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

OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

AUG 2 6 1992

MEMORANDUM

SUBJECT: Petition for Tolerances of Difenoconazole in Imported Wheat, Barley, and Rye Grain Raw Agricultural Commodities

TO: James Stone PM 22
RD (H7505C)

FROM: K. E. Whitby, Ph.D. 8/10/92
Section, II
Toxicology Branch II/(HED) (H7509C)

THRU: K. Clark Swentzel 8/11/92
Section Head
Toxicology Branch II/(HED) (H7509C)

and

for Marcia van Gemert, Ph.D. James N. Rowe 8/11/92
Chief, Toxicology Branch II/(HED) (H7509C)

128847

DP Barcode: D172066 Shaughnessy No.: 128847
Case: 283001 Submission No.: S407977
Chemical: 1-{2-[4-(-Chlorophenoxy)-2-chlorophenyl]-4-methyl-1,3-dioxolan-2-yl-methyl)-1H-1,2,4-triazole
Synonyms: Difenoconazole, CGA-169374, Dividend® 150FS

Action Requested

Review studies in support of an import tolerance request for residues of a new fungicide, Difenoconazole, in wheat, barley, and rye.

Study Title and Conclusions

000101437

§81-1: Acute Oral Toxicity Study in Rats

MRID NUMBER: 420900-06

The Acute Oral LD_{50} in males = 1,453 mg/kg
95% Confidence Limit = 933 - 2,263 mg/kg

The Acute Oral LD_{50} in females = 1,453 mg/kg
95% Confidence Limit = 933 - 2,263 mg/kg

Core Minimum / Tox Category III

§81-2: Acute Dermal Toxicity Study in Rabbits

MRID NUMBER: 420900-07

The acute dermal LD_{50} of CGA-169374 for male and female rabbits was estimated to be > 2,010 mg/kg. The report does not provide the purity of the test material. The sponsor should provide this information for the record.

Core Classification: Core Minimum **Toxicity Category: 3 Caution**

§81-3: Acute Inhalation Study in Rats

MRID NUMBER: 420900-08

The 4-hour LC_{50} for males and females was > 3,300 mg/m^3.

Core Minimum / Tox Category III - Caution

§81-4: Primary Eye Irritation Study in Rabbits

MRID NUMBER: 420900-09

CGA-169374 Technical is classified as "moderately irritating" to the ocular tissue of the rabbit based on corneal and iridal involvement and moderate-to-severe conjunctival irritation that was reversed in all rabbits by 96 hours postapplication.

Core Minimum / Tox Category III - Caution

§81-5: Primary Dermal Irritation Study in Rabbits

MRID NUMBER: 420900-10

CGA-169374 is a slight irritant to the skin of rabbits. The calculated FIFRA Primary Irritation Index for the test article was 0.1.

Core Minimum / Tox Category IV - Caution

§81-6: Dermal Sensitization Study in Guinea Pigs

MRID NUMBER: 420900-11

A 0.5 g topical application of CGA-169374 Technical did not result in delayed contact hypersensitivity in guinea pigs. The study was classified as supplementary because the purity and stability of the test material were not reported.

Core Supplementary

§82-1a: Subchronic Toxicity/Metabolism Study in Rats

MRID NUMBER: 420900-22

CGA-169374 Technical was administered orally in feed admixtures to six groups of rats of both sexes at 0, 20, 200, 750, 1500, 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 weight in both sexes, and the 200 ppm female rats showed an approximate 10% decrease in body weight relative to their controls concomitant with decreased feed consumption. There was one dose related effect of the chemical discovered during the histopathology examination, that identified modest diffuse hepatocellular enlargement, that is - 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 food intake. The somewhat compromised nutritional status of the rats could possibly and indirectly have promoted the hepatocellular enlargement as well.

The LOEL was set at 200 ppm based on the 10% decrease in body weight in the 200 ppm females (as well as a negative trend in feed consumption). The NOEL was 20 ppm.

Core Minimum

§82-1a: Subchronic Toxicity/Metabolism Study in Mice

MRID NUMBER: 420900-21

CGA 169374 technical was fed for mice at 0, 20, 200, 2500, 7500 or 15,000 ppm (both sexes with 15 animals/sex/group) for 13 weeks. The NOEL was 20 ppm. The LEL was 200 ppm based upon body weight changes and liver histopathology. Most of the mice receiving 7,500 or 15,000 ppm died during the first week of the study. Statistical analysis of feed consumption and body weight changes revealed decreases for paired 2500 ppm females. Hepatotoxicity in mice that died during the study was evidenced by hepatocellular enlargement and necrosis of individual hepatocytes. Hepatotoxicity in surviving animals was observed as hepatocellular enlargement in the 200 ppm males and the 2500 ppm groups, and as hepatocytic vacuolization of the 2500 ppm animals. In addition, coagulative necrosis was observed in the livers of 4/9 group 4 females. Because the foci were small and random this finding was not thought to be related to treatment. The unscheduled deaths in groups 5 or 6 had a high incidence of changes consistent with stress.

Core Minimum

§82-1b: 26-Week Oral Toxicity Study in Dogs

MRID NUMBER: 420900-12

NOTE: REVIEW WAS PREVIOUSLY SUBMITTED TO RD BECAUSE OF THE 6(a)2 CLASSIFICATION; WHITBY TO STONE, APRIL 17, 1992

CGA 169374 technical was administered in the diet to beagle dogs at 0, 100, 1000, 3000, or 6000 ppm for a minimum of 28 weeks. The LOEL and the NOEL respectively were, 3000 ppm and 1000 ppm. The most outstanding effect was bilateral lenticular cataracts, which was observed in 3 out of 3 animals/sex in the 6000 ppm group by ophthalmoscopic examination. The incidence in this group by microscopic exam was 3/3 females with bilateral cataracts and 2/3 males with unilateral cataracts and 1/3 males with bilateral cataracts (3/3 males were observed to have cataracts). One female fed 3000 ppm was observed to have bilateral cataracts by ophthalmoscopic examination. Microscopic examination found the incidence in males to be 1/3 with bilateral cataracts and the incidence in females to be 2/3 with unilateral cataracts and 1/3 with bilateral cataracts. Ocular effects were not observed at dietary levels $\leq$ 1000 ppm. Additional findings included iridic changes secondary to induced uveitis, reductions in mean body weight and food consumption, and alterations in hematology and clinical chemistry.

Core Minimum

§82-2: 21-Day Dermal Toxicity Study in Rabbits

MRID NUMBER: 420900-13

CGA 169374 technical was administered topically under occlusion to three groups of female and male rabbits (5/group/sex) at daily doses of 10, 100, or 1000 mg/kg for a minimum of 22 consecutive days. There were two controls used vehicle (ethanol) and naive. The NOEL was 10 mg/kg. The LEL was 100 mg/kg. Statistically significant reductions in body weight gain, and food consumption resulted from exposure to doses $\geq$ 100 mg/kg. There were no deaths. Mild to moderate skin irritation localized to the site of application of the vehicle or test article was observed via macroscopic and microscopic observation. Females exposed to 1000 mg/kg exhibited increased adrenal weights and vacuolation of hepatocytes.

Core Minimum

§83-3a Developmental Toxicity Study of CGA-169374 Technical (FL-851406) Administered Orally Via Gavage to Crl:COBS®CD®(SD)BR Presumed Pregnant Rats

MRID NUMBER: 420900-16

CGA 169347 technical was administered by gavage on days 6-15 of gestation to presumed pregnant rats at 0, 2, 20, 100, or 200 mg/kg. Significant decreases in maternal body weight gain and feed consumption were observed during the dosing period for the 100 and 200 mg/kg groups. These animals also exhibited a significant increase in the incidence of excess salivation. There was a non significant

decrease in the mean number of fetuses per dam, and non significant increases in the mean number of resorptions per dam and % postimplantation loss in the 200 mg/kg group. There was a slight (non significant) decrease in mean fetal body weight at the 200 mg/kg group. There were significant alterations in the development of fetuses in the 200 mg/kg group. The following represents these findings. The incidence of bifid or unilateral ossification of the thoracic vertebrae was significantly increased on the fetal basis. There were also significant increases in the average number of ossified hyoid and decreases in the average number of sternal centers of ossification (per fetus per litter). The average number of ribs was significantly increased (with accompanying increases in the number of thoracic vertebrae), and decreases in the number of lumbar vertebrae in this group. These findings may be related to maternal toxicity. This study may be upgraded after satisfactory review of the response to the noted deficiencies.

NOTE: Due to the relatively high percent deviation of the actual doses tested from the theoretical concentration the effect levels have been modified accordingly. This modification may be subject to change as the purity is currently unknown.

Maternal NOEL = 16 mg/kg
Maternal LOEL = 85 mg/kg
Developmental Toxicity NOEL = 85 mg/kg
Developmental Toxicity LOEL = 171 mg/kg

Core Supplementary

Study Deficiencies:

1. The purity of the test substance was not provided.

2. The stability of the test substance was not provided, although data for the stability of the test substance in the vehicle was provided.

3. It is not apparent that an analyses of the homogeneity was performed. This is of concern due to the relatively high percent deviation of the actual doses tested from the theoretical concentration.

4. There is a deficiency in reporting information for the concentration analyses. In table 1 of Appendix D (Analytical Results) the concentration found is reported in mg/g, whereas the target concentration is reported in mg/mL. The report does 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.

§83-3b: CGA-169374 Technical Teratology Study in Rabbits

MRID NUMBER: 420900-17

CGA 169347 technical was administered by gavage on days 7-19 of gestation to presumed pregnant rabbits at 0, 1, 25, or 75 mg/kg. Maternal toxicity was observed in this study as the death of one doe and abortions observed in two other high dose does. In addition, significant reductions in body weight gain of high dose does, were present days 7-10, 10-14, 7-20, and 0-29. These reductions correspond

with reduced feed consumption during these intervals (significant reductions in feed consumption in the HDT were only observed during the treatment period, not after treatment). Slight nonsignificant increases in postimplantation loss and resorptions/doe were observed in the HDT. The significant decrease in fetal weight at the HDT may have been due to treatment. The significant differences in fetal weight observed at the low and mid dose were apparently not due to treatment.

Maternal NOEL = 25 mg/kg
Maternal LOEL = 75 mg/kg
Developmental Toxicity NOEL = 25 mg/kg
Developmental Toxicity LOEL = 75 mg/kg

Core Supplementary

This study does not satisfy the guideline requirements (83-3) for a developmental toxicity study due to deficiencies. This study may be upgraded after a satisfactory review of the noted deficiencies.

Study Deficiencies:

1. The frequency of preparation of dosing solutions was not stated. The storage conditions of the test article during the study were not provided.

2. The reports for the analyses of the purity of the test article and its homogeneity in the vehicle were not provided.

3. The available historical control data only pertains to weighted fetal weight obtained from Healy Analysis. Data were not presented for the other parameters evaluated in this investigation: maternal body weight/body weight gain, feed consumption, cesarean section observations (pregnancy rates, implantation data, % pre-and post-implantation loss), gross external exam findings, soft tissue findings, or skeletal findings. Furthermore, the available data do not indicate the source or strain of animals for which the data are presented. The historical control data do not indicate the route of administration or the vehicle these animals were treated with, nor the dates of the studies.

§83-4: Two-Generation Reproductive Study in Albino Rats

MRID NUMBER: 420900-18

CGA 169347 technical was administered in the diet to male and female rats at 0, 25, 250, or 2500 ppm. Significant reductions in F_0 and F_1 male body weight gain were observed at 2500 ppm during days 0-77 and overall (terminal body weight minus day 0 body weight). Significant reductions in F_0 and F_1 body weight gain of females in the 2500 ppm group were detected during the pre-mating, gestation, and lactation periods. In addition, as indicated in table 4 the 250 ppm F_0 females had reductions (statistically nonsignificant) in body weight gain which appear to be part of a dose-related trend days 70-77 prior to mating, days 0-7 of gestation, and days 7-14 of lactation. Significant reductions in pup body weight were detected days 0, 4 (pre- and post culling) 7, 14, and 21 for males and females (day 0 female F_1 were not significant) in the 2500 ppm group of both generations. There was a significant reduction in the body weight of F_1 male pups at day 21 in the 250 ppm group.

The percentage of male pups in the F_1 generation surviving days 0-4 was significantly reduced in the 2500 ppm group.

This study may be upgraded after the registrant satisfactorily provides the purity and stability of the test article.

Parental Systemic NOEL = 25 ppm
Parental Systemic LOEL = 250 ppm
Reproductive NOEL = 25 ppm
Reproductive LOEL = 250 ppm

Core Supplementary

§83-1b: Chronic Toxicity Study in Dogs

MRID NUMBER: 420900-14

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).

Core Supplementary

§83-2: Oncogenicity Study in Mice

MRID NUMBER: 420900-15

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. 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 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).

Core Supplementary

§83-5: Combined Chronic Toxicity and Oncogenicity Study of CGA 169374 in Rats

MRID NUMBER: 420900-19

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).

Core Supplementary

§84-2: Salmonella and Escherichia/Liver Microsomal Test

MRID NUMBER: 420900-25

Five doses of CGA-169374 technical ranging from 340 to 5447 μg/plate +/- S9 were tested in two independently performed microbial/mammalian microsome plate incorporation assays, using Salmonella typhimurium strains TA1535, TA1537, TA98, and TA100, and Escherichia coli strain WP2uvrA. At doses ≥681 μg/plate, the test material precipitated and cytotoxicity results were inconsistent; it is possible that the cells could not absorb the test material at concentrations above ≈681 μg/plate. Therefore, we assess that the only valid experimental points in the initial and confirmatory trials were 340 and 681 μg/plate. A repeat assay was conducted with strains TA1537 and TA98 because of high cytotoxicity observed in the first two tests. CGA 169374 technical was tested at concentrations ranging from 85 to 1362 μg/plate, including three soluble doses. CGA-169374 technical was neither cytotoxic nor mutagenic at any soluble dose (≤340 μg/plate). Although it would have been prudent to conduct the retest with all strains over the concentration range that was reevaluated with TA1537 and TA 98, there were sufficient and valid data to conclude that CGA 169374 technical was negative in the microbial gene mutation assay.

Classification: Acceptable

§84-2: L5178Y/TK +/- Mouse Lymphoma Mutagenicity Test

MRID NUMBER: 420900-24

No conclusions can be reached from the three nonactivated and two S9-activated mouse lymphoma forward mutation assays conducted with CGA-169374 Technical. The study was seriously compromised because of the low cloning efficiencies (CEs) of the negative and solvent control cultures in all nonactivated and S9-activated trials. We assess that the suboptimal growth in the control cultures, which we assume resulted from the use of a prolonged 3-day expression time, invalidated the study. It is therefore recommended that the study be repeated using currently

recommended procedures of the mouse lymphoma assay.

Classification: Unacceptable

§84-4: Nucleus Anomaly Test in Somatic Interphase Nuclei of Chinese Hamsters

MRID NUMBER: 420900-23

Male and female Chinese hamsters (6/sex) were administered single oral doses of 250, 500, or 1000 mg/kg of CGA-169374 for 2 consecutive days. No unscheduled deaths or other clinical signs of toxicity were reported for any treatment group. Based on the analysis of bone marrow cells harvested from 3 animals /sex/group 24 hours postexposure to the second administration of the test material, there was no evidence of a cytotoxic effect on the target organ or significant increase in the frequency of nuclear anomalies (micronuclei). However, the study was compromised by design and the lack of a MTD.

Classification: Unacceptable

§84-4: Autoradiographic DNA Repair Test on Rat Hepatocytes

MRID NUMBER: 420900-27

No conclusions can be reached from the unscheduled DNA synthesis (UDS) primary rat hepatocyte assay conducted with CGA 169374 technical at concentrations ranging from 0.25 to 31.25 μg/mL. The sensitivity of the study was severely compromised by the use of an overnight attachment period and exposure of the cells to the test chemical for only 5 hours. In addition, the method used to count nuclear grains (counting the background in a cell-free portion of the slide, rather than counting cytoplasmic grains in an area adjacent to the nucleus and subtracting this value from gross nuclear counts) probably led to the unacceptably high values for nuclear grain counts and percentage of cells with ≥5 grains in the solvent (DMSO) control group. The study should be repeated using currently recommended procedures for the rat hepatocyte UDS assay. Slides should also be coded prior to analysis to eliminate bias, and cytotoxicity data should be included in the study report.

Classification: Unacceptable

§84-4: Autoradiograph DNA Repair Test on Human Fibrobiasts

MRID NUMBER: 420900-26

No conclusions can be reached from the unscheduled DNA synthesis (UDS) human fibroblast assay conducted with CGA 169374 technical at concentrations ranging from 0.08 to 10 μg/mL. While there was no evidence that a cytotoxic concentration was reached. The study should be conducted at concentrations including at least one cytotoxic dose both in the presence and absence of exogenous metabolic activation, as required by EPA Guidelines. Slides should also be coded prior to analysis to eliminate bias, and cytotoxicity data should be included in the study report.

Classification: Unacceptable

§85-1: Characterization and Identification of Major Triazole-14C and Phenyl- 14C CGA 169374 Metabolites in Rats

MRID NUMBER: 420900-28

See summary below.

§85-1: Metabolism of Triazole -14C-CGA-169374 in the Rat

MRID NUMBER: 420900-29

See summary below.

§85-1: Metabolism of Phenyl-14C CGA-169374 in the Rat

MRID NUMBER: 420900-30

See summary below.

§85-1: Metabolism of Triazole-14C and Phenyl -14C-CGA 169374 in the Rat - Distribution of Radioactivity

MRID NUMBER: 420900-31

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 groups. 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., 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 treatment related clinical effects.

Core Classification: Core Supplementary

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 the nature of the metabolites.

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

II. Tolerance Request

Dividend (the formulated end use product), is a flowable concentrate containing 150 grams of active ingredient per liter (15% a.i. by wt.). The fungicide may be applied with farm equipment such as a concrete mixer or any continuous flow seed treater which sprays the formulation onto the seed through nozzles or spinning disks in a liquid mist chamber.

Dividend is utilized in an undiluted form at 150 to 400 mL per 100 kg of seed (22.5 to 60 g of a.i..100 lb of seed).

Crop	Target Pathogen	Grams of a.i./ 100 kg of seed	Grams of a.i./ 100 kg of seed
Wheat	Tilletia caries Tilletia controversa Septoria nodorum Urocystis agropyri Ustilago tritici Cochliobolus sativus	22.5	10.2
	Gerlachia nivalis Fusarium spp. Gaeumannomyces graminis	60.0	27.2
Barley	Pyrenophora graminea	22.5	10.2
Rye	Urocystis occults	60.0	27.2

The frequency and time of application were not stated as indicated in section B of the tolerance request.

The purpose of the requested tolerance is to allow for the importation of cereal grain commodities grown from seed treated with the a.i.. A proposed tolerance of 0.1 ppm is being sought for CGA-169374 residues in the following raw agricultural commodities: wheat grain, barley grain, and rye grain. According to the tolerance request, Dividend has almost reached approval in Germany, Denmark, Sweden, Norway, and several countries in Africa.

Data Gaps

Upon initial review of the data it would appear that the Registrant has submitted the required studies in support of the toxicology data requirements. There are, however a number of studies that require submission of additional data/information to be upgraded from supplementary. Some of the studies may not be upgradable. With this in mind, the toxicology data gaps are:

§81-6	Dermal Sensitization
§83-2	Mouse Carcinogenicity Study
§83-3(a)	Developmental Toxicity Study in Rats
§83-3(b)	Developmental Toxicity Study in Rabbits
§83-4	2-Generation Reproduction Study
§84-2	L5178Y/TK^{+}/- Mouse Lymphoma Mutagenicity Test
§84-4	Structural Chromosomal Aberration Test - Nucleus Anomaly Test in Somatic Interphase Nuclei of Chinese Hamster
§84-4	Autoradiographic DNA Repair Test on Human Fibroblasts
§84-4	Autoradiographic DNA Repair Test on Rat Hepatocytes
§85-1	General Metabolism Studies

REFERENCE DOSE (R_fD)

CGA-169374 has not been evaluated by either the HED or Agency Reference Dose Committees. It is anticipated that once the chemical has been through the R_fD Committee, the recommendation will be

made that the carcinogenic potential of CGA-169374 be evaluated by the Cancer Peer Review Committee due to the positive findings in the liver during the mouse carcinogenicity study.

DRES ANALYSIS

A DRES analysis of the impact of the proposed tolerance on the R_fD should be performed once an R_fD has been established. However, if the HED Cancer Peer Review Committee classifies Difenoconazole as a carcinogen and concludes that risk should be quantitated on this basis, then DRES should calculate the cancer risk based on the proposed tolerances.

DISCUSSION

The toxicology data base for Difenoconazole is not adequate for this regulatory action due to the data gaps listed above. In addition, TOX II would not support the provision of the tolerance request until the R_fD and the Cancer Peer Review Committees have had sufficient opportunity to review the data.

