toxicology branch review of August 26, 1992 for a Import Tolerance [PP # 2E4051].

SUMMARY: The Registrant, Ciba-Geigy submitted toxicity studies on CGA-169374 [Difenoconazole, *Dividend 150FS*] in support of an Import Tolerance for Wheat, Barley and Rye [PP # 2E4051]. Toxicology Branch-II completed the review on August 26, 1992 [Memo:K. Whitby, HED to J.Stone, RD dated 8/26/92; HED Document No. 009689]. In this review, the primary dermal irritation study [81-5], the dermal sensitization study in rabbits [81-6], the chronic toxicity study in dogs [83-1b], the carcinogenicity study in mice [83-2b], the combined chronic toxicity/ carcinogenicity study in rats [83-5], the developmental toxicity study in rats [83-3a] and rabbits [83-3b], and the two-generation study in rats [83-4] were Core classified as Supplementary due to the lack of test article characterization and/or other reasons. It was stated that these studies may be upgraded after satisfactory review of raw data on the purity of the test material used and the concentration, stability and homogeneity analyses of the test diets used in these studies. Additional information was also requested for the general metabolism study [85-1] that was Core classified as unacceptable. The mutagenicity studies for structural chromosomal aberrations [84-2] and other genotoxic effects [84-4] were also Core classified as unacceptable [HED Document No. 009689]

In this submission, the Registrant provided: (1) data on the test article characterization which included the purity, stability, homogeneity, and concentration analyses; (2) the additional information requested for a metabolism study; and (3) submitted two new mutagenicity studies. The information provided in this submission are satisfactory and adequate to addres the toxicological issues raised in the original review. Consequently, the aforementioned studies Core classified supplementary are upgraded to minimum, satisfy the guideline requirements for 81-5, 81-6, 83-1b, 83-2b, 83-3a & b, 83-4, and 83-5, and are acceptable for regulatory purposes. The two new mutagenicity studies are Core classified as acceptable and satisfy the guideline requirements 84-2b and 84-4 and are acceptable for regulatory purposes. The toxicology data base is complete for technical difenoconazole.

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I. BACKGROUND

The Registrant, Ciba-Geigy, submitted toxicity studies on CGA-169374, technical [Difenoconazole, *Dividend 150FS*] in support of an Import Tolerance for Wheat, Barley and Rye [PP # 2E4051]. In the toxicology review of the submitted data, the primary irritation study [81-5], the dermal sensitization study [81-6], the chronic toxicity study in dogs [83-1b], the carcinogenicity study in mice [83-2], the combined chronic toxicity/ carcinogenicity study in rats [83-5], the developmental toxicity study in rats [83-3a] and rabbits [83-3b], and the two-generation study in rats [83-4] were Core classified as Supplementary due to the lack of test article characterization. It was stated that these studies may be upgraded after satisfactory review of raw data on the purity of the test material used and the concentration, stability and homogeneity analyses of the test diets used in these studies. In addition, the general metabolism study [85-1] and the mutagenicity studies 84-2b and 84-4 were classified as unacceptable [HED Document No. 009689; Memo:K. Whitby to C.Giles-Parker, 8/26/92].

II. AGENCY'S REVIEW OF REGISTRANT'S RESPONSE

In this submission, the registrant provided data on the test article characterization which included purity of the test article and the concentration, stability and homogeneity analyses. Additional data/information was also submitted to upgrade the general metabolism study. The MRID No. cited after the study type is the MRID No. assigned for the original study/review. The MRID No. cited after the response is the MRID No. assigned to the registrant's response in this submission.

1. § 81-5: Primary Dermal Irritation Study [MRID # 420900-10]

   Deficiency: Lack of purity of the test article and the size of treated test site.

   Response: The test article [technical, ID # FL 881994] was 91.5% pure and the test material was applied to an approximate 6.25 cm^2 area on each animal's shaved back [MRID No. 427100-03]

   Core Classification: This study classified as supplementary is upgraded to minimum and satisfies the guideline requirement [81-5] for a dermal irritation study in rabbits and is acceptable for regulatory purposes.

2. § 81-6: Dermal Sensitization Study [MRID # 420900-11]

   Deficiency: Data on the purity and stability of the test article.