Shaughnessy No. 128847 File Last Updated _____ Current Date _____

STUDY/LAB/STUDY #/DATE	MATERIAL	EPA ACCESSION NO.	RESULTS: LD50, LC50, PIS, NOEL, LEL	TOX CATEGORY	CORE GRADE/DOC. #
§81-1 Acute Oral LD50 Species: Rat CIBA-GEIGY; Summit, NJ 862119; April 13, 1987	Difenoconazole technical	420900-06	The Acute Oral LD_{50} in males = 1,453 mg/kg 95% Confidence Limit = 933 - 2,263 mg/kg The Acute Oral LD_{50} in females = 1,453 mg/kg 95% Confidence Limit = 933 - 2,263 mg/kg	3	minimum
§81-2 Acute Dermal Toxicity Species: Rabbits CIBA-GEIGY; Summit, NJ MIN 862122; April 13, 1987	Difenoconazole technical	420900-07	The acute dermal LD_{50} of Difenoconazole for male and female rabbits was estimated to be > 2,010 mg/kg. The report does not provide the purity of the test material. The sponsor should provide this information for the record.	3	minimum
§81-3 Acute Inhalation Species: Rat CIBA-GEIGY; Switzerland 901476; June 14, 1991	Difenoconazole technical	420900-08	The 4-hour LC_{50} for males and females was > 3,300 mg/m^3.	3	minimum
§81-4 Primary Eye Irritation Species: Rabbit Hazelton; Madison, WI HWI 10503688; 8/2/91	Difenoconazole technical	420900-09	Difenoconazole Technical is classified as "moderately irritating" to the ocular tissue of the rabbit based on corneal and iridal involvement and moderate-to-severe conjunctival irritation that was reversed in all rabbits by 96 hours postapplication.	3	minimum
§81-5 Primary Dermal Irritation Species: Rabbit Hazelton; Madison, WI HWI 10503687; 7/31/91	Difenoconazole technical	420900-10	Difenoconazole is a slight irritant to the skin of rabbits. The calculated FIFRA Primary Irritation Index for the test article was 0.1.	4	minimum
§81-6 Dermal Sensitization Species: Guinea Pigs CIBA-GEIGY; Summit, NJ 862076; 4/13/87	Difenoconazole technical	420900-11	A 0.5 g topical application of Difenoconazole Technical did not result in delayed contact hypersensitivity in guinea pigs. The study was classified as supplementary because the purity and stability of the test material were not reported.		supplementary
§82-1(a) Subchronic Feeding Species: Rat Hazelton; Vienna, VA HLA483242; 10/28/87	Difenoconazole technical	420900-22	Difenoconazole Technical was administered orally in feed admixtures to six groups of rats of both sexes at 0, 20, 200, 750, 1500, and 3000 ppm for 13 weeks. The LOEL was set at 200 ppm based on the 10% decrease in body weight in the 200 ppm females (as well as a negative trend in feed consumption) and increases in absolute liver weights in both sexes appearing at 750 ppm. The NOEL was 20 ppm.		minimum

§82-1(a) Subchronic Feeding Species: Mouse Hazelton; Vienna, VA HLA483241; 10/87	Difenoconazole technical	420903-21	CGA 169374 technical was fed for mice at 0, 20, 200, 2500, 7500 or 15,000 ppm (both sexes with 15 animals/sex/group) for 13 weeks. Based upon body weight changes and liver histopathology the NOEL was 20 ppm. The LEL was 200 ppm. Most of the mice receiving 7,500 or 15,000 ppm died during the first week of the study. Statistical analysis of feed consumption and body weight changes revealed decreases for paired 2500 ppm females. Hepatotoxicity in mice that died during the study was evidenced by hepatocellular enlargement and necrosis of individual hepatocytes. Hepatotoxicity in animals in surviving animals was observed as hepatocellular enlargement the in the 200 ppm males and the 2500 ppm groups, hepatocytic vacuolization of the 2500 ppm animals. In addition, coagulative necrosis was observed in the livers of 4/9 group 4 females. Because the foci were small and random this finding was not thought to be related to treatment. The unscheduled deaths in groups 5 or 6 had a high incidence of changes consistent with stress.		minimum
§82-1 (b) Subchronic Feeding Species: Dog CIBA-GEIGY: Summit, NJ 852197; 10/30/87	Difenoconazole technical	420900-12	CGA 169374 technical was administered in the diet to beagle dogs at 0, 100, 1000, 3000, or 6000 ppm for a minimum of 28 weeks. The LOEL and the NOEL respectively were, 3000 ppm and 1000 ppm. The most outstanding effect was bilateral lenticular cataracts, which was observed in 3 out of 3 animals/sex in the 6000 ppm group by ophthalmoscopic examination. The incidence in this group by microscopic exam was 3/3 females with bilateral cataracts and 2/3 males with unilateral cataracts and 1/3 males with bilateral cataracts (3/3 males were observed to have cataracts). One female fed 3000 ppm was observed to have bilateral cataracts by ophthalmoscopic examination. Microscopic examination found the incidence in males to be 1/3 with bilateral cataracts and the incidence in females to be 2/3 with unilateral cataracts and 1/3 with bilateral cataracts. Ocular effects were not observed at dietary levels ≤ 1000 ppm. Additional findings included iridic changes secondary to induced uveitis, reductions in mean body weight and food consumption, and alterations in hematology and clinical chemistry.		minimum
§82-2 21-Day Dermal Species: Rabbit CIBA-GEIGY: Summit, NJ 862014; 10/87	Difenoconazole technical	420900-13	CGA 169374 technical was administered topically under occlusion to three groups of female and male rabbits (5/group/sex) at daily doses of 10, 100, or 1000 mg/kg for a minimum of 22 consecutive days. There were two controls used vehicle (ethanol) and naive. The NOEL was 10 mg/kg. The LEL was 100 mg/kg. Statistically significant reductions in body weight gain, and food consumption resulted from exposure to doses ≥ 100 mg/kg. There were no deaths. Mild to moderate skin irritation localized to the site of application of the vehicle or test article was observed via macroscopic and microscopic observation. Females exposed to 1000 mg/kg exhibited increased adrenal weights and vacuolation of hepatocytes.		minimum

§84-2 Gene Mutation Test Species: Salmonella/E.coli CIBA-GEIGY; Switzerland 901061; 8/13/90	CGA 169374 technical 91.8%	420900-25	Five doses of Difenoconazole technical ranging from 340 to 5447 µg/plate +/- S9 were tested in two independently performed microbial/mammalian microsome plate incorporation assays, using Salmonella typhimurium strains TA1535, TA1537, TA98, and TA100, and Escherichia coli strain WP2uvra. At doses ≥681 µg/plate, the test material precipitated and cytotoxicity results were inconsistent; it is possible that the cells could not absorb the test material at concentrations above ≈681 µg/plate. Therefore, we assess that the only valid experimental points in the initial and confirmatory trials were 340 and 681 µg/plate. A repeat assay was conducted with strains TA1537 and TA98 because of high cytotoxicity observed in the first two tests. CGA 169374 technical was tested at concentrations ranging from 85 to 1362 µg/plate, including three soluble doses. Difenoconazole technical was neither cytotoxic nor mutagenic at any soluble dose (≤340 µg/plate). Although it would have been prudent to conduct the retest with all strains over the concentration range that was reevaluated with TA1537 and TA 98, there were sufficient and valid data to conclude that CGA 169374 technical was negative in the microbial gene mutation assay.		acceptable
§84-2 Gene Mutation Test Species: L5178YTK+/- Mouse Lymphoma CIBA-GEIGY: Switzerland 850570;8/6/86	Difenoconazole technical 94.5%	420900-24	No conclusions can be reached from the three nonactivated and two S9-activated mouse lymphoma forward mutation assays conducted with Difenoconazole Technical. The study was seriously compromised because of the low cloning efficiencies (CEs) of the negative and solvent control cultures in all nonactivated and S9-activated trials. We assess that the suboptimal growth in the control cultures, which we assume resulted from the use of a prolonged 3-day expression time, invalidated the study. It is therefore recommended that the study be repeated using currently recommended procedures of the mouse lymphoma assay.		unacceptable
§84-2 Structural Chromosomal Aberration Test Species: Chinese Hamster CIBA-GEIGY: Switzerland 850567; 2/3/86	Difenoconazole technical 94.5%	420900-23	Male and female Chinese hamsters (6/sex) were administered single oral doses of 250, 500, or 1000 mg/kg of Difenoconazole for 2 consecutive days. No unscheduled deaths or other clinical signs of toxicity were reported for any treatment group. Based on the analysis of bone marrow cells harvested from 3 animals /sex/group 24 hours postexposure to the second administration of the test material, there was no evidence of a cytotoxic effect on the target organ or significant increase in the frequency of nuclear anomalies (micronuclei). However, the study was compromised by design and the lack of a MTD.		unacceptable

§84-2 UDS in Primary Rat Hepatocytes CIBA-GEIGY; Switzerland 850566; 8/30/85	Difenoconazole technical 94.5%	420900-27	No conclusions can be reached from the unscheduled DNA synthesis (UDS) primary rat hepatocyte assay conducted with Difenoconazole technical at concentrations ranging from 0.25 to 31.25 µg/mL. The sensitivity of the study was severely compromised by the use of an overnight attachment period and exposure of the cells to the test chemical for only 5 hours. In addition, the method used to count nuclear grains (counting the background in a cell-free portion of the slide, rather than counting cytoplasmic grains in an area adjacent to the nucleus and subtracting this value from gross nuclear counts) probably led to the unacceptably high values for nuclear grain counts and percentage of cells with ≥5 grains in the solvent (DMSO) control group. The study should be repeated using currently recommended procedures for the rat hepatocyte UDS assay. Slides should also be coded prior to analysis to eliminate bias, and cytotoxicity data should be included in the study report.		unacceptable
§84-2 UDS in Human Fibroblasts CIBA-GEIGY; Switzerland 850568; 8/30/85	Difenoconazole technical 94.5%	420900-26	No conclusions can be reached from the unscheduled DNA synthesis (UDS) human fibroblast assay conducted with Difenoconazole technical at concentrations ranging from 0.08 to 10 µg/mL. While there was no evidence that a cytotoxic concentration was reached. The study should be conducted at concentrations including at least one cytotoxic dose both in the presence and absence of exogenous metabolic activation, as required by EPA Guidelines. Slides should also be coded prior to analysis to eliminate bias, and cytotoxicity data should be included in the study report.		unacceptable

§85-1 Metabolism Species: Rat CIBA-GEIGY; Greensboro, NC WIL Research Labs, Inc.;Ashland OH ABR 90019; 9/13/90	Difenoconazole	420900-28	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 groups. 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., 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 treatment related clinical effects.		supplementary
§85-1 Metabolism Species: Rat WIL Research Labs Inc.; Ashland OH WIL 82014; 7/20/87	Difenoconazole 94.5%	420900-29			
§85-1 Metabolism Species: Rat WIL Research Labs Inc.; Ashland OH WIL 82013; 7/20/87	Difenoconazole 94.5%	420900-30			
§85-1 Metabolism Species: Rat CIBA-GEIGY; Greensboro, NC ABR 88043; 4/25/88	Difenoconazole	420900-31			

Shaughnessy No. 128847 File Last Updated _____ Current Date _____

STUDY/LAB/STUDY #/DATE	MATERIAL	EPA ACCESSION NO.	RESULTS: LD50, LC50, PIS, NOEL, LEL	TOX CATEGORY	CORE GRADE/DOC. #
§83-2 Oncogenicity Study in Mice Hazelton Laboratories America, Inc. 483-250/ 4/3/89	Difenoconazole	420900-15	Difenoconazole was administered in the diet to male and female mice for 79 weeks at 0, 10, 30, 300, 2500 and 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 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).		supplementary

Tox Chem No. _____ File Last Updated _____ Current Date _____

STUDY/LAB/STUDY #/DATE	MATERIAL	EPA ACCESSION NO.	RESULTS: LD50, LC50, PIS, NOEL, LEL	TOX CATEGORY	CORE GRADE/DOC. #
§83-3(a) Developmental Toxicity Study Species: Rat Argus Research Laboratories 203-005;November 6, 1987	Difenoconazole technical	420900-16	Difenoconazole technical was administered to presumed pregnant rats at 0, 2, 20, 100, or 200 mg/kg by gavage. Maternal toxicity was observed at 100 and 200 mg/kg as decreased body weight gain and decreased feed consumption. Developmental toxicity was observed only at 200 mg/kg as alterations in fetal ossification. **NOTE: Due to the relatively high percent deviation of the actual doses tested from the theoretical concentration the effect levels have been modified accordingly. This modification may be subject to change as the purity is currently unknown.** Maternal NOEL = 16 mg/kg Maternal LOEL = 85 mg/kg Developmental Toxicity NOEL = 85 mg/kg Developmental Toxicity LOEL = 171 mg/kg		supplementary

Shaughnessy No. 128847 File Last Updated _____ Current Date _____

STUDY/LAB/STUDY #/DATE	MATERIAL	EPA ACCESSION NO.	RESULTS: LD50, LC50, PIS, NOEL, LEL	TOX CATEGORY	CORE GRADE/DOC. #
§83-3(b) Developmental Toxicity Study Species: Rabbit CIBA-GEIGY Corporation Research Department 87022 (MIN 862107); August 31, 1987	Difenoconazole technical	420900-17	Difenoconazole technical was administered to presumed pregnant rabbits at 0, 1, 25, or 75 mg/kg by gavage. Maternal toxicity was observed at 75 mg/kg as decreased body weight gain, decreased feed consumption. Developmental toxicity was observed at 75 mg/kg as slight nonsignificant increases in postimplantation loss and resorptions/doe and a significant decrease in fetal weight. Maternal NOEL = 25 mg/kg Maternal LOEL = 75 mg/kg Developmental Toxicity NOEL = 25 mg/kg Developmental Toxicity LOEL = 75 mg/kg		supplementary

[redacted]

Shaughnessy No.: 128847 File Last Updated _____ Current Date _____

STUDY/LAB/STUDY #/DATE	MATERIAL	EPA MRID NO.	RESULTS: LD50, LC50, PIS, NOEL, LEL	TOX CATEGORY	CORE GRADE/DOC. #
§83-4 2-Generation Reproduction Study Species: Rat Research Department CIBA-GEIGY Corporation (MIN 862091); 9/1/88	Difenoconazole technical	420900-18	Difenoconazole technical was administered was administered in the diet to male and female rats at rats at 0, 25, 250, or 2500 ppm. Significant reductions in F_0 and F_1 male body weight gain were observed at 2500 ppm during days 0-77 and overall (terminal body weight minus day 0 body weight). Significant reductions in F_0 and F_1 body weight gain of females in the 2500 ppm group were detected during the pre-mating, gestation, and lactation periods. In addition, the 250 ppm F_0 females had reductions (statistically nonsignificant) in body weight gain which appear to be part of a dose related trend days 70-77 prior to mating, days 0-7 of gestation, and days 7-14 of lactation. Significant reductions in pup body weight were detected days 0, 4 (pre- and post culling) 7, 14, and 21 for males and females (day 0 female F_1 were not significant) in the 2500 ppm group of both generations. There was a significant reduction in the body weight of F_1 male pups at day 21 in the 250 ppm group. The percentage of male pups in the F_1 generation surviving days 0-4 was significantly reduced in the 2500 ppm group. This study may be upgraded after the registrant satisfactorily provides the purity and stability of the test article. **Parental Systemic NOEL = 25 ppm** **Parental Systemic LOEL = 250 ppm** **Reproductive NOEL = 25 ppm** **Reproductive LOEL = 250 ppm**		supplementary

Shaughnessy No. 128847 File Last Updated _____ Current Date _____

STUDY/LAB/STUDY #/DATE	MATERIAL	EPA ACCESSION NO.	RESULTS: LD50, LC50, PIS, NOEL, LEL	TOX CATEGORY	CORE GRADE/DOC. #
§83-5 Combined Chronic Toxicity and Oncogenicity Study in Rats Hazelton Laboratories America, Inc. 483-249/3/31/89	Difenconazole	420900-19	Difenconazole 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).		supplementary

DOC930086

FINAL

009689

DATA EVALUATION REPORT

Difenoconozale

Study Type: Acute Oral Toxicity in Rats

Study Title: CGA-169374 Technical: Acute Oral Toxicity Study in Rats

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 Reviewer:	Betty Shindel Betty Shindel, M.P.H.	6-8-92 Date
Independent Reviewer:	John Liccione John Liccione, Ph.D.	6-8-92 Date
QA/QC Manager:	Sharon Segal Sharon Segal, Ph.D.	6/8/92 Date

Contract Number: 68D10075
Work Assignment Number: 1-79
Clement Number: 91-258
Project Officer: James E. Scott

00324

Guideline Series 81-1: Acute oral toxicity

Approved by:
EPA Reviewer: Karen Whitby, Ph.D. Signature
Review Section II, Toxicology Branch II (HED) Date 6/22/92

EPA Section Head: Clark Swentzel Signature
Review Section II, Toxicology Branch II (HED) Date 6/22/92

DATA EVALUATION REPORT

STUDY TYPE: Guideline 81-1: Acute oral toxicity in rats

EPA IDENTIFICATION NUMBERS:

Shaughnessy Number: 128847
MRID Number: 420900-06

TEST MATERIAL: CGA-169374 technical

SYNONYM: Difenoconozale

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

STUDY NUMBER: 862119

TESTING FACILITY: Division of Toxicology/Pathology, CIBA-GEIGY Corporation, Pharmaceutical Division, Summit, NJ 07901

TITLE OF REPORT: CGA-169374 Technical: Acute Oral Toxicity Study in Rats

AUTHOR: Kirk Huber

STUDY COMPLETED: April 13, 1987

CONCLUSION: Acute oral LD_{50} in males = 1,453 mg/kg
95% confidence limit = 933-2,263 mg/kg

Acute oral LD_{50} in females = 1,453 mg/kg
95% confidence limit = 933-2,263 mg/kg

CORE CLASSIFICATION: Core Minimum. This study was in conformity with Guideline Series 81-1; however, the purity of the test material in the suspension was not determined. The request should be made of the Registrant to supply the purity of the test material for the record.

TOXICITY CATEGORY: III--Caution

A. MATERIALS

1. Test Material

Test material: CGA-169374 technical
Identification number: FL-851406
Purity: Not reported, on file with sponsor
Physical description: Solid
Storage conditions: Stored at room temperature or frozen
Stability: The test material as 0.02% and 6.0% suspensions in 3% cornstarch containing 1% polysorbate 80 were determined in the study to be stable for at least 21 days when stored at room temperature or from 2°C to 8°C.
Concentration: The actual concentrations of the 2, 4, and 6% vehicle/test suspensions were 1.89, 3.78, and 5.61%, respectively (3 measurements/suspension).

2. Controls

Animals: 5 Males; 5 females
Vehicle: 3% Cornstarch containing 1% polysorbate 80

3. Test Animals

Species: Rat
Strain: Sprague Dawley
Source: Marland Farms, Hewitt, NJ
Receipt date: Not reported
Sex and numbers: 20 Males; 20 females; includes vehicle control
Age: 8 Weeks
Initial body weight: Males, 188-244 g; females, 145-201 g
Housing: Initially, housing was 1/cage. However, during actual dosing all animals were placed in groups of 5/cage. The animal room was maintained at a daily temperature of 73±5°F and a relative humidity of 50±20%. A 12-hour alternating light/dark cycle was maintained in animal room.
Feeding: Feed (Certified® Purina Rodent Chow #5002) and tap water provided ad libitum.
Animal identification: Monel eartag
Acclimation period: 7 days prior to study initiation.
Randomization: Animals assigned to cages via a computer-generated randomization table.
Health status: Animals were examined for health status during the acclimation period. Study only used animals that were judged healthy.

B. TEST PERFORMANCE

Groups of 5 rats/sex were orally administered a single dose of CGA 169374 technical by gavage. Animals were fasted approximately 16 to 18 hours before dosing, and were provided feed after dosing. The doses were 1,000, 2,000, or 3,000 mg/kg at a constant dose volume of 50 mL/kg. The solid test article was administered as a 2, 4, or 6% suspension in the vehicle (3% cornstarch containing 1% polysorbate 80). A vehicle control group of 5 rats/sex received the vehicle at a dosage volume of 50 mL/kg. Animals were frequently monitored for clinical signs of toxicity during the first day of treatment (immediately postdose, and 0.5, 1.0, 2.0, 3.0, 4.0 and 5.0 hours postdosing), and twice a day on test days 2-14. Information on the onset and duration of clinical observations was also provided. Body weights were measured once during the predosing period on test day -9 or -3, prior to dosing on test day 1, and on test days 8 and 15. All animals received a gross necropsy. For rats that died during the study, a gross necropsy was performed as soon as possible after death. For rats that survived treatment, animals were sacrificed at the end of the study and necropsied. Histopathological examinations were not performed on any of the animals.

Statistics

The method of Bliss as modified by Carmines et al. (1980)[1] was used to calculate the LD_{50}s and their 95% confidence limits.

C. QUALITY ASSURANCE MEASURE

A signed Quality Assurance Statement and a signed Good Laboratory Practice statement were included in the study report.

D. RESULTS AND STUDY AUTHOR'S CONCLUSIONS

At each dose of 1,000 mg/kg and 2,000 mg/kg, mortality was reported in 2 males and 2 females. At a dose of 3,000 mg/kg, mortality was reported in 5 males and 5 females. No mortalities were reported for any of the vehicle control animals.

Clinical signs of toxicity observed in each of the 1,000- (2 males and 2 females), 2,000- (2 males and 2 females) and 3,000-mg/kg (5 males and 5 females) dose groups consisted of hypoactivity, stains around the mouth, perineal staining, ataxia, soft feces, hypothermia, and prostration. Additional clinical observations in the 1,000-mg/kg group consisted of lacrimation (2 males and 2 females), chromorhinorrhea (2 males), and spasms (2 females). Additional clinical observations in the 2,000-mg/kg

[1]Carmines et al. A method for the evaluation of dose-effect data utilizing a programmable calculator. J. Environ. Pathol. Toxicol. 4: 23-30. 1980.

group consisted of salivation (2 males), chromodacryorrhea (2 males), lacrimation and unkempt appearance (2 females). Additional clinical observations in the 3,000-mg/kg group consisted of spasms, lacrimation, rhinorrhea, hypopnea, salivation, and ptosis. No clinical observations were observed in the vehicle control animals. The onset of clinical observations ranged from 0.5 hours postdosing to test day 7. Clinical signs of toxicity were reversed in all surviving animals by test day 10.

The percent change in body weight gain for males in the 2,000-mg/kg dose group was lower at day 8 (21% lower) and at day 15 (22% lower) than the percent change in body weight gain for the male vehicle control group at days 8 and 15. The percent change in body weight gain for females in the 2,000-mg/kg dose group was lower at day 8 (14% lower) as compared to the percent change in body weight gain for the female vehicle control group at day 8. However, the mean body weight gains in animals administered the test article were positive at days 8 and 15.

Dosage group (mg/kg)	Sex	Percent change in body weight from test day 1	
		Day 8	Day 15
0 (vehicle)	M	+40	+60
1,000	M	+32	+61
2,000	M	+19	+38
3,000	M	---*	---*
0 (vehicle)	F	+30	+42
1,000	F	+26	+42
2,000	F	+16	+37
3,000	F	---*	---*

* All animals died prior to day 8

Gross necropsy data for individual animals and a summary of necropsy findings tabulated on the basis of sex and dose level were included in the study report. No significant necropsy findings were reported for vehicle control animals or for animals administered 1,000 mg/kg or 3,000 mg/kg of the test article. At a dose level of 2,000 mg/kg, stomach lesions (dark or red color, multifocal, 0.1 x 0.1 x 0.1 cm) and/or solid red clot were reported in 2 males and 2 females that died during test days 3-5; findings were unremarkable in the 5 remaining males and females. No microscopic examinations were performed on the stomach or any other organ.

LD_{50} Determination

The estimated acute oral LD_{50} for CGA-169374 was approximately 1,453 mg/kg for males and females. By the end of the study (day 15), mortality was reported in 40% of the males and females at doses of 1,000 and 2,000 mg/kg, and 100% of the males and females at 3,000 mg/kg.

Based on these mortality results, the study author classified the acute oral toxicity of CGA-169374 technical as "low."

E. REVIEWERS' COMMENTS

The overall design and conduct of the study was acceptable for an acute oral toxicity study. However, the reviewers note that the volume of the dosing solution (i.e., 50 mL/kg) was rather large for a rodent. Generally, the volume of a dosing sclution for rodents is 5-10 mL/kg of body weight per day. With larger volumes, some of the dosing solutions can end up in the intestine or be aspirated into the lungs due to back pressure. Since control animals were asymptomatic with respect to clinical observations, aspiration into the lungs did not seem likely. However, some of the dosing solution may have entered the intestine thereby influencing the absorption of the test material. The purity of the test material in the suspensions was not reported. None of the animals that died during the study were subjected to histological examination; however, gross examination of animals did not indicate any possible target organs.

Based on the mortality results, males and females have the same acute oral LD_{50} value of 1,453 mg/kg with a 95% confidence limit of 933-2,263 mg/kg.

DOC 930087

FINAL

009689

DATA EVALUATION REPORT

Difenoconozale

Study Type: Acute Dermal Toxicity in Rabbits

Study Title: CGA-169374 Technical: Acute Dermal Toxicity Study in Rabbits

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 Reviewer	Betty Shindel, M.P.H.	7-7-92 Date
Independent Reviewer	John Liccione, Ph.D.	7-7-92 Date
QA/QC Manager	Sharon Segal, Ph.D.	7-7-92 Date

Contract Number: 68D10075
Work Assignment Number: 1-79
Clement Number: 91-259
Project Officer: James E. Scott

Guideline Series 81-2: Acute dermal toxicity

Approved by:
EPA Reviewer: Karen Whitby, Ph.D. Signature
Review Section II, Toxicology Branch II (HED) Date 7/14/92

EPA Section Head: Clark Swentzel Signature
Review Section II, Toxicology Branch II (HED) Date 7/14/92

009689

DATA EVALUATION REPORT

STUDY TYPE: 81-2 Acute dermal toxicity in rabbits

EPA IDENTIFICATION NUMBERS

Shaughnessy Number: 128847
MRID Number: 420900-07

TEST MATERIAL: CGA-169374 technical

SYNONYMS: Difenoconozale, ID no. FL 851406

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

STUDY NUMBER: MIN 862122

TESTING FACILITY: CIBA-GEIGY Corporation, Pharmaceutical Division, 556 Morris Avenue, Summit NJ, 07901

TITLE OF REPORT: CGA-169374 Technical: Acute Dermal Toxicity Study in Rabbits

AUTHOR: Kirk Huber

STUDY COMPLETED: April 13, 1987

CONCLUSIONS: The acute dermal LD_{50} of CGA-169374 technical for male and female rabbits was estimated to be >2,010 mg/kg. The report does not provide the purity of the test material. The sponsor should provide this information for the record.

CORE CLASSIFICATION: Core Minimum. This study is in conformity with Guideline Series 81-2.

TOXICITY CATEGORY: III--Caution

A. MATERIALS

1. Test Material

009689

Test material: CGA-169374 technical
Identification number: FL-851406
Purity: Not reported, on file with sponsor
Physical description: Not provided
Concentration: Mean concentration of 50% solution of test article in ethyl alcohol was 52.5 % (2 measurements).
Storage conditions: Stored frozen or at room temperature
Stability: 50% solution of test article in ethyl alcohol (200 proof dehydrated alcohol U.S.P., receipt # 19966) was stable for at least 24 hours at room temperature, and 22 days at 6°C.

2. Controls

Animals: None needed
Test Substance: None needed

3. Test Animals

Species: Rabbit
Strain: New Zealand White
Source: H.A.R.E Inc., Hewitt, NJ
Sex and numbers: 5 Males; 5 females
Age: 12-13 Weeks
Initial bodyweight: 1.82-2.42 kg
Housing: Individual. Animal room maintained at a daily temperature of 65±5°F and a relative humidity of 50±20%; a 12-hour alternating light/dark cycle was maintained.
Feeding: Feed (Purina® Certified Rabbit Chow #5322) and tap water provided ad libitum throughout predosing and study period.
Animal Identification: Monel eartag
Acclimation Period: About 9 days prior to dosing
Randomization: Animals were randomly assigned to groups via a computer-generated randomization table.
Health Status: Animals were examined for health status during the acclimation period. Study only used animals that were judged healthy.

B. TEST PERFORMANCE

Approximately 24 hours prior to application of the test article, the fur was clipped from the flank of each animal. Five male and 5 female rabbits were each dermally administered a single topical 2,010 mg/kg application of a 50% solution of CGA-169374 Technical in ethyl alcohol applied under occlusion for a 24-hour period to an area of intact skin on the shaved flank. The dimensions of the area on the flank cleared for application comprised approximately 10% (240 cm^2) of the total body surface of each rabbit. A gauze dressing secured with nonirritating tape was applied over the test site, and each animal was covered with an orthopedic stockinet. The rabbits wore a collar during the 24-hour exposure period to minimize ingestion of the test article. After the 24-hour exposure period, the wrappings and collar were removed and the treated area was rinsed with ethanol to remove residual test article and then dried with a paper towel.

A standard Draize method was used to score the severity of erythema and/or edema prior to dosing on test day 0, 30 minutes after compound removal on test day 1, and on test days 3, 7 and 14. Animals were observed for clinical signs of toxicity once during the acclimation period, and frequently (prior to dosing, immediately postdosing, and 1, 2 and 2.5 hours postdosing) on day 1 of treatment and at least once on days 2-14. Body weights were measured just prior to dosing on test day 1, and on test days 8 and 15. At the end of the study, animals were sacrificed with an unspecified euthanasia solution and necropsied.

C. QUALITY ASSURANCE MEASURE

A signed Quality Assurance Statement and a signed Good Laboratory Practice statement were included in the study report.

D. RESULTS AND STUDY AUTHOR'S CONCLUSIONS

No mortality was reported for any of the treated animals administered 2,010 mg/kg of CGA-169374 technical. Based on these results, the acute dermal LD_{50} of CGA-169374 technical was >2,010 mg/kg for male and female rabbits. The study author classified CGA-169374 technical as nontoxic when applied under occlusion to intact rabbit skin.

The only reporting of erythema (very slight; Draize Grade 1) was at 30 minutes after compound removal on test day 1 at the application site of 2 males and 1 female. Other dermal irritation consisted of fissuring in 1 male at test day 3, desquamation in all 10 animals at test day 7, a scab in 1 male at test day 7 (cervical area, considered by the study author to be related to mechanical injury rather than treatment), and desquamation in 4 males and 2 females at test day 14. The study author stated that dermal changes were minimal, and were probably the result of occluding the test substance in an alcohol vehicle. The author based this conclusion on results obtained in a primary dermal irritation study (study # 86065) and a 21-day dermal toxicity study (study # 86191) in rabbits administered CGA 169374.

Clinical observations were reported as unremarkable for all animals during the study period. Body weight gain was observed in all rabbits at test days 8 and 15. Mean percent changes in absolute body weights for males at days 8 and day 15 when compared to their initial body weights were +9% and +18%, respectively. Mean percent changes in absolute body weights for females at days 8 and day 15 when compared to their initial body weights were +10% and +19%, respectively. No significant findings were reported upon gross necropsy. Histopathological examinations were not performed.

E. REVIEWERS' COMMENTS

This study is in conformity with Guideline Series 81-2 and is classified as Core Minimum. The report does not provide the purity of the test material.

The estimated acute dermal LD_{50} of CGA-169374 technical for male and female rabbits is >2,010 mg/kg. The highest dose administered in the study (2,010 mg/kg) slightly exceeds the limit dose of 2,000 mg/kg specified in Guideline Series 81-2. Based on this LD_{50}, the Toxicity Category is III--Caution.

DOC 930087

FINAL

009689

DATA EVALUATION REPORT

Difenoconozale

Study Type: Acute Dermal Toxicity in Rabbits

Study Title: CGA-169374 Technical: Acute Dermal Toxicity Study in Rabbits

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 Reviewer	Betty Shindel, M.P.H.	7-7-92 Date
Independent Reviewer	John Liccione, Ph.D.	7-7-92 Date
QA/QC Manager	Sharon Segal, Ph.D.	7-7-92 Date

Contract Number: 68D10075
Work Assignment Number: 1-79
Clement Number: 91-259
Project Officer: James E. Scott

00334

Guideline Series 81-2: Acute dermal toxicity

Approved by:
EPA Reviewer: Karen Whitby, Ph.D. Signature _____
Review Section II, Toxicology Branch II (HED) Date 7/16/92

EPA Section Head: Clark Swentzel Signature _____
Review Section II, Toxicology Branch II (HED) Date 7/15/92

DATA EVALUATION REPORT

STUDY TYPE: 81-2 Acute dermal toxicity in rabbits

EPA IDENTIFICATION NUMBERS

Shaughnessy Number: 128847
MRID Number: 420900-07

TEST MATERIAL: CGA-169374 technical

SYNONYMS: Difenoconozale, ID no. FL 851406

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

STUDY NUMBER: MIN 862122

TESTING FACILITY: CIBA-GEIGY Corporation, Pharmaceutical Division, 556 Morris Avenue, Summit NJ, 07901

TITLE OF REPORT: CGA-169374 Technical: Acute Dermal Toxicity Study in Rabbits

AUTHOR: Kirk Huber

STUDY COMPLETED: April 13, 1987

CONCLUSIONS: The acute dermal LD_{50} of CGA-169374 technical for male and female rabbits was estimated to be >2,010 mg/kg. The report does not provide the purity of the test material. The sponsor should provide this information for the record.

CORE CLASSIFICATION: Core Minimum. This study is in conformity with Guideline Series 81-2.

TOXICITY CATEGORY: III--Caution

A. MATERIALS

1. Test Material

Test material: CGA-169374 technical
Identification number: FL-851406
Purity: Not reported, on file with sponsor
Physical description: Not provided
Concentration: Mean concentration of 50% solution of test article in ethyl alcohol was 52.5 % (2 measurements).
Storage conditions: Stored frozen or at room temperature
Stability: 50% solution of test article in ethyl alcohol (200 proof dehydrated alcohol U.S.P., receipt # 19966) was stable for at least 24 hours at room temperature, and 22 days at 6°C.

2. Controls

Animals: None needed
Test Substance: None needed

3. Test Animals

Species: Rabbit
Strain: New Zealand White
Source: H.A.R.E Inc., Hewitt, NJ
Sex and numbers: 5 Males; 5 females
Age: 12-13 Weeks
Initial bodyweight: 1.82-2.42 kg
Housing: Individual. Animal room maintained at a daily temperature of 65±5°F and a relative humidity of 50±20%; a 12-hour alternating light/dark cycle was maintained.
Feeding: Feed (Purina® Certified Rabbit Chow #5322) and tap water provided ad libitum throughout predosing and study period.
Animal Identification: Monel eartag
Acclimation Period: About 9 days prior to dosing
Randomization: Animals were randomly assigned to groups via a computer-generated randomization table.
Health Status: Animals were examined for health status during the acclimation period. Study only used animals that were judged healthy.

B. TEST PERFORMANCE

Approximately 24 hours prior to application of the test article, the fur was clipped from the flank of each animal. Five male and 5 female rabbits were each dermally administered a single topical 2,010 mg/kg application of a 50% solution of CGA-169374 Technical in ethyl alcohol applied under occlusion for a 24-hour period to an area of intact skin on the shaved flank. The dimensions of the area on the flank cleared for application comprised approximately 10% (240 cm^2) of the total body surface of each rabbit. A gauze dressing secured with nonirritating tape was applied over the test site, and each animal was covered with an orthopedic stockinet. The rabbits wore a collar during the 24-hour exposure period to minimize ingestion of the test article. After the 24-hour exposure period, the wrappings and collar were removed and the treated area was rinsed with ethanol to remove residual test article and then dried with a paper towel.

A standard Draize method was used to score the severity of erythema and/or edema prior to dosing on test day 0, 30 minutes after compound removal on test day 1, and on test days 3, 7 and 14. Animals were observed for clinical signs of toxicity once during the acclimation period, and frequently (prior to dosing, immediately postdosing, and 1, 2 and 2.5 hours postdosing) on day 1 of treatment and at least once on days 2-14. Body weights were measured just prior to dosing on test day 1, and on test days 8 and 15. At the end of the study, animals were sacrificed with an unspecified euthanasia solution and necropsied.

C. QUALITY ASSURANCE MEASURE

A signed Quality Assurance Statement and a signed Good Laboratory Practice statement were included in the study report.

D. RESULTS AND STUDY AUTHOR'S CONCLUSIONS

No mortality was reported for any of the treated animals administered 2,010 mg/kg of CGA-169374 technical. Based on these results, the acute dermal LD_{50} of CGA-169374 technical was >2,010 mg/kg for male and female rabbits. The study author classified CGA-169374 technical as nontoxic when applied under occlusion to intact rabbit skin.

The only reporting of erythema (very slight; Draize Grade 1) was at 30 minutes after compound removal on test day 1 at the application site of 2 males and 1 female. Other dermal irritation consisted of fissuring in 1 male at test day 3, desquamation in all 10 animals at test day 7, a scab in 1 male at test day 7 (cervical area, considered by the study author to be related to mechanical injury rather than treatment), and desquamation in 4 males and 2 females at test day 14. The study author stated that dermal changes were minimal, and were probably the result of occluding the test substance in an alcohol vehicle. The author based this conclusion on results obtained in a primary dermal irritation study (study # 86065) and a 21-day dermal toxicity study (study # 86191) in rabbits administered CGA 169374.

Clinical observations were reported as unremarkable for all animals during the study period. Body weight gain was observed in all rabbits at test days 8 and 15. Mean percent changes in absolute body weights for males at days 8 and day 15 when compared to their initial body weights were +9% and +18%, respectively. Mean percent changes in absolute body weights for females at days 8 and day 15 when compared to their initial body weights were +10% and +19%, respectively. No significant findings were reported upon gross necropsy. Histopathological examinations were not performed.

E. REVIEWERS' COMMENTS

This study is in conformity with Guideline Series 81-2 and is classified as Core Minimum. The report does not provide the purity of the test material.

The estimated acute dermal LD_{50} of CGA-169374 technical for male and female rabbits is >2,010 mg/kg. The highest dose administered in the study (2,010 mg/kg) slightly exceeds the limit dose of 2,000 mg/kg specified in Guideline Series 81-2. Based on this LD_{50}, the Toxicity Category is III--Caution.

DOC 930090

FINAL

009689

DATA EVALUATION REPORT

Difenoconozale

Study Type: Primary Dermal Irritation Study in Rabbits

Study Title: Primary Dermal Irritation Study of CGA-169374 Technical in Rabbits

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 Reviewer	Betty Shindel, M.P.H.	6-8-92 Date
Independent Reviewer	John Licciione, Ph.D.	6-8-92 Date
QA/QC Manager	Sharon Segal, Ph.D.	6/8/92 Date

Contract Number: 68D10075
Work Assignment Number: 1-79
Clement Number: 91-262
Project Officer: James E. Scott

00038

Guideline Series 81-5: Primary Dermal Irritation

009689

Approved by:
EPA Reviewer: Karen Whitby Signature
Review Section II, Toxicology Branch II (HED) Date 6/17/92

EPA Section Head: Clark Swentzel Signature
Review Section II, Toxicology Branch II (HED) Date 6/22/92

DATA EVALUATION REPORT

STUDY TYPE: 81-5 Primary dermal irritation study in rabbits

EPA IDENTIFICATION NUMBERS

Shaugnessy Number: 128847
MRID Number: 420900-10

TEST MATERIAL: CGA-169374 Technical

SYNONYM: Difenoconozale

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

STUDY NUMBER: HWI 10503687

TESTING FACILITY: Hazleton Wisconsin, Inc., 3301 Kinsman Boulevard, Madison, WI 53704

TITLE OF REPORT: Primary Dermal Irritation Study of CGA-169374 Technical in Rabbits

AUTHOR: Steven M. Glaza

STUDY COMPLETED: July 31, 1991

CONCLUSIONS: CGA-169374 Technical is a slight irritant to the skin of rabbits. The calculated FIFRA Primary Irritation Index for the test article was 0.1.

CORE CLASSIFICATION: Core Minimum. The purity (as well as any possible impurities) and stability of the test material were not reported. The size of the test site was not specified. The sponsor should supply the information on the test material for the record.

TOXICITY CATEGORY: IV--Caution

009689

Guideline Series 81-5: Primary Dermal Irritation

A. MATERIALS

1. Test Material

Test material: CGA-169374 Technical
Identification number: FL-881994
Purity: Not reported, on file with sponsor
Physical description: Light beige powder
Storage conditions: Stored at room temperature
Stability: Not reported, on file with sponsor.

2. Controls

Animals: None needed
Test Substance: None needed

3. Test Animals

Species: Rabbit
Strain: New Zealand White albino; Hra: (NZW) SPF
Source: Hazleton Research Products, Inc., Kalamazoo MI
Sex and numbers: 3 Males; 3 females
Age: Adult
Initial body weight: 2,404-2,688 g
Housing: Individual; animal room maintained at a daily temperature of 21-25°C and a relative humidity of 66-77%.
Feeding: Feed (Purina® Mills High Fiber Rabbit Chow #5326) and tap water provided ad libitum throughout predosing and study period.
Animal identification: Animal number and corresponding eartag
Acclimation period: At least 7 days prior to dosing

B. TEST PERFORMANCE

The back and flanks (unspecified dimensions) of each rabbit were clipped free of fur approximately 24 hours prior to study initiation. A dose of 0.5 g of CGA-169374 moistened with 0.9% saline was topically applied to shaved intact skin on the back of each animal. The test site was covered with a semi-occlusive dressing consisting of a gauze patch measuring 6.25 cm^2 that was secured with paper tape, loosely wrapped with Saran Wrap, and secured with Elastoplast tape. After an exposure period of 4 hours, the gauze dressing was removed and the residual test article was washed from the treatment site using tap water and disposable paper towels. Animals were weighed just prior to administration of the test article.

Dermal irritation was reported at 30 minutes following patch removal on day 1 (referred to as a 4-hour score), and 24, 48, and 72 hours after patch removal. The study was terminated at 3 days post-treatment. At each interval, erythema and/or edema responses were scored according to a standard Draize method.

C. QUALITY ASSURANCE MEASURE

A signed Quality Assurance Statement and a signed Good Laboratory Practice statement were included in the study report.

D. RESULTS AND STUDY AUTHOR'S CONCLUSIONS

The only reported dermal irritation consisted of very slight erythema (Draize Score-1) in 1 female at the 4-hour scoring interval. The average Primary Irritation Index (P.I.I.) for the 4-hour interval was 0.2.

$$\text{P.I.I.} = \frac{\text{total erythema and edema scores for all animals}}{\text{number of test sites (6) at each observation period}} = \frac{1}{6} = 0.2$$

The average P.I.I. of the 4-, 24,-, 48- and 72-hour scoring intervals was 0.1 (1/24).

E. REVIEWERS' COMMENTS

The study report did not specify the dimensions of the test site. However, based on the size of the gauze patch (6.25 cm^2) covering the test site it is possible that the test site area was of adequate size to ensure proper conduct of the study. According to Guideline Series 81-5, the area of the test site should be approximately 6 cm^2. Nevertheless, the sponsor should clarify if the size of the test site was 6.25 cm^2.

Based on a Primary Irritation Index of 0.1, the reviewers agree with the study author's descriptive rating for CGA-169374 Technical as "slightly irritating" to the skin of albino rabbits. Based on these results, the reviewers assign CGA-169374 technical a dermal irritation toxicity category (40 CFR 162.10) of IV--Caution.

This study is classified as Core Supplementary since the size of the test site and the purity and the stability of the test material were not reported.

009689

DOC 930091
FINAL

DATA EVALUATION REPORT

Difenoconozale

Study Type: Dermal Sensitization

Study Title: CGA-169374 Technical Dermal Sensitization Study in Guinea Pigs

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 Reviewer	Betty Shindel, M.P.H.	6-8-92 Date
Independent Reviewer	John Liccione, Ph.D.	6-8-92 Date
QA/QC Manager	Sharon Segal, Ph.D.	6/8/92 Date

Contract Number: 68D10075
Work Assignment Number: 1-79
Clement Number: 91-263
Project Officer: James E. Scott

00042

Guideline Series 81-6: Dermal sensitization

Approved by:
EPA Reviewer: Karen Whitby, Ph.D. Signature
Review Section II, Toxicology Branch II (HED) Date 4/17/92

EPA Section Head: Clark Swentzel Signature
Review Section II, Toxicology Branch II (HED) Date 8/22/92

DATA EVALUATION REPORT

STUDY TYPE: 81-6 Dermal sensitization

EPA IDENTIFICATION NUMBERS

Shaughnessy Number: 128847
MRID Number: 420900-11

TEST MATERIAL: CGA-169374 Technical

SYNONYM: Difenoconozale

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

STUDY NUMBER: MIN 862076

TESTING FACILITY: Ciba-Geigy Corporation, Pharmaceutical Division, 556 Morris Avenue, Summit, NJ 07901

TITLE OF REPORT: CGA-169374 Technical Dermal Sensitization Study in Guinea Pigs

AUTHOR: Kirk Huber

FINAL REPORT: April 13, 1987

CONCLUSIONS: A 0.5 g topical application of CGA 169374 technical did not result in delayed contact hypersensitivity in guinea pigs.

CORE CLASSIFICATION: Core Supplementary. This study is classified as Core Supplementary because the purity (as well as any impurities) and stability of the test material were not reported.

TOXICITY CATEGORY: Not applicable

A. MATERIALS

1. Test Material

Test material: CGA-169374 technical
Identification number: FL-851406
Purity: Not reported, on file with sponsor
Physical description: Resinous material
Storage conditions: Stored frozen or at room temperature
Stability: Not reported, on file with sponsor

2. Test Animals

Species: Guinea pig
Strain: Hartley
Sex and numbers: 40 Females
Source: Elm Hill Breeding Laboratories, Chelmsford, Massachusetts
Receipt date: Not reported
Age: Not reported
Initial body weight: 278-385 g
Feeding: Feed (Certified Agway® Guinea Pig Chow) and tap water provided ad libitum.
Housing: Group housing, 2 animals per cage. Temperature = 73±5°F and relative humidity = 50±20%. A 12-hour photocycle was maintained.
Randomization: Animals randomly assigned to groups via a computer-generated randomization table.
Acclimation: At least 2 weeks prior to initiation of the study
Animal identification: Monel eartag
Health status: Study used animals judged healthy during acclimation period

Prelimimary dermal irritation screen: A single topical application of 0.5 g of CGA-169374 technical was applied to a gauze patch and secured to the shaved flank (dimensions of test site not specified) of 4 guinea pigs for 6 hours with occlusive tape. The patch was removed after the 6-hour exposure period, and the test site was wiped with 95% alcohol and water to remove residual test article. The severity of erythema and edema was scored by use of the Draize method. Results of the dermal irritation screen were not provided.

B. TEST PERFORMANCE

A modified Buehler method was used to determine the potential of CGA-169374 technical to produce delayed contact hypersensitivity. Ten 6-hour topical inductions of 0.5 g of CGA 169374 technical or 0.5 mL of 0.05% DNCB (1-chloro-2,4-dinitrobenzene) were applied followed by a topical challenge (induction and challenge phases discussed in detail below). There were 10 female guinea pigs in both the test group and the positive control group from a concurrent study (MIN 862075). In addition, two nonsensitized groups (10 guinea pigs/group) each received only a challenge dose of test material or DCNB. Body weights were measured on test day 1 prior to dosing and on test days 8, 15, 22, 29 and 36. The physical condition of the animals was monitored at least once a day during the predosing and dosing periods, and once prior to sacrifice on test day 38. Animals received a physical and auditory examination prior to the

start of the study on test day -3. All surviving animals were sacrificed at the end of the study; however, gross necropsies and histopathological examinations were not performed.