   Response: The test article [technical, ID # FL 851406] was 94.5% pure and stability analyses indicated the material to be stable at room temperature for up to 24 months [MRID No. 427100-04]

   Core Classification: This study classified as supplementary is upgraded to minimum and satisfies the guideline requirement [81-6] for a dermal sensitization study in guinea pigs and is acceptable for regulatory purposes.

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3. § 83-1(b): Chronic Toxicity Study in Non-Rodents [MRID # 420900-14]

Deficiencies: Data on the purity and stability of the test article and individual [raw] data for the clinical observations.

Response: The test article [technical, ID # FL 851406] was 94.5% pure and stability analyses indicated the material to be stable at room temperature for up to 24 months. Individual clinical observations presented showed no treatment-related clinical signs; dogs in all groups including the controls exhibited emesis, mucoid feces, and/or diarrhea [MRID No. 427100-05].

Core Classification: This study classified as supplementary is upgraded to minimum and satisfies the guideline requirement [83-1b] for a chronic toxicity study in non-rodents and is acceptable for regulatory purposes.

4. § 83-2(b): Carcinogenicity Study in Mice [MRID # 420900-15]

Deficiencies: Data on the purity, concentration, stability and homogeneity analyses of the test diets, omission of cageside observation on eight different days and lack of slides for differential counts for six mice.

Response: The purity of the test article [technical] was 94.5% for ID # FL 851406 and 95.2% for ID # FL861408. Results of the concentration analyses are shown below:

Target Dose [ppm]	% Target		Mean
	Low	High	
10	87	118	101 ± 8
30	85	119	99 ± 8
300	93	112	100 ± 5
2500	80	111	97 ± 6
4500	85	108	99 ± 5

Stability analyses indicated that a 10-ppm level was stable in the diet stored at room temperature for at least 16 days [diets were prepared fresh weekly during the study]. The Day 16 analysis were 98% and 97% of target levels of the A and B samples, respectively. Homogeneity analyses indicated that each mix, i.e., top, middle, and bottom, were less than 10% of the target levels, which was indicative of homogenous mixes for all but the 10 ppm mix. The sample variability for the 10-ppm mix was 12%. Homogeneity analyses ranged from 92.9 to 107.1% of the target levels. The oversight in cageside observations and the slide preparations were indicated as Protocol Deviations in the original report in Appendix 11 and were not considered to have an adverse effect on the outcome of the study [MRID No.427100-06].

Core Classification: This study classified as supplementary is upgraded to minimum and satisfies the guideline requirement [83-2b] for a carcinogenicity study in mice and is acceptable for regulatory purposes.

5. § 83-3(a): Developmental Toxicity Study in Rats [MRID # 420900-16]

Deficiencies: Lack of purity, stability and homogeneity analyses and a deficiency in reporting information for the concentrations analyses. Under analytical results, the concentration found was reported in mg/g, while the target concentration was reported in mg/mL. The report did not provide a means of conversion [i.e., density or the weight of suspension which is equal to 1 mL]. This in addition to the purity, would permit a more accurate assessment of the % deviation from the target dose.

Response: The test article [technical, ID # FL 851406] was 94.5% pure and stability analyses indicated the material to be stable at room temperature for up to 24 months. The dosing suspensions were prepared weekly for each of two weeks of dosing and were analyzed on the first and last day of dosing. The suspensions were stirred continuously during dosing. Analytical data showed consistent results for both the first and the last day and between the first and second week suspensions for each dose. With the exception of the 20 mg/L concentration which changed from -19.5% on the first day to -3.5% on the last day, the percent deviation from target for the samples on the first and last day of use did not differ substantially. Had the suspension not been homogenous, the difference between the two days of sampling would have been much larger. Inadequacy in the analytical data were due to difficulties experienced during the analyses. For example, the preparation of the sample for analyses involved resuspension of a small sample and pipetting of this sample. The freezing and thawing of the sample in a capped container allowed some of the test article to deposit on the cap and sides of the sample bottle which were not rinsed into the bottle prior to sampling for analyses. These procedures resulted in providing levels that were below target. Had the procedure included a rinse of the sample vial with a solvent to ensure that the entire sample was analyzed [i.e., based on weight of the entire sample], the analytical results would have been with a few percent of target value. In addition, the conversion of the mg/kg target value for analytical results to the target mg/mL target value would result in 2 to 5% variation below target value because there was no measurement of specific gravity to make the conversion. It is clear that the difficulties associated with the analyses of the samples of the dosing suspensions resulted in analytical values below target values; but the consistency of the analytical results showed that the suspensions were homogeneous, the test article was stable at room temperature, and there were no questions of study conduct or results [MRID No. 427100-07].