Induction phase: 0.5 g of CGA 169374 technical or 0.5 mL of 0.05% DNCB were applied to the shaved left flank of 20 guinea pigs (10/group) on test days 1, 3, 6, 8, 10, 13, 15, 17, 20, and 22 for a 6-hour exposure period.

Scoring of the skin for erythema and edema was performed 24 hours after each induction dose using the Draize method.

Challenge phase: Guinea pigs were challenged with 0.5 g of CGA 169374 technical or 0.5 mL of 0.05% DNCB on test day 36. The test material or DNCB were topically applied in the same manner used in the induction phase to the shaved right flank (dimensions of test site not specified) of all 40 guinea pigs. Following the 6-hour exposure period, the patch was removed and the test site was wiped with 95% alcohol and water to remove residual test article.

At 24 and 48 hours after the challenge exposure, all test sites were examined for a sensitization response. The criteria for a positive allergic reaction was redness that was greater than that produced in the respective nonsensitized groups. A mean skin sensitization score was obtained for each group at each scoring period after challenge. The mean sensitization score was calculated by adding the erythema scores for all animals and dividing by the total number of animals evaluated.

C. QUALITY ASSURANCE MEASURE

A signed Quality Assurance Statement and a signed Good Laboratory Practice Compliance Statement were included.

D. RESULTS AND STUDY AUTHOR'S CONCLUSIONS

There was no dermal irritation reported in any of the animals receiving CGA 169374 technical during the induction phase. No dermal response was observed in sensitized animals at 24 or 48 hours after challenge with CGA 169374 technical. For the positive control group, slight erythema (Draize grade 1) was occasionally reported during the induction phase in 6/9 of the DNCB sensitized animals. In sensitized animals challenged with the positive control material, the mean sensitization score at 28 and 48 hours postdosing was 0.7 and 0.4, respectively. In nonsensitized animals challenged with the test article or positive control material, there was no dermal response.

One of the animals that was induced and challenged with CGA 169374 technical died on day 12 of the study. However, the study author considered this death to be spontaneous and not treatment-related. Clinical signs of toxicity were unremarkable for this animal.

Administration of the test article had no significant effect on body weights.

E. REVIEWERS' COMMENTS

The reviewers agree with the study author's conclusion that a topical application of 0.5 g did not result in delayed contact hypersensitivity in guinea pigs. This study is classified as Core Supplementary because the purity (as well as any impurities) and stability of the test material were not reported.

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

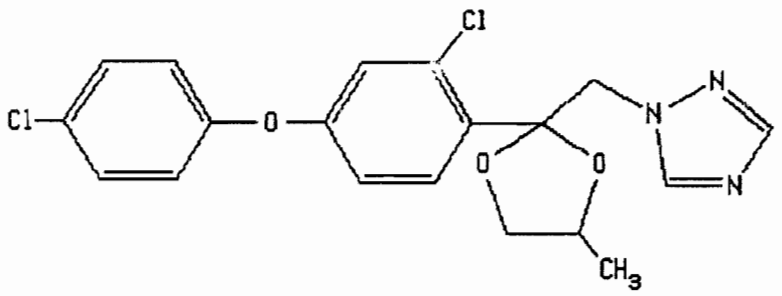
CAS REG. NO.: 119 446-68-3

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 taker 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

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.

Appendix 1

MRID NO 420900-22

RIN 1360-95

TOX REVIEW- 009689 DIFENOCONAZOLE

Page____ is not included in this copy.

Pages 61 through 93 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.

__V__ 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.

009689

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

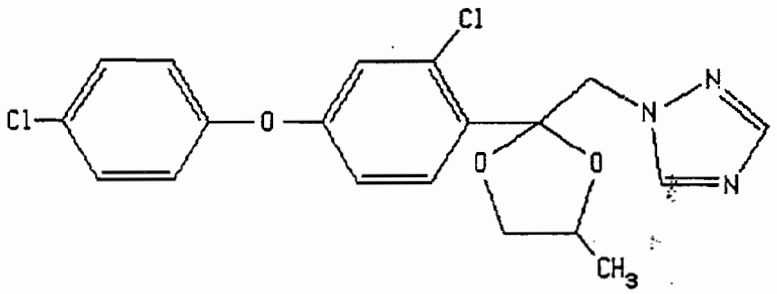
CAS REG. NO.: 119 446-68-3

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

00004

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.

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.

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* Vagina	x	Skin, abdominal mammary region*#
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.

Appendix 1

MRID NO. 420900-21

RIN 1360-95

TOX REVIEW 009689 DIFENOCONAZOLE

Page____ is not included in this copy.

Pages 106 through 124 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.

✓ FIFRA registration data.

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Tox. Chem. No. N/A File Last Updated N/A Current Date 04-10-92 Document # ______

Study/Lab/Study #/Date	Material	EPA MRID No.	Results: LD50, LC50, PIS, NOEL, LOEL	Tox Cat	Core-Grade
13 Week Oral feeding study in mice (§82-1)/Hazleton Labs. America, Incorporated 9200 Leesburg Turnpike Vienna, Virginia 22180/ 483-2411/October 20,1987	CGA-169374 Technical (94.5% 1-[2-(2-chloro-4-(4-chloro phenoxy)phenyl)-4-methyl-1, 3-dioxolan-2-yl)-methyl]-1H-1,2,4-triazole/dark brown solid/Dividend/fungicide/ difenoconazole	420900-21	Mice were fed dose levels of 20, 200, 2500, 7500, or 15000 ppm test article offered in feed admixtures. Most of the mice fed 7500 or 15000 ppm died during the first week. Test article-related effects of statistical significance were reduced body weight gain in the 2500 ppm paired female mice as well as a negative trend. Hepatotoxicity was the principal finding in mice that DOS evidenced by hepatocellular enlargement and necrosis of individual hepatocytes. Terminal necropsies also showed hepatotoxicity that included hepatocellular enlargement in group 4 animals and group 3 males and hepatocytic vacuolization in group 4 animals. 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 ≤ 7500 ppm.	N/A	Minimum

Reviewed by: Dan W. Hanke, Ph. D. Dan W Hanke 04 March 1992
Section III, Tox. Branch II (H7509C)
Secondary Reviewer: James N. Rowe, Ph. D. James N. Rowe 3/4/92
Section III, Tox. Branch II (H7509C)

DATA EVALUATION RECORD

STUDY TYPE: Repeated dose dermal toxicity: 21 day study (§82-2)

TOX CHEM NO (CASWELL NO):

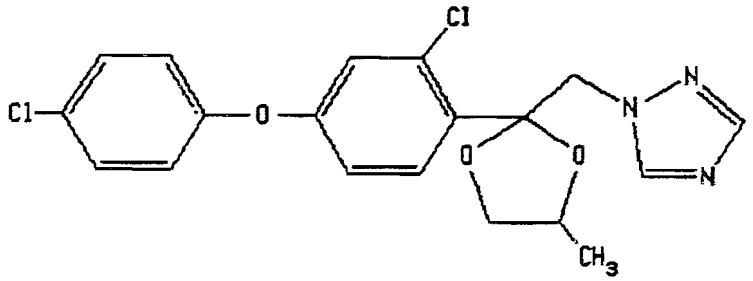
CAS REG NO: 119 446-68-3

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

HED PROJECT NO: 2-0696

MRID NO.: 420900-13

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; 1H-1,2,4-Triazole;

STUDY NUMBER: 862014

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

TESTING FACILITY: Research Department
Pharmaceuticals Division
Ciba-Geigy Corporation
556 Morris Avenue
Summit, NJ 07901

TITLE OF REPORT: 21-Day Dermal Toxicity Study in Rabbits

AUTHOR(S): D.M. Schiavo, J.R. Hazelette, and J.D. Green

REPORT ISSUED: October 28, 1987 (study completed)

SUMMARY & CONCLUSIONS:
CGA169374 Technical was dissolved in absolute ethanol and administered topically under occlusion to three groups of female and male rabbits (5/sex/group) at daily doses of 10 mg/kg, 100 mg/kg, or 1000 mg/kg for at least 22 consecutive days. An additional group of rabbits (5/sex) served as vehicle (100 % ethanol) controls exposed to volumes of ethanol comparable to the rabbits treated with the test material dissolved in the ethanol. Another group of rabbits (5/sex) served as untreated controls. None of the rabbits died on study. The test mixture is quite viscous. No mention was made of the relative viscosity of the test mixture in ethanol to the "viscosity" of ethanol. Perhaps the viscosities of the test mixture and the vehicle control should have been made similar. The vehicle-solubilized test material or vehicle alone was applied daily to intact skin for six hours.

On the strength of the available data it is possible to conclude that statistically significant decrements in body weight, body weight gain, and food consumption resulted in rabbits exposed to CGA-169374 at doses $\geq$ 100 mg/kg. None of the rabbits died on study. Macroscopic and microscopic observations ranged from mild to moderate skin irritation localized to the site of application of vehicle or test article-treated rabbits. The HDT females had increased adrenal weights and vacuolation of hepatocytes. Based on decrements in body weight, body weight gain, and food consumption, the NOEL of CGA-169374 was determined to be 10 mg/kg/day.

A signed quality assurance statement was present.

Core Classification: Minimum

This study satisfies the guideline requirements (§82-2) for a repeated dose dermal toxicity: 21 day study.

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 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

determination was made on a representative sample after termination of the study via gas chromatography equipped with a nitrogen-phosphorus detector. The test mixture was not detected in the control samples.

2. Test animals: Species: white rabbit; Strain: New Zealand; Age: approximately 14 to 16 weeks; Weight: females ranged 2.31 kg to 3.21 kg, males ranged 2.41 kg to 3.41 kg; Source: H.A.R.E. Inc, Hewitt, New Jersey.

METHODS:
Prior to application of the test substance, and as needed during the study, each rabbit was prepared by clipping the skin of the flank and back free of hair. One group of rabbits did not receive the test substance or the vehicle and served as an untreated control. A solution of CGA 169374 Technical (FL 850406) or the vehicle at ambient temperature was applied daily for 22 consecutive days to an area of intact skin comprising approximately 10 % (approximately 240 sq. cm.) of the total body surface of the rabbits according to the Standard Operating Procedure of the Safety Evaluation Facility. A gauze dressing was applied over the test site and secured to the animal with a non-irritating type adhesive tape (Zonas® Porous Tape, Johnson & Johnson). Gauze dressing and tape only were applied to untreated control animals. Following application each animal was fitted with an Elizabethan collar and returned to its cage. The compound remained in contact with the skin for ar least 6 hours daily. Following the exposure period the gauze dressing was removed and the treatment site was gently washed with 95 % aqueous ethanol and tap water and dried thoroughly with a paper towel. Each animal was then returned to its cage overnight.

The procedures for statistical treatment of the data and animal assignment, etc appear in appendices 1 and 2 respectively of this DER and were taken from pp 7, 23-26 and pp 3-4 respectively of the submission.

RESULTS and DISCUSSION.

Clinical Observations.
Clinical signs, physical and ophthalmoscopic examination, and gross observations did not support treatment-related effects and were unremarkable. In fact the only clinical sign reported was "few feces present" in both sexes at the HDT.

Signs of localized dermal irritation included erythema (Draize grade 1), wrinkled skin, desquamation, dry skin, fissuring, thickened skin, or scab formation and were observed in the vehicle controls as well as all treatment groups. Additionally, localized erythema of moderate severity (Draize grade 2) was

noted in the HDT females. See appendix 3 of this DER taken from pp 28-29 of the study report.

An historical note is John Draize, who published the dermal irritation scoring system in 1944, died late in 1991.

Microscopically, the dermal changes reported in this study were characterized as hyperkeratosis, acanthosis, congestion, hemorrhage, parakeratosis, and/or inflammation (purulent, hyperplastic). No dermal signs were observed in any of the untreated control rabbits throughout the study.

Clinical Pathology.
There were no significant treatment-related clinical signs observed at termination of the study for either sex with regard to hematology or biochemistry. There were, however, some clinical signs noted as unrelated to systemic toxicity of the test article, because they were not supported by gross or microscopic pathology and fell within the range of normal biological variation as determined before treatment. The signs were an increase in mean percent neutrophils and a reduction in mean percent lymphocytes in the HDT females on day 19. The authors proffer these deviations may be related to elevation of circulating corticosteroid levels, increased adrenal weights, and/or the inflammatory reactions occurring in the skin. In support of their views the study report authors make reference to a textbook on endocrinology (reference # 32 on p 30 of the study report).

With regard to biochemical parameters, a decrease in mean chloride levels and an increase in mean total bilirubin levels were seen in the HDT females on day 19. Also, a decrease in mean potassium levels was noted in HDT males on day 19. The individual values were stated to fall within historical control ranges for chloride, 98-112 milliequivalents per litre (mEq/l); total bilirubin, 0.07-0.60 mg/dl; and potassium, 4.0-6.9 mEq/l.

Summary data for both hematology and biochemical parameters appear in appendix 4 of this DER and were taken from pp 41-65 of the submission.

Mortality.
None of the animals died on study (DOS), and all the rabbits were sacrificed at term.

Food consumption and body weight.
Paired decreases in mean body weight of 15 %, only in female rabbits, and decrements in percent body weight gain were observed at the highest dose tested (HDT) in both female and male rabbits. There was a positive correlation at the HDT between the decrement in body weight gain and resultant body weight loss while the rabbits were on test. At the mid dose tested (MDT) a transient

decrease was observed in percent body weight gain for females. There was a positive correlation comparing the decrements in body weight gain and body weight with modest reductions in mean food consumption throughout the bulk of the study. There was, however, a slight increase in mean food consumption unrelated to treatment noted on test day 21 in the lowest dose tested (LDT) males.

Summary data appear in appendix 5 of this DER and were taken from pp 34-39 of the submission.

Macroscopic and microscopic observations and organ weights at terminal sacrifice.
Microscopically, vacuolation of hepatocytes was observed in four out of five HDT female rabbits. The authors infer from the appearance of the vacuoles, that fatty metamorphosis in the liver occurred, not from circulating test material, but rather resultant to the test article precipitating changes in the skin. Once again these changes, therefore, were not deemed to mirror compound-related systemic toxicity, and the authors cite a text on the physiological and pharmacological effects of corticosteroids (reference # 33 on p 30 of the study report) suggesting increased adrenal corticosteroid levels could have given rise to fatty metamorphosis. Summary data appear in appendix 6 of this DER and were taken from p 173 of the submission.

With regard to absolute or relative organ weight changes, there was no gross pathology attributed to the test article. The increases in mean absolute and relative adrenal weight noted in the majority of the HDT females may have been related to adrenal hypertrophy. The study authors lumped changes in mean relative heart, liver, and kidney weights together as being secondary to body weight loss, because there was no meaningful correlation with other indices of test article effects. The increases in mean absolute and relative ovary weights in the MDT females were not deemed toxicologically significant. Select summary data appear in appendix 7 of this DER and were taken from pp 74-79 of the submission.

MRID NO. 420900-13

Appendix 1

RIN 1360-95

TOX REVIEW 009689 DIFENOCONAZOLE

Page____ is not included in this copy.

Pages 132 through 163 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.

____ Information about a pending registration action.

✓ FIFRA registration data.

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

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

Tox. Chem. No. N/A File Last Updated N/A Current Date 02-28-92 Document # _____

Study/Lab/Study #/Date	Material	EPA MRID No.	Results: LD50, LC50, PIS, NOEL, LEL	Tox Cat	Core-Grade
Repeated dose dermal toxicity in rabbits: 21 day study (§82-2)/ Research Depart Pharmaceuticals Div Ciba Geigy 556 Morris Ave Summit NJ 07901/862014/28 Oct87	CGA-169374 Technical (94.5% 1-[2-(2-chloro-4-(4-chloro phenoxy)phenyl)-4-methyl-1, 3-dioxolan-2-yl)-methyl]-1H-1,2,4-triazole/dark brown solid/Dividend/fungicide/ difenoconazole	420900-13	None of the rabbits DOS at doses of 10, 100, or 1000 mg/kg/day. There were no test material-related signs of gross, microscopic, or biochemical toxicity at any dose tested. The NOEL was 10 mg/kg/day (the LDT) based on statistically significant decrements in body weight, body weight gain, and food consumption at doses ≥ 100 mg/kg/day.	N/A	Minimum

Primary Review by: K.E. Whitby, Ph.D. 4/16/92
Toxicologist, Review Section II, Tox. Branch (H7509C)
Secondary Review by: K. Clark Swentzel 4/16/92
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	17.44	14.64	16.09
49	19.45	14.37	22.54	19.76	19.29	22.30	19.97	19.97	16.09	18.82
56	21.54	17.50	24.19	20.70	19.65	23.91	23.23	23.23	17.77	21.34
63	24.10	19.40	27.86	23.84	23.12	26.53	25.01	25.01	20.08	22.56
70	25.63	20.73	30.55	26.48	24.02	29.43	27.60	27.60	22.05	24.67
77	26.32	22.23	31.17	27.13	24.39	28.62	27.30	27.30	21.59	24.27
84	28.53	23.46	33.23	28.53	27.02	32.49	28.54	28.54	23.73	25.93
91	28.94	25.06	34.88	30.59	27.91	33.30	29.21	29.21	25.24	27.23
105	27.64	27.23	36.29	31.97	28.43	32.94	32.73	32.73	22.38	25.80
133	28.79	33.19	41.83	38.41	32.94	39.54	39.84	39.84	25.20	31.05
161	28.13	35.66	43.11	39.63	33.62	40.88	41.31	41.31	24.54	29.73
189	30.27	38.43	45.42	40.77	36.30	42.35	40.66	40.66	27.58	32.36
217	27.87	38.43	46.02	39.71	38.55	40.77	37.45	37.45	26.08	29.76
245	34.35	44.13	49.98	43.38	41.70	46.01	42.25	42.25	32.50	33.34
273	35.86	46.08	51.68	45.44	43.34	44.21	45.55	45.55	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.

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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.

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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

[redacted]

APPENDIX I
Materials and Methods

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009689

Primary Review by: K.E. Whitby, Ph.D. 6/22/92
Toxicologist, Review Section II, Tox. Branch (H7509C)
Secondary Review by: K. Clark Swentzel
Section Head, Review Section II, Tox. Branch (H7509C)

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

00131

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.

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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.

RIN 1360-95

TOX REVIEW 009689 DIFENOCONAZOLE

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Pages 203 through 204 are not included.

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____ The product confidential statement of formula.

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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

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	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

APPENDIX I

Statistical Analyses

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GUIDELINE: 83-3

Primary Review by: K. E. Whitby, Ph.D. 1/3/92
Toxicologist, Review Section II, Toxicology Branch II/HED
(H7509C)

Secondary Review by: K. Clark Swentzel 2/4/92
Section Head, Review Section II, Toxicology Branch II/HED
(H7509C)

DATA EVALUATION RECORD

Study Type: Teratology - Developmental Toxicity
Species: Rat
Guideline: 83-3(a)

EPA Identification Nos.: EPA MRID No.: 420900-16
Shaughnessy No.: 128847
HED Project No. 2-0696

Test Material: CGA 169374 Technical

Synonyms: Difenconazole

Sponsor: Agricultural Division
CIBA-GEIGY Corporation
Post Office Box 18300
Greensboro, NC 27419

Study Number(s): Argus 203-005

Testing Facility: Argus Research Laboratories, Inc.
2025 Ridge Rd.
Perkasie, PA 18944

Title of Report: Developmental Toxicity Study of CGA-169374 Technical (FL-851406) Administered Orally Via Gavage to Crl:COBS®CD®(SD)BR Presumed Pregnant Rats

Author(s): Elizabeth A. Lochry, Ph.D.

Report Issued: November 6, 1987

Study Dates: February 3, 1986 (initiation of cohabitation)
February 28, 1986 (last cesarean section)

Conclusions: (summary of findings)

CGA 169347 technical was administered by gavage on days 6-15 of gestation to presumed pregnant rats at 0, 2, 20, 100, or 200 mg/kg. Significant decreases in maternal body weight gain and feed consumption were observed during the dosing period for the

feed consumption were observed during the dosing period for the 100 and 200 mg/kg groups. These animals also exhibited a significant increase in the incidence of excess salivation. There was a non significant decrease in the mean number of fetuses per dam, and non significant increases in the mean number of resorptions per dam and % postimplantation loss in the 200 mg/kg group. There was a slight (non significant) decrease in mean fetal body weight at the 200 mg/kg group. The following represents the significant alterations in the development of fetuses in the 200 mg/kg group. The incidence of bifid or unilateral ossification of the thoracic vertebrae was significantly increased on the fetal basis. There were also significant increases in the average number of ossified hyoid and decreases in the average number of sternal centers of ossification (per fetus per litter). The average number of ribs was significantly increased (with accompanying increases in the number of thoracic vertebrae), and decreases in the number of lumbar vertebrae in this group. These findings may be related to maternal toxicity. This study may be upgraded after satisfactory review of the response to the noted deficiencies.

Core Classification: supplementary

NOTE: Due to the relatively high percent deviation of the actual doses tested from the theoretical concentration the effect levels have been modified accordingly. This modification may be subject to change as the purity is currently unknown.

Maternal NOEL = 16 mg/kg
Maternal LOEL = 85 mg/kg
Developmental Toxicity NOEL = 85 mg/kg
Developmental Toxicity LOEL = 171 mg/kg

RANGE-FINDING STUDY

The main developmental toxicity study was preceded by a pilot study, Argus Research Project 203-005P.

Results

The doses tested and the results were not provided in the current report.

MAIN STUDY

A. Materials

1. **Test Compound**:

Test Substance: CGA 169374
Purity: not provided
Description: not provided
Lot No.: FL 851406
Stability: not provided
Storage Conditions: room temperature, prepared test substance suspensions were refrigerated

During the preparation of the dosing solutions the purity of the test substance was assumed to be 100%. The report indicates that the actual purity of the test substance is on file with the sponsor. The concentrations used for dosage administration were prepared on February 10 and 17, 1986. The concentrations were resuspended prior to use each day and stirred constantly while dosing.

Page 16 of the report states in a footnote that "all original records documenting this procedure (test substance preparation) are contained in the raw data for 203-005PA. CGA 169374 technical in liquid form was combined with HiSil using acetone as a solvent to reduce the test substance to a powder. The concentrations were prepared as 0, 0.2, 2.0, 10.0, and 20.0 mg/mL (the dosing volume was 10 mL/kg). The suspensions were prepared weekly at volumes sufficient for a minimum of seven days of use. The prepared concentrations were stored in clear glass 2 liter beakers covered with Parafilm®. The beakers and the vehicle were stored under refrigeration.

The formulated test substance was assumed to be a 50% mixture of CGA 16934 and HiSil®. Analysis of the formulated test substance at the beginning and end of the dosing period found the mixture was between 44 and 49% test substance. Samples of the dosing formulation were analyzed for concentration and stability.

2. **Vehicle(s)**:

Vehicle: aqueous 0.5% carboxymethylcellulose (CMC) suspended in R.O. water
Purity: not stated
Lot No.: 114F-0414
Stability: not stated
Storage Conditions: not stated

The vehicle [0.5% (w/v) CMC] was suspended in R.O. water was combined with 30 mg/mL HiSil using acetone as a solvent. This was done to assure that an equal amount of HiSil was present in both the vehicle and the highest concentration of the test substance used.

3. **Test Animal(s)**:

Species: rat
Strain: Crl:COBS®CD®(SD)BR
Source: Charles River, Kingston, NY
Age: 71 days on receipt
Weight: 200 - 246 g on the day after arrival

One hundred-eighty Crl:COBS®CD®(SD)BR virgin female rats were received on January 7, 1986 from the Kingston, NY Facility of Charles River Breeding Laboratories, Inc.. One hundred-fifty were assigned to this study. The remaining 30 were assigned to another study.

On January 21, 1986, 160 male rats of the same strain and source were received for the purpose of breeding. The males were 86 days old (DOB: November 9, 1985) just prior to cohabitation, and weighed 333 - 433 g.

B. Methods

1. **Animal Husbandry**

On arrival from the breeder the females were assigned to individual cages based on a computer generated randomization procedure. Females were assigned a temporary number which was used for identification during acclimation. Male rats were randomized in a similar manner and assigned permanent numbers and ear tags.

The animal study room was independently supplied with 12 changes per hour of 100% fresh hepa filtered air. The room had approximately 173 sq. ft. of floor space.

The room temperature was maintained at 74±4°F (except for a 15

hour interval 2/15-16/86 where the temperature was 68°F). The humidity in the study room was recorded once each week and ranged from 42 - 58%. The study room was maintained on a 12 hour light/dark cycle (7:00 a.m. - 7:00 p.m.).

The animals drank local water ad libitum from an automatic watering system which had been passed through a reverse osmosis membrane. The water is analyzed annually for possible chemical contamination and monthly for bacterial contamination. Chlorine was added to the processed water as a bacteriostat. The water contained approximately 0.0 to 0.3 ppm chlorine and was determined to be acceptable for consumption.

The rats were fed Purina Rodent Chow Meal® #5002 ad libitum.

2. Mating

After approximately 4 weeks of acclimation, 150 virgin female rats were mated with males in a 1:1 ratio for a maximum of five days. Females were presumed pregnant (day 0 of gestation) when sperm was observed in a vaginal smear or a copulatory plug was found in the cage pan. Males were sacrificed at the completion of the mating period (February 13, 1986). Females that did not mate were assigned to the test facility general population.

3. Study Design

This study was designed to assess the developmental toxicity potential of CGA 169374 when administered by gavage to presumed pregnant rats on gestation days 6 through 15, inclusive.

4. Group Arrangement:

Test Group	Dose Level (mg/kg)	Number Assigned
Control	0	25
Low Dose	2	25
Low Mid Dose	20	25
High Mid Dose	100	25
High Dose	200	25

Day 0 of gestation was February 4 - 8, 1986 for the animals assigned to the study. The animals weighed 229 - 306 on day 0 of gestation. Animals were assigned to groups using a second computer generated (weight ordered) randomization procedure based on day 0 of gestation body weights.

5. Dosing:

All doses were administered orally in a volume of 10 mL/kg of body weight/day prepared during the dosing period. The dosing

solutions were analyzed for concentration. Dosing was adjusted daily for observed body weight.

6. **Observations**:

Dams were observed daily during the dosing and post dosing period for clinical signs, treatment related toxicity, abortions, or death. The body weight of dams were recorded on days 0 and 6-20 of gestation. Feed consumption was recorded days 0-6, and then daily days 6-20.

Dams were euthanized by carbon dioxide asphyxiation on day 20 of gestation. During the necropsy the abdomen and uterus of each rat was opened and examined for the number and placement of implantations, early and late resorptions, and live and dead fetuses. Corpora lutea were counted, and each fetus was weighed, sexed and examined for external alterations. Approximately one half of the fetuses in each litter were assigned to either soft tissue examination [using a modification of Wilson's sectioning technique (Wilson 1965)] or to skeletal examination [after clearing and staining with alizarin red-S (Staples and Schnell, 1963)].

Historical control data were provided to allow comparison with concurrent controls.

7. **Statistical Analysis**

The statistical analysis section of the report is included in the methods section which is appended. Maternal physical sign data and the maternal and fetal incidence data were analyzed using the variance test for homogeneity of the binomial distribution. Body weight (maternal and fetal), feed consumption, fetal ossification sites, percent dead or resorbed conceptuses, percent male fetuses and percent fetuses with alterations were analyzed using Bartlett's test of homogeneity of variances and ANOVA. If the ANOVA was significant and appropriate then Dunnett's test was used. If the ANOVA was not appropriate, the Kruskal-Wallis test was used; in cases where statistical significance occurred, Dunn's method of multiple comparisons was used to identify significance.

Analysis of Covariance was used to evaluate maternal body weight change from days 0 to days 6 and 20 of gestation. Average maternal body weight change from day 16 to days 17 through 20 of gestation was analyzed using the statistical regimen described above (using Bartlett's test of homogeneity of variance and ANOVA). Enumeration data from cesarean sections were evaluated Kruskal-Wallis test and Dunn's method of multiple comparisons (used to identify the statistical significance).

8. **Compliance**

A signed Statement of Confidentiality Claim was provided (p. 2) and dated June 7, 1990.

A signed Statement of Compliance with EPA GLP's was provided (p. 7) and dated November 6, 1987.

A signed Quality Assurance Statement was provided (pp. 211-214) and dated November 6, 1987.

A signed Flagging Criteria Statement was provided (p. 4) which indicated that the data neither meet nor exceeds the applicable criteria. The statement was dated June 15, 1990.

C. Results

1. Analyses of the Test Substance

The analytical report for concentration analyses was prepared by Lancaster Laboratories, Inc..

a. Purity

This reviewer was unable to locate data pertaining to purity in this report. The report indicates that the assumption was made that the purity of the test substance was 100% and that the actual purity of the test substance is on file with the sponsor.

b. Stability

The report indicates that the stability documentation is available in the sponsor's records (p. 16).

c. Concentration

In table 1 of Appendix D (Analytical Results) the concentration found is reported in mg/g, whereas the target concentration is reported in mg/mL. The report does 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.

Based upon the available information, the concentration of the LDT (0.2 mg/mL) used on the first and last days of use indicated the actual concentration ranged from 75 - 70% of the theoretical concentration. The 2.0 mg/mL concentrations analyzed from the beginning and end of use of each of the two batches prepared for dosing found this suspension to be within 84-70% of the theoretical concentration. The 10 and 20 mg/mL concentrations were reported to be within 96-80% of their theoretical concentrations.

Target Concentration mg/mL	Mean Concentration Found mg/g	% Deviation of Mean Concentration Found From Target	Actual Dose Tested mg/kg
0.2	0.1425	-28.75	1.425
2.0	1.5525	-22.375	15.525
10.0	8.4525	-15.475	84.525
20.0	17.125	-14.375	171.25

d. Homogeneity

The report states that samples taken of the first and last days of use for each batch prepared demonstrated that the suspensions were homogeneous. Analyses of homogeneity (samples from the top, middle and bottom of the vessel containing the dosing suspensions) was not apparently performed.

2. Analyses of Feed and Water

The R.O. water used was analyzed and found to be suitable for use.

The report indicates that the routine analyses by the feed supplier did not reveal any contaminants or deviations from expected nutritional requirements.

3. Maternal Toxicity

a. Mortality

There was no maternal mortality in this investigation.

b. Clinical Observations

A kinked tail was observed for one control dam (26,572). Another dam (26,594) in the control group had a missing or misaligned upper incisor. One dam in the 20 mg/kg group (26,644) had a split in the lower margin of the mouth. The dams receiving 100 and 200 mg/kg exhibited significantly increased incidence of excess salivation. Relative to the control and two lowest doses tested, the higher two doses tested showed a slightly higher incidence of red vaginal exudate. The authors relate this observation to the resorption of 10 or 16 conceptuses for one of the three rats in each of the dosage groups. None of the other

findings observed during the clinical observation of the dams appeared to be related to treatment (e.g.- alopecia, chromodacryorrhea, chromorrhinorrhea, localized skin lesions, etc.).

c. **Body Weight**

The investigators supplied the following data:

TABLE 1: Mean Maternal Body Weight Gain (grams)[a]

Dose (mg/kg)	0	2	20	100	200
Days 0-6	19.6	21.2	18.4	19.5	18.4
Days 6-15	37.8	39.5	37.7	29.0**	16.7**
Days 16-20	61.7	63.6	64.5	68.6	70.1
Days 0-20	128.9	134.2	131.7	127.6	113.0**

* Significantly different from control ($p \leq 0.05$).
** Significantly different from control ($p \leq 0.01$).
Data extracted from Argus Report 203-005, table 3 pp. 47-48.
a = Corrected Maternal body weight gain was not calculated since gravid uterine weights were not measured.

There was a treatment related significant inhibition of maternal body weight gain at the 100 and 200 mg/kg groups. Weight loss was first observed the first day of treatment and lasted until day 8 for the 100 mg/kg group and until day 10 for the 200 mg/kg group. Inhibition of maternal weight gain was present through the remainder of the dosing period for these groups relative to the control group. Days 16-19 of gestation there was a dose related rebound or increase in maternal body weight gain, which was significant at the 200 mg/kg level. Although the table on p. 48 does not indicate it, there was a significant decrease in overall maternal body weight gain (days 0-20) in the HDT according to the text on p. 29.

d. **Feed Consumption**

Maternal feed consumption was analyzed and tabulated on a daily basis after the predosing period (days 0-6). Intermittent dose dependent reductions in maternal feed consumption were observed during the dosing period in the 100 and 200 mg/kg groups relative to the control group. Significance was detected in the 100 mg/kg group days 6-7, 7-8, 8-9, and 11-12. Significance was found in the 200 mg/kg group days 6-7, 7-8, 8-9, and 9-10. A significant dose dependent rebound/increase in maternal feed consumption was observed days 16-20 in the 100 and 200 mg/kg groups.

f. **Gross Pathological Observations**

Observations recorded during necropsy did not reveal treatment related findings.

g. **Cesarean Section Observations**

The 200 mg/kg group had a non significant decrease in the mean number of live fetuses/dam, and a nonsignificant increase in % postimplantation loss and the mean number of resorptions per dam. Also at the HDT, there was a slight (nonsignificant) decrease in fetal body weight.

TABLE 3: Cesarean Section Observations[a]

Dose (mg/kg):	**0**	**2**	**20**	**100**	**200**
#Animals Assigned	25	25	25	25	25
Pregnancy Rate (%)	100	100	96	100[b]	100
Maternal Wastage					
#Died	0	0	0	0	0
#Non pregnant	0	0	1	0	0
N	25	25	24	23[b]	25
Corpora Lutea/Dam	16.1	17.4	15.9	17.5	15.6
Implantations/Dam	14.8	14.7	14.7	15.1	14.0
Total Live Fetuses	352	341	335	324	306
Live Fetuses/Dam	14.1	13.6	14.0	14.1	12.2[c]
Total Resorptions	18	27	18	24	44
Early	18	27	17	24	42
Late	0	0	1	0	2
Resorptions/Dam	0.72	0.93	0.75	1.04	1.76
Total Dead Fetuses	0	0	0	0	0
Dead Fetuses/Dam	0	0	0	0	0
Mean Fetal Wt. (g)	3.39	3.48	3.41	3.38	3.25
♂	3.50	3.58	3.47	3.49	3.32
♀	3.28	3.39	3.34	3.28	3.20
Preimpl. Loss(%)[d]	9.3	14.6	6.9	13.3	10.6
Postimpl. Loss(%)	4.8	7.4	4.8	6.6	13.5[c]
Sex Ratio (% Male)	52.0	49.0	51.5	46.9	46.6

a= Data extracted from Argus report 203-005 tables 7, 8 and 17.
b= Two dams were excluded from tabulation because the permanent numbers assigned to these rats were inadvertently reversed during

the eartagging-procedure. These dams were not sacrificed on day 20 of gestation (one was sacrificed on day 17 and the other on day 21 - both were pregnant). Examination of the fetuses (gross external, soft-tissue, and skeletal) found the fetuses to "appear normal".
c= This value includes dam 26,689 for which the entire litter was resorbed (16 implants), Table 7 p. 54 does not include this litter in the calculation of the mean number of live fetuses or % resorbed conceptuses/litter and reports the group means for these values as 12.8 and 9.8 respectively. Therefore, these endpoints were not statistically analyzed with this data point included.
d= % Preimplantation loss was calculated by this reviewer.

4. Developmental Toxicity

TABLE 4: External Examination of Fetuses

Dose (mg/kg):	**0**	**2**	**20**	**100**	**200**
#pups(litters) examined	352(25)	341(25)	335(24)	324(23)	306(24)
Eyes					
Bulge, Depressed Unilateral	-	-	-	1(1)	-
Body					
Umbilical Hernia	-	-	-	-	1(1)

Data extracted from Argus report 203-005 table 9 p. 56.

There were no treatment related findings during the gross external exam.

TABLE 5: Visceral Examinations

Dose (mg/kg):	**0**	**2**	**20**	**100**	**200**
#pups(litters) examined	170(25)	165(25)	163(24)	156(23)	146(24)
Brain Lateral &/or Third Ventricles, Slight Dilation	2(2)	-	-	-	1(1)
Eyes Microphthalmia, Bulge Depressed, Unilateral	-	-	-	1(1)	-
Lungs Diaphragmatic Lobe, Agenesis	-	-	-	1(1)	-
Kidneys Pelvis, Slight or Moderate Dilation, Unilat. or Bilat.	-	1(1)	4(2)	1(1)	1(1)

Data extracted from Argus report 203-005 table 10 p. 57.

There were no statistically significant or increased incidences of treatment related findings during the soft tissue exam.

TABLE 6: Skeletal Examinations

Dose (mg/kg):	0	2	20	100	200
#pups(litters) examined	182(25)	176(25)	172(24)	168(23)	160(24)[a]
Vertebrae					
Cervical Rib					
Present	2(2)	0(0)	1(1)	1(1)	1(1)
Thoracic Centra					
Unossified	0(0)	0(0)	0(0)	0(0)	1(1)
Bifid	0(0)	1(1)	0(0)	2(2)	5(4)[b]
Unilat. Ossif.	0(0)	0(0)	0(0)	0(0)	3(1)[b]
Lumbar, Arch, Inc. Ossif.	2(2)	0(0)	0(0)	0(0)	1(1)
Ribs					
Wavy	5(3)	0(0)**	1(1)[b]	0(0)**	0(0)**
Hypoplastic	6(3)	0(0)**	0(0)**	0(0)**	0(0)**
Fused	0(0)	0(0)	0(0)	0(0)	1(1)
Manubrium					
Inc. or Unossified	2(2)	0(0)	1(1)	0(0)	1(1)
Sternebrae					
1+ Inc. or Unossified	10(7,	0(0)**	4(2)**	5(3)*	10(7)
Front Limb					
Polydactyly	1(1)	0(0)	0(0)	0(0)	0(0)
Pelvis					
Pubes, Inc. or Unossified, Bi- or Uni-lateral	8(6)	4(3)	6(4)	1(1)	1(1)
Ischia, Inc. or Unossified, Bi- or Uni-lateral	6(4)	0(0)**	0(0)**	0(0)**	1(1)**
Mean Ossification Sites- per fetus/litter					
Hyoid	0.72	0.76	0.88	0.90	0.95*
Vertebrae, Thoracic	13.0	13.0	13.0	13.0	13.2**
Vertebrae, Lumbar	6.0	6.0	6.0	6.0	5.8**
Ribs	13.0	13.0	13.0	13.0	13.2**
Sternal	3.73	3.73	3.63	3.53	3.40*

Data extracted from Argus report 203-005 tables 11 and 12 pp. 58-61, and p. 62.

a= Excludes litter #26,689; all conceptuses were resorbed.
b= Significantly different from control on the fetal basis ($p \leq 0.01$).
* Significantly different from control on the litter or litter and fetal basis ($p \leq 0.05$).
** Significantly different from control on the litter or litter and fetal basis ($p \leq 0.01$).

There were significant alterations in the development of fetuses in the 200 mg/kg group. The following represents these findings. The incidence of bifid or unilateral ossification of the thoracic vertebrae was significantly increased on the fetal basis. There were also significant increases in the average number of ossified hyoid and decreases in the average number of sternal centers of ossification (per fetus per litter). The average number of ribs was significantly increased (with accompanying increases in the number of thoracic vertebrae), and decreases in the number of lumbar vertebrae in this group. These findings may be related to maternal toxicity.

D. **Discussion/Conclusions**

1. Maternal Toxicity:

Significant decreases in maternal body weight gain and feed consumption were observed during the dosing period for the 100 and 200 mg/kg groups. These animals also exhibited a significant increase in the incidence of excess salivation.

2. Developmental Toxicity:

a. Deaths/Resorptions:

There was a non significant decrease in the mean number of fetuses per dam, and non significant increases in the mean number of resorptions per dam and % postimplantation loss in the 200 mg/kg group.

b. Altered Growth:

There was a slight (non significant) decrease in mean fetal body weight at the 200 mg/kg group.

c. Developmental Anomalies:

There were significant alterations in the development of fetuses in the 200 mg/kg group. The following represents these findings. The incidence of bifid or unilateral ossification of the thoracic vertebrae was significantly increased on the fetal basis. There were also significant increases in the average number of ossified hyoid and decreases in the average number of sternal centers of ossification (per fetus per litter). The average number of ribs

was significantly increased (with accompanying increases in the number of thoracic vertebrae), and decreases in the number of lumbar vertebrae in this group. These findings may be related to maternal toxicity.

E. **Study Deficiencies**:

1. The purity of the test substance was not provided.

2. The stability of the test substance was not provided, although data for the stability of the test substance in the vehicle was provided.

3. It is not apparent that an analyses of the homogeneity was performed. This is of concern due to the relatively high percent deviation of the actual doses tested from the theoretical concentration.

4. There is a deficiency in reporting information for the concentration analyses. In table 1 of Appendix D (Analytical Results) the concentration found is reported in mg/g, whereas the target concentration is reported in mg/mL. The report does 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.

F. **Core Classification**: supplementary

This study may be upgraded upon satisfactory review of the response to the deficiencies noted above.

NOTE: Due to the relatively high percent deviation of the actual doses tested from the theoretical concentration the effect levels have been modified accordingly. This modification may be subject to change as the purity is currently unknown.

Maternal NOEL = 16 mg/kg
Maternal LOEL = 85 mg/kg
Developmental Toxicity NOEL = 85 mg/kg
Developmental Toxicity LOEL = 171 mg/kg

APPENDIX I

Analytical Results

APPENDIX D

ANALYTICAL RESULTS

RIN 1360-95

TOX REVIEW. 009689 DIFENOCONAZOLE

Page_____ is not included in this copy.

Pages 242 through 254 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.

__✓__ 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.

GUIDELINE: 83-3

Primary Review by: K. E. Whitby, Ph.D. 1/31/92
Toxicologist, Review Section II, Toxicology Branch II/HED
(H7509C)

Secondary Review by: K. Clark Swentzel 2/6/92
Section Head, Review Section II, Toxicology Branch II/HED
(H7509C)

DATA EVALUATION RECORD

Study Type: Teratology - Developmental Toxicity
Species: Rabbit
Guideline: 83-3(b)

EPA Identification Nos.: EPA MRID No.: 420900-17
Shaughnessy No.: 128847
HED Project No.: 2-0696

Test Material: CGA-169374 Technical

Synonyms: Difenconazole

Sponsor: Agricultural Division
CIBA-GEIGY Corporation
Post Office Box 18300
Greensboro, NC 27419

Study Number(s): Toxicology/Pathology Report 87022 (MIN 862107)

Testing Facility: CIBA-GEIGY Corporation
Research Department
Toxicology/Pathology Division
Reproductive Toxicology Subdivision
Safety Evaluation Facility
556 Morris Avenue
Summit, NJ 07901

Title of Report: CGA-169374 Technical Teratology Study in Rabbits

Author(s): H.E. Hummel, M.A. Yourneff, M.L.A. Giknis, and E.T. Yau

Report Issued: August 31, 1987

Study Dates: 05/24/86 (first day of dosing)
06/20/86 (last day of necropsy)

Conclusions: (summary of findings)

CGA 169347 technical was administered by gavage on days 7-19 of

gestation to presumed pregnant rabbits at 0, 1, 25, or 75 mg/kg. Maternal toxicity was observed in this study as the death of one doe and abortions observed in two other high dose does. In addition, significant reductions in body weight gain of high dose does, were present days 7-10, 10-14, 7-20, and 0-29. These reductions correspond with reduced feed consumption during these intervals (significant reductions in feed consumption in the HDT were only observed during the treatment period, not after treatment). Slight nonsignificant increases in postimplantation loss and resorptions/doe were observed in the HDT. The significant decrease in fetal weight at the HDT may have been due to treatment. The significant differences in fetal weight observed at the low and mid dose were apparently not due to treatment.

Core Classification: supplementary

This study does not satisfy the guideline requirements (83-3) for a developmental toxicity study due to deficiencies. This study may be upgraded after a satisfactory review of the noted deficiencies.

Maternal NOEL = 25 mg/kg
Maternal LOEL = 75 mg/kg
Developmental Toxicity NOEL = 25 mg/kg
Developmental Toxicity LOEL = 75 mg/kg

RANGE-FINDING STUDY

The main developmental toxicity study was preceded by a pilot study, which was performed to determine the dosage levels to be used in the main study. The details of this study were not provided.

MAIN STUDY

A. Materials

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

1. **Test Compound**:

Test Substance: CGA 169347 technical
Purity: not provided
Description: not provided
Batch No.: FL 851406
Contaminant: not provided
Stability: 21 days (no data)
Storage Conditions: not stated

The protocol, page 18, states that the retention, purity, stability, and characterization of the test article remains the responsibility of CIBA-GEIGY, Agricultural Division, located in Greensboro, North Carolina.

2. **Vehicle(s)**:

The control article (vehicle) was 3% aqueous corn starch containing 1.0% Tween 80. The vehicle has been determined to be stable for at least 21 days at either room temperature or 2-8°C.

3. **Test Animal(s)**:

Species:	Rabbit
Strain:	New Zealand White S.P.F.
Source:	H.A.R.E. - Marland Hewitt, NJ
Age:	Not Stated
Weight:	2.94 - 3.0 kg

The number of animals obtained form the breeder for the purpose of this study was not stated. The fate of the animals not mated was also not stated.

B. Methods

1. **Animal Husbandry**

All animals received Purina Certified Rabbit Chow #5325 and water (via an automatic watering system), ad libitum. The feed and drinking water were monitored for levels of contaminants. Does were caged individually in mesh bottom stainless steel cages which were changed almost biweekly. The animal room was maintained at 65±5° F with a relative humidity of 50±20%; the room had 14 hours of light and 10 hours of dark. On one day the room temperature exceeded the acceptable range by 1°F, which did not compromise the integrity of the study.

2. **Mating**

Approximately 3.5 weeks were permitted for acclimation of 76 sexually mature, virgin female rabbits to the test facility. The animals were artificially inseminated over four days (19 females/day). Insemination was performed using semen for the buck colony (same strain) which is maintained at this facility.