Core Classification: This study classified as supplementary is upgraded to minimum and satisfies the guideline requirement [83-3a] for a developmental toxicity study in rats and is acceptable for regulatory purposes.

6. § 83-3(b): Developmental Toxicity Study in Rabbits [MRID # 420900-17]

Deficiencies: The following information was not provided in the original report: frequency of the preparation of the dosing solution; storage conditions for the test article during the study; purity of the test compound; homogeneity analyses; historical control data [with the exception of fetal weights]; route of administration for the historical control animals; and source or strain of animals used in the historical control data.

Response: The test article [technical, FL 851406] was 94.5% pure and stability analyses indicated the material to be stable at room temperature for up to 24 months.

The dosing suspensions were prepared weekly for each of two weeks of dosing and were analyzed on the first and last day of dosing. The suspensions were stirred continuously during dosing. Three samples were collected from each concentration of test article and the results indicated that the suspensions were uniform. Historical control data were provided for fetal weights only to justify the statement made in the report that the fetal control values obtained in this study were higher than those normally seen in the test laboratory. Historical control data are not routinely provided unless they are needed to justify statement made in the study report. Rabbits used in the studies identified in the historical control data were New Zealand White obtained from HARE-Marlan,I Hewitt, N.J. The route of administration was gavage and the studies were conducted between 1985 and 1987 [MRID No. 427100-08].

In the final report the Healy analysis employed for fetal body weights showed the probability value for the F statistics to be 0.072 for male fetal weights and 0.104 for female fetal weights. However, this analysis utilized a procedure of performing pairwise comparisons between treated and control groups irrespective of whether the overall F test was statistically significant. Therefore, results of an ANOVA on fetal body weights using litter size as the covariable was submitted. The probability was 0.0842 for male fetal weights and 0.0689 for female weights, indicating no statistical significant difference when litter size was considered. Consequently, there were no treatment-related effects on fetal body weights, and any apparent differences in weights were likely due to the effects of the variation in average litter size [MRID No. 427100-08].

Core Classification: This study classified as supplementary is upgraded to minimum and satisfies the guideline requirement [83-3b] for a developmental toxicity study in rabbits and is acceptable for regulatory purposes.

7. § 83-4: Two-Generation Reproductive Toxicity Study in Rats [MRID # 420900-18]

Deficiencies: Lack of purity and stability data.

Response: The test article [technical, ID # FL 851406] was 95.5% pure and stability analyses indicated the material to be stable at room temperature for upto 24 months [MRID No.427100-09].

Core Classification: This study classified as supplementary is upgraded to minimum and satisfies the guideline requirement [83-4] for a two generation reproductive toxicity study in rats and is acceptable for regulatory purposes.

8. § 83-5: Combined Chronic Toxicity/Carcinogenicity Study in Rats [MRID # 420900-19]

Deficiencies: Data on the purity, concentration, stability and homogeneity analyses of the test diets were not provide. Bone was not examined histologically. Thyroid/parathyroid was not weighed and blood creatinine level was not measured.

Response: The purity of the test article [CGA-169374, technical, ID # FL851406] was 94.5%. Results of the concentration analyses are shown below:

Target Dose [ppm]	% Target		Mean
	Low	High	
10	75	112	96 ± 8
20	87	109	97 ± 5
500	84	111	97 ± 4
2500	89	111	99 ± 5

Results of the stability analyses indicated that a 10-ppm level was stable in the diet stored at room temperature for at least 16 days [diets were prepared fresh weekly during the study. Results of the Day 16 analysis were 98% and 97% of target levels of the A and B samples, respectively. Homogeneity analyses indicated that each mix, i.e., top, middle, and bottom, ranged from 87 to 106% of the target levels. The Agency does not concur with the registrant that the bone is not required for histopathology. As stated in the Subdivision F Guidelines [1984, Page 144, EE,-- sternum and/or femur with bone marrow], the bone is a required tissue for histopathological examination. However, the lack of histopathology of the bone in this study does not affect the study since no gross lesions were seen to indicate any treatment-related effects. Concur with the registrant that the Thyroid/parathyroid is not required as an organ weight and creatinine is supplemental and may not be required for every study. [MRID No.427100-10].