Buck I.D.	Ear Punch	Semen Used for Insemination:
E04	100	05/19/86
G01	902	05/20/86
G07	98	05/21/86
G04	2	05/22/86

For unknown reasons a number of females lost body weight between their last weighing during acclimation and the day of artificial insemination. Because the animals appeared to be in good health they were placed on the study. All animals gained weight prior to dosing.

3. **Study Design**

This study was designed to assess the developmental toxicity potential of CGA169374 when administered by gavage to presumed pregnant rabbits on gestation days 7 through 19, inclusive.

4. **Group Arrangement**:

Test Group	Dose Level (mg/kg)	Number Assigned
Control	0	19
Low Dose	1	19
Mid Dose	25	19
High Dose	75	19

5. **Dosing:**

The test article was administered by gavage as a 0.02, 0.05, 1.5% suspension in 3% aqueous cornstarch containing 1.0% Tween 80 (also known as polysorbate 80) All doses were in a volume of 5 mL/kg of body weight/day. Dosing was based on the animals most recent body weight recorded on gestation days 7, 10, and 14. The frequency of preparation of the dosing solutions was not stated. prepared during the dosing period. The dosing solutions were analyzed for concentration and stability (Appendix 7.24, p. 372-375). These data report that the test article is stable in 3% corn starch suspension with 1.0% polysorbate 80 when stored at room temperature or at 2 - 8° C.

6. **Observations:**

The animals were checked twice daily for mortality and once daily for altered appearance or behavior. Does were weighed on days 0, 7, 10, 14, 20, 24, and 29 of gestation. Feed consumption was measured daily on gestation days 5 - 29. All does were given ophthalmoscopic examinations prior to initiation of the study and again following the completion of dosing by the Staff Ophthalmologist. Does were sacrificed on day 29 of gestation.

Does were euthanized on day 29 of gestation by an i.v. injection of T-61 euthanasia solution. All does were examined for gross pathological changes at necropsy. The ovaries were examined, the corpora lutea were counted, the uterus and its contents were weighed, live fetuses, dead fetuses, and intrauterine resorption sites were counted. Viable fetuses were euthanized with an i.p. injection of T-61 euthanasia solution and placed in 95% ethanol, after being weighed and examined for gross abnormalities.

On the day of necropsy, the viscera of each viable fetus was examined by a modification of the Staples technique (Staples, 1974) and the sex determined. The exam included: the brain, heart, major blood vessels, trachea, lungs, diaphragm, oral cavity (including palate), tongue, esophagus, stomach, intestines, liver, gall bladder, pancreas, thymus, spleen, kidneys, ureters, bladder, adrenals, ovaries and uterus or testicles. Visceral data was recorded manually on forms.

Following this exam, the fetuses were returned to 95% ethanol and subsequently prepared for skeletal exam by clearing in KOH and staining with Alizarin red S (Staples, 1964). An in-house developed and validated IBM data base management system was employed to collect data from the skeletal readings.

Historical control data for rabbit fetal weights obtained from studies conducted at CIBA-GEIGY Corporation SEF at Summit, NJ were provided to allow comparison with concurrent controls. The dates for the studies, and the vehicles used were not reported.

7. **Statistical Analysis**

The methods employed for statistical analyses of the data are appended.

8. **Compliance**

A signed Statement of No Confidentiality Claim dated June 7, 1990, was provided (p.2).

A signed Statement of compliance with EPA GLP's dated August 31, 1987, was provided (p.5).

A signed Quality Assurance Statement dated August 28, 1987, was provided (p. 6).

A signed Flagging Criteria Statement dated June 11, 1990, was provided.

C. Results

1. Analyses of the Test Substance

a. Purity -

The protocol, page 18, states that the retention, purity, stability, and characterization of the test article remains the responsibility of CIBA-GEIGY, Agricultural Division, located in Greensboro, North Carolina. This reviewer was unable o locate the purity within this report.

b. Stability -

Appendix 7.24 p. 374-375 indicate that 0.02% and 6.0% concentrations were tested and "based on the data obtained, the above mixtures exhibit satisfactory chemical stability for the indicated time periods and storage conditions (room temperature for 21 days)". The appendix does not provide the actual concentrations of the test material found on day 0 and day 21 or report the % lost during the 21 day period.

c. Concentration -

Appendix 7.24 p. 373 indicates that the concentration was tested with formulations prepared on 5/21/86 and 5/27/86. The dates the formulations were tested were not given. The concentrations found were within 10% of the target concentration.

d. Homogeneity -

The protocol, page 8, states that the uniformity, concentration, and stability of the test article as formulated in the control article is the responsibility of Toxicology/Pathology Administration and Technical Operations (TPATO) in Summit, NJ. The results of testing the uniformity of the test material were not reported.

2. Maternal Toxicity

a. Mortality

During the conduct of this investigation three does died. A control doe died on gestation day 15; a low dose doe died on gestation day 16. These deaths were due to dosing accidents. Doe # 26 died on gestation day 18 after what the authors termed "apparent treatment related anorexia". Page 379 (appendix 7.26 Maternal Pathology Report) provides a diagnosis for doe #26 of acute toxic hepatitis, possibly due to toxemia of pregnancy.

Two other high dose does aborted on gestational days 18 and 24 and were therefore, euthanized.

b. Clinical Observations

There was a statistically significant dose related increase in the incidence of stool variation (decreased/soft/no) in the 25 and 75 mg/kg groups. The study authors note that this is a highly variable parameter in the rabbit and was thought to be due to variations in feed consumption which are often observed in rabbit studies (the finding was assumed to be unrelated to treatment).

Additional clinical signs included alopecia, anogenital stains, and blood in the pan.

c. Body Weight

The investigators supplied the following data:

TABLE 1: Mean Maternal Body Weight Gain (grams)[a]

Dose (mg/kg)	0	1	25	75
Days 0-7	262.5	235.0	207.5	222.0
Days 7-20	200.7	179.2	171.9	-6.2*
Days 20-29	146.0	118.5	102.5	165.0
Days 0-29	613.3	538.5	481.9	404.2*
Corrected Body Weight Gain	190.0	80.8	-14.8*	14.3

* Significantly different from control ($p \leq 0.05$)
corrected body weight gain for entire gestation period = body weight gain days 0-29 minus gravid uterus weight.
Data extracted from report number MIN 862107 appendices 7.9, 7.10, and 7.11.

Significant reductions in the body weight gain of high dose does, were present days 7-10, 10-14, 7-20, and 0-29. These reductions correspond with reduced feed consumption during these intervals (significant reductions in feed consumption in the HDT were only observed during the treatment period, not after treatment). The overall corrected body weight gain was significantly reduced in the mid dose group, and nonsignificantly reduced in the high dose group. The mid dose group had a slight nonsignificant decrease in body weight gain (and a significant reduction in feed consumption) during the post dosing period (days 20-29) which is apparently responsible for the significant reduction in the corrected body weight gain.

d. <u>Feed Consumption</u>

The investigators supplied the following data:

TABLE 2: Mean Maternal Feed Consumption (g)[a]

Dose (mg/kg)	0	1	25	75
Days 5-7	389	393	405	423
Days 7-19	2314	2299	2146	1481*
Days 19-29	1799	1277*	1284*	1632

* Significantly different from control ($p \leq 0.05$)
a = Data extracted from report MIN 862107 table 6.3 p. 35.

Significant reductions in feed consumption were observed in the high dose group throughout the dosing period. No significant reductions in feed consumption were observed in the low or mid

dose groups during treatment. However, occasional significant differences were observed in these groups during the post treatment interval. The reduction in feed consumption of the mid dose group during the post treatment interval corresponds to a nonsignificant reduction in maternal body weight gain.

f. Gross Pathological Observations

In general, necropsy did not reveal treatment related findings. Two specimens were obtained at necropsy which appeared abnormal and were submitted for histological exam. The caudal lobe of the liver of doe #26 (high dose) was mottled, discolored and hardened in appearance. This doe also had distended intestines. Upon completion of histopathological exam, a diagnosis of acute toxic hepatitis was made. The authors proposed that this may have been due to toxemia of pregnancy. The left lobe of the lung of the intermediate dose doe #68 at necropsy was found to be white and caseous. The diagnosis of acute pneumonia and pleuritis possibly due to aspiration was made after histopathological exam. Neither of these findings were attributed to treatment. One doe in the 1 mg/kg group was found to have a double amniotic sac at necropsy. Partial agenesis (both horns including cervix) was observed in one 75 mg/kg doe.

Ophthalmoscopic examinations of the does did not reveal any treatment related ocular changes.

g. **Cesarean Section Observations**

TABLE 3: Cesarean Section Observations[a]

Dose (mg/kg):	0	1	25	75
#Animals Inseminated	19	19	19	19
# Pregnant	16	14	16	15
Pregnancy Rate (%)	84.2	73.7	84.2	78.9
Maternal Wastage				
#Died	1	1	0	1
#Non pregnant	3	5	3	4
#Aborted	0	0	0	2
#Premature Delivery	0	0	0	0
N	15	13	16	12
Total Corpora Lutea	151	143	173	123
Corpora Lutea/Doe	10.1	11.0	10.8	10.3
Total Implantation	103	104	144	87
Implantations/Doe	6.9	8.0	9.0	7.3
Total Live Fetuses	96	98	136	76
Live Fetuses/Doe	6.4	7.5	8.5	6.3
Total Resorptions	7	6	8	11
Early	4	5	6	7
Late	3	1	2	4
Resorptions/Doe	0.5	0.5	0.5	0.9
Total Dead Fetuses	0	0	0	0
Mean Fetal Weight (gm)				
♂	47.28	42.70	41.33*	41.94*
♀	45.56	40.96*	39.11*	41.40
Preimplantation Loss(%)	31.6	29.5	16.5	30.5
Postimplantation Loss(%)	7.39	7.76	6.62	12.86
Sex Ratio (% Male)	57.3	53.1	50.0	51.3

* Significantly different from control ($p \leq 0.05$)
[a] = Data extracted from report MIN 862107 tables 6.6 & 6.7 and appendix 7.14.

One high dose doe aborted on gestational day 18; another aborted on day 24.

Slight nonsignificant increases in postimplantation loss and resorptions/doe were observed in the HDT. Significant reductions

in fetal weight were observed in all treatment groups. The authors did not believe that the significant differences in fetal weight observed at the low and mid dose were due to treatment. They reported that the mean control fetal weight values in the current study were greater than any ever reported in this test facility, or their historical control data. The authors also note that the mean litter size was greater in these groups (which would influence fetal weights) and that there were no correlated gross, visceral or skeletal anomalies.

3. **Developmental Toxicity**

Fetus #1 of doe #45 (control animal) was examined externally and viscerally but was not present in the jar at the time of skeletal examination. The report states that "it is thought that this fetus was inadvertently discarded prior to skeletal processing." The loss of one fetus would not jeopardize the interpretation or outcome of this study.

TABLE 4: External Examination of Fetuses

Dose (mg/kg)	0	1	25	75
#pups(litters) examined	96(15)	98(13)	136(16)	76(12)
Raised, discolored area ventral thorax	0	0	1	0

Data extracted from report MIN 862107 table 6.8 p. 40.

TABLE 5: Visceral Examinations

Dose (mg/kg)	0	1	25	75
#pups(litters) examined	96(15)	98(13)	136(16)	76(12)
Microcephaly	0	0	1	0
Horseshoe Kidney	0	0	0	1
Crytophthalmos - partial	0	0	0	1
Total # Fetuses with Visceral Malformations	0	0	1	2
Total # Litters with Visceral Malformations	0	0	1	2

Data extracted from report MIN 862107 table 6.8 p. 40.

TABLE 6: Skeletal Examinations

#pups(litters) examined	95(15)[a]	98(13)	136(16)	76(12)
Hyoid-				
bowed	1(1)	-	-	1(1)
incompletely ossif.	-	-	3(1)	1(1)
Centrum/Vertebrae-				
additional	3(1)	4(1)	2(1)	2(1)
Ribs-				
fully formed (13th)	50(15)	48(10)	53(14)	49(12)
floating rudimentary (13th)	8(6)	3(3)	5(5)	4(4)
rudimentary (13th)	32(13)	18(9)	38(14)	13(8)
localized thickening	-	1(1)	-	-
Sternebrae-				
bipartite	2(2)	2(1)	-	1(1)
misaligned	2(2)	-	-	-
incompletely ossif.	18(9)	7(6)	25(11)	11(7)
unossified	22(9)	19(8)	24(9)	26(8)
fused	-	1(1)	-	-
Forepaw-				
middle phalange, inc. ossif.	-	1(1)	1(1)	-
middle phalange, unossified	2(1)	1(1)	3(1)	1(1)
proximal phalange, inc. ossif.	-	-	-	1(1)
metacarpal, inc. ossif.	1(1)	-	-	-
metacarpal, unossifed	-	-	-	3(1)
Hindleg-				
patella, inc. ossif.	20(10)	14(7)	37(11)	5(4)
unossifed	4(1)	15(6)	18(8)	5(2)
Hindpaw-				
middle phalange, unossified	-	-	-	1(1)
tarsus	-	-	-	1(1)
SUMMARY				
Total No. w 1+ variation	86(15)	81(12)	118(16)	68(12)
Total No. w 1+ variation excluding forepaw, hindpaw, and patella variations	84(15)	73(12)	106(16)	68(12)

[a] Doe #45, fetus #1 was lost following visceral exam, and consequently did not have skeletal exam.

Data extracted from report MIN 862107 tables 6.9 and 6.10 pp. 41-42.

D. Discussion/Conclusions

1. Maternal Toxicity:

Maternal toxicity was observed in this study as the death of one doe and the abortions observed in two other high dose does. In addition, significant reductions in body weight gain of high dose does, were present days 7-10, 10-14, 7-20, and 0-29 of gestation. These reductions correspond with reduced feed consumption during these intervals (significant reductions in feed consumption in the HDT were only observed during the treatment period, not after treatment).

2. Developmental Toxicity:

a. Deaths/Resorptions:

Slight (nonsignificant) increases in postimplantation loss and resorptions/doe were observed in the HDT.

b. Altered Growth:

Significant reductions in fetal weight were observed in all treatment groups. The decrease in fetal weight at the HDT may have been due to treatment. The significant differences in fetal weight observed at the low and mid dose were apparently not due to treatment.

c. Developmental Anomalies:

Based upon the available information, CGA 169374 as tested in this investigation did not result in a significant increase in treatment induced developmental anomalies.

E. Study Deficiencies:

1. The frequency of preparation of dosing solutions was not stated. The storage conditions of the test article during the study were not provided.

2. The reports for the analyses of the purity of the test article and its homogeneity in the vehicle were not provided.

3. The available historical control data only pertains to weighted fetal weight obtained from Healy Analysis. Data were not presented for the other parameters evaluated in this investigation: maternal body weight/body weight gain, feed consumption, cesarean section observations (pregnancy rates, implantation data, % pre-and post-implantation loss), gross external exam findings, soft tissue findings, or

skeletal findings. Furthermore, the available data do not indicate the source or strain of animals for which the data are presented. The historical control data do not indicate the route of administration or the vehicle these animals were treated with, nor the dates of the studies.

F. Core Classification: supplementary

This study does not satisfy the guideline requirements (83-3) for a developmental toxicity study due to the deficiencies stated above. This study may be upgraded after a satisfactory response to these deficiencies.

Maternal NOEL = 25 mg/kg
Maternal LOEL = 75 mg/kg
Developmental Toxicity NOEL = 25 mg/kg
Developmental Toxicity LOEL = 75 mg/kg

APPENDIX I

Materials and Methods

RIN 1360-95

TOX REVIEW. 009689 DIFENOCONAZOLE

Page____ is not included in this copy.

Pages 270 through 281 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.

✓ 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.

GUIDELINE: §83-4

Primary Review by: K. E. Whitby, Ph.D. 4/1/92
Toxicologist, Review Section II, Toxicology Branch II/HED (H7509C)

Secondary Review by: K. Clark Swentzel 4/1/92
Section Head, Review Section II, Toxicology Branch II/HED (H7509C)

DATA EVALUATION REPORT

STUDY TYPE: 2 Generation Reproduction - Rat (83-4)

EPA Identification Nos.: EPA MRID No.: 420900-18
Shaughnessy No.: 128847
HED Project No. 2-0696

TEST MATERIAL: CGA 169374 Technical

SYNONYMS: Difenconazole

STUDY NUMBERS: Master File Index Number (MIN 862091)
Toxicology/Pathology Report 88045

SPONSOR: Agricultural Division
CIBA-GEIGY Corporation
Post Office 18300
Greensboro, NC 27419

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

TITLE OF REPORT: A Two-Generation Reproductive Study in Albino Rats

AUTHOR(S): M.L.A. Giknis

REPORT ISSUED: September 1, 1988

STUDY DATES: April 7, 1986 (initiation of F_0 ♂ test article exposure)
April 8, 1986 (initiation of F_0 ♀ test article exposure)
February 18, 1987 (last necropsy)

CONCLUSION:

CGA 169347 technical was administered in the diet to male and female rats at 0, 25, 250, or 2500 ppm. Significant reductions in F_0 and F_1 male body weight gain were observed at 2500 ppm during days 0-77 and overall (terminal body weight minus day 0 body

weight). Significant reductions in F_0 and F_1 body weight gain of females in the 2500 ppm group were detected during the pre-mating, gestation, and lactation periods. In addition, as indicated in table 4 the 250 ppm F_0 females had reductions (statistically nonsignificant) in body weight gain which appear to be part of a dose-related trend days 70-77 prior to mating, days 0-7 of gestation, and days 7-14 of lactation. Significant reductions in pup body weight were detected days 0, 4 (pre- and post culling) 7, 14, and 21 for males and females (day 0 female F_1 were not significant) in the 2500 ppm group of both generations. There was a significant reduction in the body weight of F_1 male pups at day 21 in the 250 ppm group.

The percentage of male pups in the F_1 generation surviving days 0-4 was significantly reduced in the 2500 ppm group.

This study may be upgraded after the registrant satisfactorily provides the purity and stability of the test article.

Parental Systemic NOEL = 25 ppm
Parental Systemic LOEL = 250 ppm
Reproductive NOEL = 25 ppm
Reproductive LOEL = 250 ppm

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:	not stated
Description:	white powder
Batch No.:	FL851406
Stability:	see below
Storage Conditions:	not stated

Page 30 of the report states that the stability, purity, characterization, and retention of the reserve samples of the test article are the responsibility of the CIBA-GEIGY Corporation, Agricultural Division, Greensboro, NC. The report indicates that the sponsor verified that the test article is stable to 70°C (duration not stated).

2. **Test Animal(s)**:

Species:	rat
Strain:	Sprague Dawley (CRCD, VAF/PLUS)
Source:	Charles River Laboratory, Ltd., Kingston, NY

Date: April 2, 1986
Age: ♂ 38 days at receipt
♀ 37 days at receipt
Weight: 160-192 g (F_o ♂ at beginning of treatment)
127-172 g (F_o ♀ at beginning of treatment)

One hundred thirty male and equal number of female rats were obtained from the breeder for the purpose of this study. The animals were acclimated for 5-6 days (time in-house prior to initiation of exposure).

B. Methods

1. Animal Husbandry

All animals received ground Purina #5002 Certified Rodent Chow® without (control) or with the test article, and tap water (via water bottle) ad libitum. The drinking water was monitored periodically for potability and suspected environmental contaminants present in either feed or water. No known contaminants were found to be present in feed or water that the author felt were likely to interfere with the purpose or conduct of the study.

Animals were caged individually (except during mating, lactation, and weaning) in solid bottom cages containing hard-wood chips (Beta-Chips) which were changed weekly. The room temperature was maintained at 73±5°F with a relative humidity of 50±20%. On one day (8/2/86) due to technical error the environmental conditions (temp. and humidity) were not recorded for the F_o animals. In addition, transient increases in temperature (1/26/86) and humidity (2/17/86) were noted in the room housing the parental F_1 dams and their offspring.
The animals were maintained on a 14 hour light, 10 hour dark cycle. An additional hour of darkness was furnished as the result of a technical error on October 10, 1986. In addition, lighting was disrupted for approximately 15 minutes due to a power failure on February 9, 1987.

2. Mating

Eleven weeks after the beginning of treatment the F_o animals in each group were mated in a 1:1 ratio. The animals were permitted 3 weeks for mating and were separated on the day that evidence of mating was found (sperm/and or copulatory plug). The females were allowed to litter.

After the weaning of the last litter thirty male and female F_1 pups were selected in each group to continue as the second parental generation. When possible one

pup/sex/litter was selected from each available litter. Litters from which more than one pup per sex were used were randomly selected. These animals were permitted 14 weeks of dietary exposure prior to mating. The animals were mated in the same manner as the previous generation.

3. **Study Design**

Each animal was assigned a unique TEROS® acclimation number and cage position. One hundred twenty of each sex from the acclimation colony were randomly assigned a TEROS® temporary id number and were randomly distributed into 4 treatment groups consisting of 30 of each sex. Prior to the initiation of treatment, the temporary animal number became the permanent number. The animals were eartagged with this number if the animals was considered to be suitable for the study.

Table 1: Assignment of Animals

Dose (ppm)	F_0 Parents		F_1 Parents	
	♂	♀	♂	♀
0	1 - 30	31 - 60	401 - 430	431 - 460
25	101 - 130	131 - 160	501 - 530	531 - 560
250	201 - 230	231 - 260	601 - 630	631 - 660
2500	301 - 330	331 - 360	701 - 730	731 - 760

The control animals received ground Purina #5002 Certified Rodent Chow®. The test article [CGA 169374 technical (FL 851406)] was administered to 3 groups of animals at 25, 250, or 2500 ppm continuously for 2 generations. After 11 weeks of exposure to the test diets, the F_0 animals in each group were mated.

After weaning the last litter 30 males and females were selected to serve as the second parental generation. The F_0 males were sacrificed on day 141 of the study. The F_0 females were sacrificed on day 141 of the study after weaning their offspring.

After mating the F_1 animals, the dams were permitted to litter and all F_2 offspring were sacrificed on lactation day 21 and 40 F_2 pups (5/sex/group) were presented to the Pathology subdivision for necropsy and tissue preservation.

A third of the F_1 males were euthanized after 144 days on the study, a second third after 145 days, and the remaining

third of the animals after 146 days. One half of the F_1 females were sacrificed after 167 days and the remaining half were sacrificed after 168 days.

3. **Diet Preparation**

Mixing of the test article with the diet was performed by the Test Article Formulation Laboratory, Toxicology/Pathology Administration and Technical Operations (T/PATO) in Summit, NJ. T/PATO was also responsible for determination of the stability, homogeneity, and concentration of CGA 169374 in the feed admixture.

Test diet was prepared every two weeks and stored at room temperature in closed containers until used. It was tested for homogeneity and chemical stability. Samples of freshly prepared treated food were analyzed for chemical presence and concentration at the beginning, middle and end of the study.

4. **Observations**

All animals were observed daily for changes in appearance and behavior and twice daily for mortality. Feed consumption and body weight were recorded weekly for the males prior to mating. Body weight for males was recorded weekly thereafter and at sacrifice.

Body weight and feed consumption for the dams were recorded weekly during the premating period and on days 0, 7, 14, and 20 of gestation. Female body weights during lactation were recorded days 0, 4, 7, 14, and 21. Feed consumption and therefore test article intake were not provided for the lactation period.

Females that did not show evidence of mating continued to be weighed at weekly intervals during the mating period until delivery or scheduled sacrifice. F_0 females that showed positive mating signs but failed to deliver a litter were weighed as scheduled during their presumed gestation and at sacrifice on August 27, 1986. The same procedure was followed for the F_1 females that failed to deliver until their sacrifice on February 17 or 18, 1987.

Due to technical errors the following parameters were not recorded during the in-life phase of data collection:

1) parental body weight-
   F_0 male no. 306 (2500 ppm) day 35 premating
   F_0 female no. 46 (0 ppm) day 7 lactation
   F_1 female no. 536 (25 ppm) days 0 and 21 lactation

--F_1 female no. 545 (25 ppm) day 0 lactation

2) feed consumption-
F_1 female [illegible]. (25 ppm) day 20 gestation

Ophthalmoscopic examinations (indirect ophthalmoscopy) were performed by the staff ophthalmologist on F_0 animals prior to test article administration and 10 weeks after the beginning of administration. The F_1 animals were examined in the same manner at 20-28 days of age. The F_1 selected for mating were re-examined approximately 2 weeks before cohabitation.

NECROPSY

All parental animals and 40 (5/sex/group) randomly selected F_2 pups were euthanized and necropsied by the Pathology Subdivision. At necropsy grossly abnormal organs and the number of uterine implantation sites were recorded. Specimens of the vagina, cervix, uterus, ovaries, testes, epididymides, seminal vesicles, prostate, pituitary and coagulating gland were collected from the parental animals and fixed in 10% neutral buffered formalin. The tissues of the control and high dose animals were examined microscopically, as well as any grossly abnormal tissues. Paired ovaries and testes were weighed.

Due to technical error organ weights were not recorded for:

1) F_0 male no. 207 (250 ppm)
2) F_1 male no. 708 (2500 ppm)
3) F_1 female no. 449 (0 ppm)

POSTNATAL OBSERVATIONS

On postnatal day 0 the number of viable pups, and their sex was recorded. The number of stillborn pups was also recorded. Neonates were examined for gross abnormalities. Litters were culled to 8 (4 ♀ and 4 ♂) where possible on postnatal day 4. The culled pups were euthanized and a gross internal examination was performed. Pup body weight was recorded on postnatal days 0, 4, 7, 14, and 21.

Due to technical error the following litters were not weighed:

1) F_1 female no. 536 (25 ppm) - day 0
2) F_0 female no. 46 (0 ppm) - day 7
3) F_1 female 536 (25 ppm) - day 21

Page 1251 of the report indicate that in the F_1 generation 2 litters in the control, 1 litter in the 250, and 1 in the

2500 ppm groups were missexed on day 0. One litter in the control group was missexed on day 14. The report indicates that the mean litter weights in these instances are correct. The body weight of one pup in the control group was omitted due to an inaccurate recording on day 0. Page 1344 of the report indicates that one litter in the F_2 2500 ppm group was missexed on day 0; however the mean litter weight is correct.

Changes in behavior and appearance were observed throughout the lactation period. Deaths noted during the lactation period were recorded.

5. **Statistics** - The statistical procedures used for analyzing the numerical data are in appendix 1.

6. **Compliance**

A signed Statement of No Confidentiality Claim dated September 1, 1988 was provided (p. 2).

A signed Statement of Compliance with EPA GLP's dated September 1, 1988 was provided (p. 5)

A signed Quality Assurance Statement dated August 30, 1988 was provided (p. 6 and p. 1553).

A signed Flagging Criteria Statement dated June 7, 1990 was provided (p. 4).

C. **Results**

1. **Analyses of the Test Substance**

a. **Purity**

Page 30 of the report indicates that the purity stability and characterization of the test article is the responsibility of the sponsor.

b. **Stability**

Page 124 of the report indicates that stability of the 25 and 2500 ppm admixtures was established by the analytical Chemistry Laboratory (T/PATO). These admixtures were found to be stable for 30 days at room temperature (p. 128). Page 30 of the report indicates that the sponsor verified that CGA 169374 is "stable to at least 70°C.

c. **Concentration**

Concentrations of the dietary admixture were verified at weeks 1, 5, 9, 18, 21, 33, and 45. The concentration found for the different admixtures of the test article in the feed was within ± 10% of the target dose.

d. Homogeneity

Homogeneity was analyzed from three sub-samples (top, middle, and bottom) from each dose level at weeks 1 and 18 of the study. The mean (for the top, middle, bottom) concentration found was within ± 10% of the target concentration.

3. Parental Toxicity

a. Mortality

F_0 Generation

In the F_0 generation there were 2 unscheduled deaths. One control male (no.19) was found dead on day 109; one 25 ppm female (no. 159) was found dead on day 118. Based on the necropsy findings, the cause of death was not related to treatment in either case.

F_1 Generation

One 25 ppm female (no. 549) and two 2500 ppm females (nos. 739 and 752) died on lactation days 2, 1, and 5 respectively.

b. Clinical Observations

F_0 Generation

There were no treatment related clinical observations in this generation.

F_1 Generation

There were no treatment related clinical observations in this generation.

Table 2: Clinical Observations

Observation	Dietary Level (ppm)							
	0		25		250		2500	
	♂	♀	♂	♀	♂	♀	♂	♀
$F_0^{\dagger}$ Generation (N)	30	30	30	30	30	30	30	30
Alopecia	7	2	4	6	7	4	1	2
Anogenital Stains	-	-	-	-	1	-	-	-
Chromodacryorrhea	-	1	-	-	2	-	2	1
Death	1	-	-	1	-	-	-	-
Inflammation (ears)	-	3	-	3	1	4	1	4
Mass	-	-	-	1	-	1	1	1
Sore/Scab	2	-	2	-	5	1	-	1
Umbilical Hernia	-	-	-	-	-	-	-	1
Unthrifty	-	-	-	-	1	-	-	-

Observation	Dietary Level (ppm)							
	0		25		250		2500	
	♂	♀	♂	♀	♂	♀	♂	♀
$F_1^{\dagger}$ Generation (N)	30	30	30	30	30	30	30	30
Alopecia	1	6	6	14	3	6	2	4
Ataxia	-	-	-	1	-	-	-	-
Chromodacryorrhea	2	1	1	-	1	1	-	-
Death	-	-	-	-	-	-	-	2
Dystocia	-	1	-	-	-	-	-	1[a]
Hypothermia	-	-	-	-	-	-	-	1[a]
Inflamed Ears	-	-	-	1	-	2	-	1
Moribund	-	-	-	-	-	-	-	1[a]
Nasal, Discharge	-	-	-	-	-	-	1	-
Pale	-	-	-	-	-	-	-	1[a]
Sore/Scab	1	2	2	2	1	1	3	1
Stained Pubic Area	-	-	-	-	-	-	-	1[a]
Tail - Kinked/ Truncated	1	-	-	1	-	-	-	-
Teeth - Overgrown/Missing/ Crooked	-	-	2	-	-	1	-	-

Data extracted from report MIN 862091, appendices 7.2.1, 7.2.2, 7.2.3, 7.2.4, 7.2.5, and 7.2.6 (pp. 131-207).

† Observations from pre-mating, mating, and post-mating periods.
a These observations were for female 752 during the lactation period. This female died day 5 of lactation.

c. **Body Weight Data**

F_0 Males

Prior to the initiation of treatment, F_0 male body weights were comparable. Significant treatment related reductions in the body weight of 2500 ppm males were observed days 7-77 (pre-mating). Reduced body weight gain was observed days 0-7, 21-42, and 63-70 and overall (0-77) in the HDT. Sporadic statistically significant increases in body weight gain of the 25 ppm (days 63-70 and 70-77) and 250 ppm (days 42-49 and 70-77) males. Overall body weight gain (days 0-141) was significantly reduced in the 2500 ppm group males.

Mean body weight of the males receiving 2500 ppm was significantly reduced during mating (days 84, 91, and 98), postmating (days 105, 112, 119, 126, and 133), and at terminal necropsy (day 141).

F_1 Males

The mean body weight of the males receiving 250 ppm was significantly reduced days 0-21 and 35. The 2500 ppm group had a significant reduction in body weight during days 0-140 and at termination.

Significant reductions in body weight gain were observed in the males receiving 25 ppm days 0-7 and 63-70. Males receiving 250 ppm had significant reductions in body weight gain days 0-7, 28-42, and days 63-70. The 2500 ppm males also had significant reductions in mean body weight gain days 0-7, 28-35, 63-70, 0-98 (pre-mating) and 0-terminal. This decreased body weight gain persisted into the mating (days 105-119) and post-mating (days 119-terminal) periods.

Table 3: Selected Paternal Body Weight Gain Data (g)

Dose (ppm)	0	25	250	2500
F_0 Generation (N)	30	30	30	30
Days 0-7	57.8	54.4	56.4	44.1*
Days 21-28	44.3	46.3	44.8	38.5*
Days 63-70	16.9	11.6*	13.9	12.0*
Days 0-77	367.3	365.1	373.0	322.0*
0-TBW[a]	434.9	438.6	441.4	375.7*
F_1 Generation (N)	30	30	30	30
Days 0-7	67.3	63.3*	63.2*	56.8*
Days 28-35	29.0	24.7	23.0*	22.1*
Days 63-70	22.4	14.5*	14.7*	14.4*
Days 0-98	404.6	384.1	394.1	365.3*
0-TBW[a]	446.1	417.6	430.0	403.6*

a Overall terminal body weight gain = Terminal Body Weight (TBW) - day 0 weight.
* Significantly different from control ($p \leq 0.05$).
Data extracted from report MIN 862091 tables 6.5.1 and 6.13.1 pp. 65-67 and 102-103.

F_0 Females

Females receiving 2500 ppm had significant reductions in mean body weight days 7-77, prior to mating. Significant reductions in mean body weight gain were observed in the group receiving 25 ppm prior to mating days 14-21. Significant reductions in body weight gain prior to mating were also observed in the group receiving 2500 ppm days 0-7, 14-21, 35-42, 49-56, and 70-77.

Mean gestation body weight was reduced in the group receiving 2500 ppm days 0, 7, 14, and 20. Mean gestation body weight gain was significantly reduced in the MDT and the HDT days 0-7.

During the lactation period (days 0, 4, 7, and 14) the dams receiving 2500 ppm had significantly reduced mean body weight. Mean body weight gain was significantly reduced days 7-14 and 14-21.

F_1 Females

Significant reductions in body weight were observed during the premating period, gestation, lactation, and at termination in the group receiving 2500 ppm. Mean body weight gain was significantly reduced during premating days 21-28, 42-49, 77-84, and during gestation days 0-7.

Dams receiving 250 ppm had reductions in mean body weight on premating day 7; and a reduction in body weight gain on days 42-49 prior to mating.

Dams receiving 25 ppm had reductions in body weight gain on premating days 42-49 and 70-77.

Table 4: Selected Maternal Body Weight Gain Data (g)

Dose (ppm)	0	25	250	2500
F_0 Generation	Pre-Mating			
Days 0-7	29.1	28.1	27.9	19.6*
Days 70-77	9.4	3.6	6.7	3.1*
	Gestation			
Days 0-7	36.3	34.4	30.2*	24.0*
	Lactation			
Days 7-14	21.5	19.6	14.0	10.2*
Days 14-21	-19.9	-24.8	-18.6	-8.7*
F_1 Generation	Pre-Mating			
Days 21-28	20.6	18.0	20.9	15.2*
Days 42-49	14.3	9.0*	8.3*	9.6*
Days 77-84	8.6	6.9	7.5	1.4*
	Gestation			
Days 0-7	27.9	28.4	26.7	19.6*
	Lactation			
Days 14-21	-18.7	-18.9	-19.0	-3.0*

* Significantly different from control ($p \leq 0.05$).
Data extracted from report MIN 862091 tables 6.5.2, 6.5.3, 6.5.4, 6.13.2, 6.13.3, and 6.13.4 pp. 68-71 and 104-107.

d. **Feed Consumption and Compound Intake**

A copy of the feed consumption tables from the investigators report is appended (Appendix II).

F_0 Males

For the entire premating period there was a significant reduction in feed consumption for the males receiving 2500 ppm. A significant reduction in feed consumption was not observed in the males receiving 250 or 25 ppm.

F_1 Males

Significant reductions in feed consumption were found in the males receiving 2500 ppm during the premating period days 0-98. Significant alterations in feed consumption were not reported for the other treatment groups.

F_0 Females

Feed consumption was significantly reduced in the 2500 ppm females during the premating period day 0-7 and 21-77. During the gestation period significant reductions in feed consumption were observed days 0-7 in the 2500 ppm group.

F_1 Females

The females receiving 2500 ppm had significant reductions in feed consumption during the premating (days 0-98) and gestation (days 0-20) periods. The group receiving 250 ppm had a significant reduction in feed consumption prior to mating days 0-7 and during gestation days 7-14.

e. **Test Article Intake**

Test article intake was calculated by:

$$\frac{\text{Mean daily food consumption for interval}}{\text{Average mean body weight for interval}\dagger} \times \text{Dietary Concentration}$$

† Average mean body weight = $\frac{\text{mean body weight beginning of interval plus mean body weight end of interval}}{2}$

Table 5: Selected Test Article Consumption Intervals (mg/kg)

Days	Dietary Level (ppm)					
	25		250		2500	
	♂	♀	♂	♀	♂	♀
F_0 Generation Pre-Mating						
0-7	2.75	2.65	27.44	26.28	247.52	227.60
21-28	1.87	2.01	18.39	20.31	178.17	195.82
42-49	1.52	1.87	15.14	18.74	150.99	180.27
70-77	1.31	1.65	13.28	16.21	132.09	166.32
F_0 Gestation						
7-14	-	1.72	-	17.13	-	173.90
14-20	-	1.52	-	15.52	-	168.22
F_1 Generation Pre-Mating						
0-7	2.56	2.54	27.58	25.63	300.93	269.68
28-35	1.59	1.86	16.41	18.91	171.81	184.65
56-63	1.30	1.58	13.14	15.79	140.24	166.87
91-98	1.15	1.41	11.46	13.65	122.65	149.31
F_1 Gestation						
7-14	-	1.57	-	14.10	-	150.47
14-20	-	1.56	-	14.14	-	158.03

Data extracted from report MIN 862091 tables 6.3.1, 6.3.2, 6.11.1, and 6.11.2 pp. 57-58 and 93-94.

f. **Reproductive Parameters**

Based on the available data, treatment did not appear to have a significant effect on the duration of gestation, the number of days required for successful mating, or the mating fertility, and gestation indices.

No significant changes in absolute organ weight were detected. The total ovary/testes weight as a percentage of total body weight was significantly increased for the F_0 males, and the F_1 males and females at 2500 ppm. This finding is apparently related to significant reductions in terminal body weight.

Treatment did not cause a significant effect on the clinical

observations and gross external malformations in the offspring of either generation. One F_1 2500 ppm pup had agenesis of the tail. The same gross malformation was observed in one F_2 pup in the 25 ppm group.

The percentage of male pups in the F_1 generation (not shown by sex in Table 6) surviving days 0-4 was significantly reduced in the 2500 ppm group as compared to the control (98.7 vs. 95.2). In the F_2 generation there also appeared to be a decrease relative to the control for this group (98.3 vs. 93.8), however this was not reported as significant.

F_1 male pup body weight was significantly reduced at 2500 ppm days 0-21. The body weight of F_1 male pups in the 250 ppm group were significantly reduced compared to controls at day 21. F_1 female pup body weight at 2500 ppm was also significantly reduced days 4-21. In the F_2 generation both male and female pups in the 2500 ppm group had significantly reduced body weight days 0-21.

The implantation data contains instances where the number of live pups exceeds the number of implants (see means in table 6). Therefore it would appear that the data pertaining to the mean number of implants, the post implantation loss, and the % postimplantation loss are inaccurate.

Page 120 (table 6.16.5) of the report shows that the incidence of F_2 pups with hollow kidneys was 5/196, 4/195, 6/225, and 10/221 for the 0, 25, 250, and 2500 ppm groups respectively. This finding was not observed in a dose related manner in the F_1 pups.

RIN 1360-95

TOX REVIEW 009689 DIFENOCONAZOLE

Page 298 is not included in this copy.

Pages _____ through _____ 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.

✓ 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.

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7. Sacrifice and Pathology

No significant treatment related changes were observed in the parental animals or progeny at necropsy.

a. Organ weight (Parental) -

No significant changes in absolute organ weight were detected. The total ovary/testes weight as a percentage of total body weight was significantly increased for the F_0 males, and the F_1 males and females at 2500 ppm. This finding is apparently related to significant reductions in terminal body weight.

b. Gross and Microscopic Pathology - Parental -

No significant treatment related alterations were observed via gross or microscopic examination.

D. **Discussion/Conclusions**

1. **Systemic Toxicity**:

Significant reductions in F_0 and F_1 male body weight gain were observed at 2500 ppm during days 0-77 and overall (terminal body weight minus day 0 body weight).

Significant reductions in F_0 and F_1 body weight gain of females in the 2500 ppm group were detected during the pre-mating, gestation, and lactation periods. In addition, as indicated in table 4 the 250 ppm F_0 females had reductions (statistically nonsignificant) in body weight gain which appear to be part of a dose related trend days 70-77 prior to mating, days 0-7 of gestation, and days 7-14 of lactation.

2. **Reproductive Toxicity**:

a. Deaths/Survival:

There were no treatment related deaths.

b. Altered Growth:

Significant reductions in pup body weight were detected days 0, 4 (pre- and post culling) 7, 14, and 21 for males and females (day 0 female F_1 were not significant) in the 2500 ppm group of both generations. There was a significant reduction in the body weight of F_1 male pups at day 21 in the 250 ppm group.

c. Reproductive Indices:

The percentage of male pups in the F_1 generation surviving days 0-4 was significantly reduced in the 2500 ppm group.

E. **Study Deficiencies**:

1. The purity and stability of the test article was not provided.

F. Core Classification: Core Supplementary Data.

This study does not satisfy the guideline requirements (§83-4) for a multigeneration reproductive toxicity study. However, this study, may be upgraded upon satisfactory review of the registrants response.

Parental Systemic NOEL = 25 ppm
Parental Systemic LOEL = 250 ppm
Reproductive NOEL = 25 ppm
Reproductive LOEL = 250 ppm

APPENDIX I

Materials and Methods

RIN 1360-95

TOX REVIEW. 009689 DIFENOCONAZOLE

Page_____ is not included in this copy.

Pages 302 through 318 are not included.

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____ The product confidential statement of formula.

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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)

009689

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
862852	April 29, 1987	65-82
871909	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.

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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	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.

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b. Clinical Chemistry

X	Electrolytes:	X	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.

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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, in addition, were weighed (paired organs were weighed as pairs).

X	Digestive system	X	Cardiovasc./Hemat.	X	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

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.

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

APPENDIX I
Statistical Methods

RIN 1360-95

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DOC930094

FINAL

DATA EVALUATION REPORT

DIFENOCONOZALE (CGA 169374)

Study Type: Mutagenicity: Gene Mutation in Cultured Mammalian Cells (Mouse Lymphoma Cells)

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 Reviewer Nancy E. McCarroll, B.S Date 6/11/92

Independent Reviewer Lynne T. Haber, Ph.D Date 6/11/92

QA/QC Manager Sharon Segal, Ph.D. Date 6/11/92

Contract Number: 68D10075
Work Assignment Number: 1-79
Clement Number: 91-265
Project Officer: James Scott

00002

GUIDELINE § 84: MUTAGENICITY
MAMMALIAN CELLS IN CULTURE GENE MUTATION

MUTAGENICITY STUDIES

Approved by:

EPA Reviewer: Karen E. Whitby, Ph.D. Signature:
Review Section II, Toxicology Branch (II)/HED Date:

EPA Section Head: Clark Swentzel Signature:
Review Section II, Toxicology Branch (II)/HED Date: 6/22/92

DATA EVALUATION REPORT

STUDY TYPE: Mutagenicity: Gene mutation in cultured mammalian cells (mouse lymphoma cells)

EPA IDENTIFICATION Numbers:

Shaughnessy Number: 128847

MRID Number: 420900-24

TEST MATERIAL: CGA-169374 technical

SYNONYMS: Difenoconozale; (±)-cis-trans-1-[2-[4-(4-chlorophenoxy)-2-chlorophenyl]-4-methyl-1,3-dioxolan-2-yl-methyl]-1H-1,2,4-triazole; $C_{19}H_{17}Cl_2N_3O_3$

SPONSOR: Ciba-Geigy, Corp., Greensboro, NC

STUDY NUMBER: 850570

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

TITLE OF REPORT: CGA-169374 Technical Gene Mutations Test L5178Y/$Tk^{+/-}$ Mouse Lymphoma Mutagenicity Test

AUTHORS: P. Dollenmeier

REPORT ISSUED: August 6, 1986

CONCLUSIONS-EXECUTIVE SUMMARY: No conclusions can be reached from the three nonactivated and two S9-activated mouse lymphoma forward mutation assays conducted with CGA-169374 technical. The study was seriously compromised because of the low cloning efficiencies (CEs) of the negative and solvent control cultures in all nonactivated and S9-activated trials. We assess that the suboptimal growth in control cultures, which we assume resulted from the use of a prolonged 3-day expression time, invalidated the study (see Sections C and D for detailed discussions). It is, therefore, recommended

that the study be repeated using currently recommended procedures for the mouse lymphoma assay.[1]

STUDY CLASSIFICATION: Unacceptable. The study does not satisfy Guideline requirements (§84.2) for genetic effects Category I, Gene Mutations.

A. MATERIALS:

1. Test Material: CGA 169374 technical

Description: Not provided
Identification Number: Batch number P. 503001
Purity: 94.5%
Receipt date: Not reported
Stability: Reported to be ensured by the sponsor
Contaminants: None listed
Solvent used: Dimethyl sulfoxide (DMSO)
Other provide information: Neither the storage conditions nor the frequency of test material solution preparation were reported.

2. Control Materials:

Negative: Fischer's medium containing antibiotics and 10% horse sperm (F_{10p})

Solvent/final concentration: DMSO/1%

Positive: Nonactivation (concentrations, solvent): Ethyl methanesulfonate (EMS) was used at 0.75 µL/mL in an unspecified solvent

Activation (concentrations, solvent): Dimethylnitrosamine (DMN) was used at 8 µL/mL in an unspecified solvent.

3. Activation: S9 derived from male Tif:RAIf(SPF)

x	Aroclor 1254	x	induced	x	rat	x	liver
_____	phenobarbital	_____	noninduced	_____	mouse	_____	lung
_____	none			_____	hamster	_____	other
_____	other			_____	other		

The S9 liver homogenate was prepared by the testing laboratory.