Core Classification: This study classified as supplementary is upgraded to minimum and satisfies the guideline requirement [83-5] for a combine chronic toxicity/ carcinogenicity study in rats and is acceptable for regulatory purposes.

9. § 85-1 General Metabolism [420900-28/29/30/31]

Deficiencies: 1) Determination of the fraction of dose excreted in bile after oral dosing. 2) Determination of metabolites present in the excreted bile of low and high dose animals since different metabolites might be formed at the high dose. 3) Identification of major peaks in urine or evidence that the metabolite identification were indicated in the study, in spite of the presence of at least two peaks in high-dose female urine in which each peak contained 4-5% of the dose.

Response: The registrant submitted studies describing the absorption, distribution, and excretion, as well as the pharmacokinetics of the test article after a sing oral gavage doses of 0.5 or 300 mg/kg in rats [MRID No. 427100-13] and isolated and identified urinary metabolites in three females after a single oral gavage dose of 300 mg/kg [MRID No. 427100-14]. A Data Evaluation Report is attached.

Core Classification: This study classified as supplementary is upgraded to acceptable and satisfies the guideline requirement [85-1] for a general metabolism study in rats and is acceptable for regulatory purposes.

III. REVIEW OF THE NEW MUTAGENICITY STUDIES

Since an UDS assay in rat hepatocytes [MRID No. 420900-27] and a UDS assay in human fibroblasts [MRID No. 420900-26] were Core classified as unacceptable [HED Document No. 009689], the registrant submitted two new mutagenicity studies to satisfy guideline requirement 84-2(b) & 84-4. A Data Evaluation Report for these two new studies are attached and a summary is provided below:

1. § 84-2(b) *In Vivo* Mammalian Bone Marrow Cytogenetic Test: Chromosomal Analysis [MRID No. 427100-11

In an *In Vivo* micronucleus assay, no increase in micronucleated polychromatic erythrocytes occurred in mice given oral administration of difenoconazole [technical, 91.2%] at 0, 400, 800 or 1600 mg/kg and sacrificed at 16, 24 or 48 hours post-treatment.

Core Classification: This study classified as acceptable and satisfies the guideline requirement [84-2b] for Category II, Structural Chromosomal Aberration and is acceptable for regulatory purposes.

2. §84-4 Other Genotoxic Effects: UDS Assay in Rat Hepatocytes [MRID No. 427100-12]

Difenconazole [technical, 92.2%] was negative in an UDS assay with primary rat hepatocytes as measured by an autoradiographic methods at concentrations up to 50.0 μg/mL/.

Core Classification: This study classified as acceptable and satisfies the guideline requirement [84-4] for Category III, Other Genotoxic effects and is acceptable for regulatory purposes.

IV. CONCLUSIONS

The information provided in this submission are satisfactory and adequate to address the toxicological issues raised during the initial review. The studies Core classified supplementary for guideline requirements 81-5, 81-6, 83-1b, 83-2b, 83-3a & b, 83-4, 83-5] are upgraded to minimum and are acceptable for regulatory purposes. The two new mutagenicity studies for Guideline requirements 84-2b and 84-4 are Core classified as acceptable and are acceptable for regulatory purposes. In all, the toxicology data base is complete for technical difenoconazole. There are no data caps.

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DIFENOCONAZOLE

429728-03

ASSESSMENT OF THE LIVER TUMORS OBSERVED IN CD-1 MICE FED EXCESSIVE LEVELS OF DIFENOCONAZOLE (CGA 169374): A MITOGENIC RESPONSE

AUTHOR - J.T. STEVENS

COMPLETED ON OCTOBER 7, 1993

VOLUME 1 OF 1 OF STUDY

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