S9 mix composition:

Component	Final concentration in cultures
NADP	0.5 mM
Isocitrate	2.92 mM
Tris (hydroxymethyl)-aminomethane	2.33 mM
$MgCL_2$	0.17 mM
S9 homogenate	8.33 µL/mL

[1]Caspary, W.J., Lee, Y.J., Poulton, S., Myhr, B.C., Mitchell, A.D., Rudd, C.J. (1988). Evaluation of the L5178Y mouse lymphoma cell mutagenesis assay: Quality-control guidelines and response categories. Environ. Mol. Mutagen 12:19-36.

MAMMALIAN CELLS IN CULTURE GENE MUTATION

4. Test Cells: Mammalian cells in culture

__x__ mouse lymphoma L5178Y cells
_____ Chinese hamster ovary (CHO) cells
_____ V79 cells (Chinese hamster lung fibroblasts)
_____ other (list):

Properly maintained? Storage and cell maintenance conditions were not reported.
Periodically checked for mycoplasma contamination? Not reported.
Periodically checked for karyotype stability? Not reported.
Periodically "cleansed" against high spontaneous background? Yes.

5. Locus Examined:

__x__ thymidine kinase (TK)
selection agent: (give concentration)
__50 µg/mL__ bromodeoxyuridine (BrdU)
_____ fluorodeoxyuridine (FdU)
_____ trifluorothymidine (TFT)

_____ hypoxanthine-guanine-phosphoribosyl transferase (HGPRT)
selection agent: (give concentration)
_____ 8-azaguanine (8-AG)
_____ 6-thioguanine (6-TG)

_____ Na^+/K^+ATPase
selection agent: (give concentration)
_____ ouabain

_____ other (locus and/or selection agent; give details):

6. Test Compound Concentrations Used:

(a) Preliminary cytotoxicity assay: Seven doses (5, 10, 20, 40, 80, 160, and 320 µg/mL) were evaluated in the presence and absence of S9 activation.

(b) Mutation assay:

(1) Nonactivated conditions:

- Trial 1: 8, 16, 32, 48, 64, 72, and 80 µg/mL
- Trial 2: 15, 30, 60, 90, 120, 135, and 150 µg/mL
- Trial 3: 12, 24, 48, 72, 96, 108, and 120 µg/mL

(2) S9-activated conditions:

- Trial 1: 5, 10, 20, 30, 40, 45, and 50 µg/mL
- Trial 2: 3, 6, 12, 18, 24, 27, and 30 µg/mL

B. TEST PERFORMANCE:

1. Cell Treatments:

(a) Cells exposed to test compound for:
__4__ hours (nonactivated) __4__ hours (activated)

(b) Cells exposed to positive controls for:
__4__ hours (nonactivated) __4__ hours (activated)

(c) Cells exposed to negative and/or solvent controls for:
__4__ hours (nonactivated) __4__ hours (activated)

(d) After washing, cells cultured for __3__ days (expression period) before cell selection

Note: No explanation was given for the use of a 3-day expression period.

(e) After expression, $4x10^5$ cells/plate in __8__ plates were incubated for 14-16 days in selection medium to determine numbers of mutants; and 200 cells/plate in __4__ plates were incubated for __12__ days in incubated nonselection medium to determine cloning efficiency (CE).

2. Statistical Methods: The data were not evaluated for statistical significance.

3. Evaluation Criteria: The test material was considered positive if the mutation frequency (MF) at any test concentration exceeded the MF of the concurrent solvent control by a factor of 2.5.

4. Protocol: None provided

C. REPORTED RESULTS:

1. Preliminary Cytotoxicity Test: The study author stated that the two highest doses of the test material (160 and 320 µg/mL) precipitated. Percent relative suspension growth (RSG) for these levels and also 80 µg/mL with and without S9 activation was ≤7.3%. For the remaining nonactivated doses, RPS ranged from 55.2% at 40 µg/mL to 108.6% at 10 µg/mL. With S9 activation, RPS ranged from 18.8% at 40 µg/mL to 93.8% at 5 µg/mL. Based on these results, the initial nonactivated assay was conducted with 8 to 80 µg/mL and the initial S9-activated assay was performed with 5 to 50 µg/mL.

2. Nonactivated Assays: Representative results from the three nonactivated trials with CGA 169374 technical are presented in Table 1. As shown, recalculation of the data by our reviewers using standard methods[2] revealed that with the exception of the DMSO-treated

[2]Caspary, W.J., et al. (1988). Environ. Mol. Mutagen. 12:19-36.

RIN 1360-95

TOX REVIEW 009689 DIFENOCONAZOLE

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cultures in Trial 3, the absolute CEs for the negative and solvent control cultures fell below the minimum required for an acceptable assay (50%).[3] These findings suggest that cultures were beyond the peak of optimum cell growth when they were plated for mutants and survivors. We assume that the less than adequate growth probably resulted from the prolonged 3-day expression period. Representative daily cell counts from the nonactivated trials (Appendix A) tend to support this assumption. As shown, suspension growth for days 1 and 2, in both negative and solvent control cultures, generally proceeded as expected (i.e., 3- to 4-fold increase on day 1 and 4- to 5-fold in day 2) for cultures adjusted each day to $3x10^5$ cells/mL. By day 3, however, the rate of cell growth was clearly declining. Based on these considerations, we assess that the nonactivated trials were severely compromised; therefore, the findings with the test material cannot be evaluated. It was noted, however, that while a doubling of the MF occurred at 120 µg/mL in Trial 2, CGA 169374 technical was severely cytotoxic at this dose. There also appeared to be an overall lack of agreement in the cytotoxicity data from the three trials.

3. S9-Activated Assay: Similarly, the findings from the two S9-activated trials (Table 2) could not be evaluated because of the less than adequate absolute CE for the negative and solvent controls, presumably related to the extended expression time.

From the overall findings, the study author stated:

> "It is concluded that, under the given experimental conditions, no evidence of mutagenic effects of CGA 169 374 technical was observed in this mammalian forward mutation system."

D. REVIEWERS' DISCUSSION AND INTERPRETATION OF RESULTS: As previously stated, the unacceptable absolute CE for the negative and solvent controls both with and without S9 activation severely compromised the study. We further assess that the less than adequate results obtained with background controls probably resulted from the prolonged expression time. Although the recommended 2-day expression time represents a compromise and may, to some extent, limit the detection of slow growing mutants the daily cell counts for this study (see representative daily cell count data, Appendix A) did suggest that the maximum yield of mutants and survivors would have been obtained from cloning the cultures ~48 hours after exposure. Similarly, there was no indication in the report that small mutant colonies were seen in any treatment group; therefore, the rationale for the prolonged expression time cannot be justified.

The positive controls (0.75 µL/mL EMS -S9; 8 µL/mL DMN +S9) induced powerful mutagenic responses. However, based on our reviewers' experience with this assay, the concentrations that were selected to demonstrate assay sensitivity were excessive. For example, MFs within the range of $300-500x10^{-6}$ (50% relative total growth) are generally achieved when

[3]Caspary, W.J., et al. (1988). Environ. Mol. Mutagen. 12:19-36.

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0.25 µL/mL EMS is used as the positive control.[4] Additionally, the wide variation in the MFs seen in the currently reviewed study (326.1-3016.7x10^{-6} with EMS; 223.6-1349.2x10^{-6} with DMN) was generally related to the erratic results obtained from the viability plates rather than marked differences in recovered mutants. With the exception of the final nonactivated trial, mutant colonies recovered following exposure to both mutagens were relatively constant. We contend that the inability of the performing laboratory to reproduce comparable results with the positive controls adds additional support to our assessment that assay conditions were not optimal. Based on the above considerations, we conclude that the study is unacceptable and should be repeated using currently recommended procedures for the mouse lymphoma assay.

E. QUALITY ASSURANCE MEASURES: Was test performed under GLPs? Yes. (A quality assurance statement was signed and dated July 29, 1986).

F. CBI APPENDIX: Appendix A, Representative Daily Cell Count Data, CBI pp. 20 and 23; Appendix B, Materials and Methods, CBI pp. 10-12.

CORE CLASSIFICATION: Unacceptable. The study does not satisfy Guideline requirements (§84.2) for genetic effects Category I, Gene Mutations.

[4] Myhr, B., Bowers, L., Caspary, W.J. (1985). Assays for the induction of gene mutations at the thymidine kinase locus in L5178Y mouse lymphoma cells in culture. In: J. Ashby, F.J. de Serres et al., eds. Progress in Mutation Research. Vol. 5. Elsevier Science Publishers, Amsterdam, pp. 555-568.

APPENDIX A

REPRESENTATIVE DAILY CELL COUNT DATA
CBI pp. 20 and 23

APPENDIX B

MATERIALS AND METHODS
CBI pp. 10-12

RIN 1360-95

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Pages 373 through 376 are not included.

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DOC930093

FINAL

DATA EVALUATION REPORT

DIFENOCONOZALE (CGA-169374)

Study Type: Mutagenicity: Nuclear Anomaly Test in Chinese Hamsters

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 Reviewer Nancy E. McCarroll, B.S Date 6/11/92

Independent Reviewer Lynne Haber, Ph.D. Date 6/11/92

QA/QC Manager Sharon Segal, Ph.D. Date 6/11/92

Contract Number: 68D10075
Work Assignment Number: 1-79
Clement Number: 91-264
Project Officer: James Scott

CCC?

GUIDELINE § 84: MUTAGENICITY
MICRONUCLEUS

MUTAGENICITY STUDIES

Approved by:

EPA Reviewer: Karen E. Whitby, Ph.D.
Review Section II, Toxicology
Branch II/HED (H-7509C)

Signature: _____
Date: 6/17/92

EPA Section Head: Clark Swentzel
Review Section II, Toxicology
Branch II/HED (H-7509C)

Signature: _____
Date: 6/22/92

DATA EVALUATION REPORT

STUDY TYPE: Nuclear anomaly test in Chinese hamsters

EPA IDENTIFICATION Numbers:

Shaughnessy Number: 128847

MRID Number: 420900-23

TEST MATERIAL: CGA-169374 technical

SYNONYMS: Difenoconozale: (±)-cis-trans-1-[2-[4-(4-chlorophenoxy)-2-chlorophenyl]-4-methyl-1,3-dioxolan-2-yl-methyl]-1H-1,2,4-triazole: $C_{19}H_{17}Cl_2N_3O_3$

SPONSOR: Ciba-Geigy Corp., Greensboro, NC

STUDY NUMBER: 850567

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

TITLE OF REPORT: CGA-169374 Technical Structural Chromosomal Aberration Test Nucleus Anomaly Test in Somatic Interphase Nuclei of Chinese Hamster

AUTHOR: F. Strasser

REPORT ISSUED: February 3, 1986

CONCLUSIONS--EXECUTIVE SUMMARY: Male and female Chinese hamsters (6/sex) were administered single oral doses of 250, 500, or 1000 mg/kg CGA-16 374 for 2 consecutive days. No unscheduled deaths or other clinical signs of toxicity were reported for any treatment group. Based on the analysis of bone marrow cells harvested from 3 animals/sex/group 24 hours postexposure to the second administration of the test material, there was no evidence of a cytotoxic effect on the target organ or significant increase in the frequency of nuclear anomalies (micronuclei). However, the study was compromised for the following reasons:

1. According to Federal Register, Vol. 52:19080-19081 (dated May 20, 1987), using a repeated treatment schedule, "bone marrow samples should be taken at least three times, starting not earlier than 12 hours after the last treatment...but not extending beyond 72 hours."

2. The lack of overt toxicity for the animals or cytotoxicity on the target organ renders the data insufficient to establish that the maximum tolerated dose (MTD) was achieved.

3. Less than the number of animals per group recommended by Guidelines (i.e., 5 males and 5 females group) were analyzed for micronuclei induction.

4. There was no indication that slides were coded prior to analysis.

STUDY CLASSIFICATION: Unacceptable. The study does not satisfy Guideline requirements (§84.2) for genetic effects Category II, Structural Chromosome Aberrations.

A. MATERIALS:

1. Test Material: CGA 169374 technical

Description: Not provided
Identification number: Batch number P. 503001
Purity: 94.5%
Receipt date: Not reported
Stability: Reported to be ensured by the sponsor
Contaminants: None listed
Vehicle used: Sesame oil
Other provided information: Neither the storage conditions nor the frequency of test material solution preparation was reported.

2. Control Materials:

Negative/route of administration: None

Vehicle/final concentration/route of administration: Sesame oil at a dosing volume of 10 mL/kg was administered by oral gavage once daily for 2 consecutive days.

Positive/final concentration/route of administration: Cyclophosphamide (CP), prepared in sesame oil was administered by oral gavage once daily for 2 consecutive days at 128 mg/kg; dosing volume = 10 mL/kg.

3. Test Compound:

Route of administration: Oral gavage

Dose levels used:

- Preliminary toxicity tests:
  - Trial 1: 200, 1000, and 5000 mg/kg/day (one treatment)
  - Trial 2: 200, 1000, and 5000 mg/kg/day (two consecutive daily dosings)
- Nuclear anomaly test: 250, 500, and 1000 mg/kg/day (two consecutive daily dosings)

4. Test Animals:

(a) Species: Chinese hamsters Strain: Random, outbred Age: 4-9 weeks (males); 6-10 weeks (females)
Weight range: 24-35 g (males); 21-30 g (females)
Source: Tierfarm, Sisseln

(b) Number of animals used per dose:

- Preliminary toxicity test: 2 males; 2 females per treatment group
- Nuclear anomaly test: 6 males; 6 females, per treatment group; bone marrow cells were only scored from 3 males and 3 females per group.

Note: The report did not indicate whether dosing was based on individual body weights.

(c) Properly maintained? Yes.

B. TEST PERFORMANCE:

1. Treatment and Sampling Times:

(a) Test compound:
Dosing: _____ once __x__ twice (24 hr apart)
_____ other (describe): _____
Sampling (after last dose): _____ 6 hr _____ 12 hr
__x__ 24 hr _____ 48 hr _____ 72 hr

(b) Vehicle control:
Dosing: _____ once __x__ twice (24 hr apart)
_____ other (describe): _____
Sampling (after last dose): __x__ 24 hr _____ 48 hr
_____ 72 hr

(c) Positive control:
Dosing: _____ once __x__ twice (24 hr apart)
_____ other (describe): _____
Sampling (after last dose): __x__ 24 hr _____ 48 hr
_____ 72 hr

2. Tissues and Cells Examined:

___x___ bone marrow _____ others (list):
Number of bone marrow cells examined per animal: __1000__ were scored for the following nuclear anomalies: single Jolly bodies, fragments of nuclei in erythrocytes, micronuclei in erythroblasts, micronuclei in leukopoietic cells, and polyploid cells.

3. Details of Slide Preparation: At 24 hours after administration of the second dose of the test material, vehicle, or positive control, animals were sacrificed by cervical dislocation. Bone marrow cells were harvested from both femurs, mixed with 0.5 µL rat serum, and spread onto slides. Prepared slides were successively stained with undiluted and diluted (1:1) May-Grunwald solutions and 40% Giemsa, coverslipped and scored. The report did not indicate whether slides were coded prior to analysis.

4. Statistical Methods: The data were analyzed for significance using the X^2 test at $p<0.05$.

5. Evaluation Criteria: No criteria for a positive response, the validity of the assay, or the biological significance of the findings were presented.

6. Protocol: A protocol was not provided.

C. REPORTED RESULTS:

1. Preliminary Toxicity Tests: No data were presented from the preliminary assessment of compound toxicity. The study author stated that "In this experiment the dose of 1000 mg/kg was determined as the highest applicable in the mutagenicity assay, together with further two doses, diminishing by a factor of 0.5."

2. Nuclear Anomaly Test: No deaths or clinical signs of compound toxicity were reported for males and females receiving the 2 daily administrations of 250, 500, or 1000 mg/kg CGA 169374 technical. There were also no appreciable increases in the percentage of cells with nuclear anomalies in bone marrow cells of 3 males and three females evaluated per treatment group (Table 1). By contrast, a significant increase ($p<0.05$) in the total percent of cells with nuclear anomalies was noted in the positive control (CP) group.

Based on the overall results, the study author concluded "Under the conditions of this experiment, no evidence of mutagenic effects was obtained in Chinese hamsters treated with CGA 169374 technical."

TABLE 1. Results of the Nuclear Anomaly Assay (Micronucleus Test) in Chinese Hamsters Treated with CGA-169374 Technical

Substance	Dose	Animals Exposed per Group[a,b]	No. of Animals Analyzed per Group	Number of Cells Analyzed per Group	Average Percent Cells with Nuclear Anomalies[a]					
					Jolly Bodies	Nuclear Fragments in Erythrocytes	Micronuclei in Erythroblasts	Micronuclei in Leukopoietic Cells	Polyploid Cells	Total
Vehicle Control										
Sesame oil	10 mL/kg/day	6M	3M	3000	0.00	0.00	0.00	0.00	0.00	0.00
		6F	3F	3000	0.23	0.00	0.07	0.03	0.00	0.33 (0.17)[c]
Positive Control										
Cyclophosphamide	128 mg/kg/day	6M	3M	3000	7.00	0.87	0.63	0.63	0.03	9.17
		6F	3F	3000	6.87	1.27	1.57	0.23	0.07	10.0 (9.59)*
Test Material										
CGA-169374 technical	250 mg/kg/day	6M	3M	3000	0.23	0.00	0.07	0.00	0.00	0.30
		6F	3F	3000	0.27	0.00	0.00	0.00	0.00	0.27 (0.29)
	500 mg/kg/day	6M	3M	3000	0.03	0.00	0.00	0.00	0.03	0.07
		6F	3F	3000	0.13	0.00	0.00	0.00	0.00	0.13 (0.1)
	1000 mg/kg/day	6M	3M	3000	0.07	0.00	0.00	0.00	0.00	0.07
		6F	3F	3000	0.17	0.00	0.00	0.00	0.00	0.17 (0.12)

[a]Average values calculated by our reviewers.
[b]Animals were sacrificed 24 hours after administration of the second of two daily oral gavage doses.

[c]Values in () = Average values combined for both sexes.

*Significantly higher ($p<0.05$) than the corresponding vehicle control by X^2 test.

D. REVIEWERS' DISCUSSION/CONCLUSIONS: We assess that the study as performed does not provide acceptable evidence of a negative response for the following reasons:

1. There were no signs of overt compound toxicity in the treated animals or cytotoxic effects on the target organ. The data are, therefore, insufficient to establish that the MTD of CGA 169374 technical was tested.

2. According to Federal Register, Vol. 52:19080-19081 (dated May 20, 1987), using a repeated treatment schedule, "bone marrow samples should be taken at least three times, starting not earlier than 12 hours after the last treatment...but not extending beyond 72 hours."

3. The number of animals evaluated for micronuclei induction (3 males and 3 females/treatment group) does not conform with EPA (Federal Register, Vol. 52:19080-19081, May 20, 1987) or OECD guidelines, or the Gene-Tox recommendation of at least 10 animals/group (5 males/5 females).[1]

4. Slides were not coded prior to analysis.

We conclude, therefore, that the study is unacceptable.

E. QUALITY ASSURANCE MEASURES: Was the test performed under GLPs? Yes. (A quality assurance statement was signed and dated January 24, 1986).

F. CBI APPENDIX: Appendix A, Materials and Methods, CBI pp. 9-11.

CORE CLASSIFICATION: Unacceptable. The study does not satisfy Guideline requirements (§84.2) for genetic effect Category II, Structural Chromosome Aberrations.

[1]MacGregor, J.T., Heddle, J.A. Hite, M., Margolin, B.H., Ramel, C., Salamone, M.F., Tice, R.R., and Wild, D. (1987). Guidelines for the conduct of the micronucleus assays in mammalian bone marrow erythrocytes. Mutat. Res. 189:103-112.

APPENDIX A

MATERIALS AND METHODS
CBI pp. 9-11

APPENDIX A

MATERIALS AND METHODS
CBI pp. 9-11

RIN 1360-95

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FINAL

DATA EVALUATION REPORT

DIFENOCONOZALE (CGA-169374 TECHNICAL)

Study Type: Mutagenicity: Unscheduled DNA Synthesis Assay in Primary Rat Hepatocytes

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 Reviewer Lynne T. Haber, Ph.D. Date 6/8/92

Independent Reviewer Nancy E. McCarroll, B.S. Date 6/8/92

QA/QC Manager Sharon Segal, Ph.D Date 6/8/92

Contract Number: 68D10075
Work Assignment Number: 1-79
Clement Number: 91-268
Project Officer: James Scott

00039

GUIDELINE §84: MUTAGENICITY
UDS

MUTAGENICITY STUDIES

Approved by:

EPA Reviewer: Karen Whitby, Ph.D. Signature: _____
Review Section II,
Toxicology Branch II/HED H7509C Date: 6/17/92
EPA Section Head: Clark Swentzel Signature: _____
Review Section II,
Toxicology Branch II/HED H7509C Date: 6/22/92

DATA EVALUATION REPORT

STUDY TYPE: Mutagenicity: In vitro unscheduled DNA synthesis assay in primary rat hepatocytes.

EPA IDENTIFICATION Numbers:

Shaughnessy No.: 128847

MRID Number: 420900-27

TEST MATERIAL: CGA-169374 technical

SYNONYMS: Difenoconozale; (+)-cis-trans-1-[2-[4-(4-chlorophenoxy)-2-chlorophenyl]-4-methyl-1,3-dioxolan-2-yl-methyl]-1H-1,2,4-triazole; $C_{19}H_{17}Cl_2N_3O_3$

SPONSOR: Ciba-Geigy Corp., Greensboro, NC

STUDY NUMBER: 850566

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

TITLE OF REPORT: CGA-169374 Technical Test for Other Genotoxic Effects Autoradiographic DNA Repair Test on Rat Hepatocytes

AUTHOR: E. Puri

REPORT ISSUED: August 30, 1985

CONCLUSIONS-EXECUTIVE SUMMARY: No conclusions can be reached from the unscheduled DNA synthesis (UDS) primary rat hepatocyte assay conducted with CGA-169374 technical at concentrations ranging from 0.25 to 31.25 µg/mL. The sensitivity of the study was severely compromised by the use of an overnight attachment period and exposure of the cells to the test chemical for only 5 hours. In addition, the method used to count nuclear grains (counting the background in a cell-free portion of the slide, rather than counting cytoplasmic grains in an area adjacent to the nucleus and subtracting this

value from gross nuclear counts) probably led to the unacceptably high values for nuclear grain counts and percentage of cells with ≥5 grains in the solvent (dimethyl sulfoxide) control group. The study should be repeated using currently recommended procedures for the rat hepatocyte UDS assay.[1] Slides should also be coded prior to analysis to eliminate bias, and cytotoxicity data should be included in the study report.

STUDY CLASSIFICATION: Unacceptable. The study does not satisfy Guideline requirements (§84.4) for genetic effects Category III, Other Mutagenic Mechanisms.

A. MATERIALS:

1. Test Material: CGA-169374 technical

   Description: Not provided
   Lot number: Batch number P. 503001
   Purity: 94.5%
   Receipt date: Not reported
   Stability: Reported to be stable; however, no data were provided to support this statement.
   Contaminants: None listed
   Solvent used: Dimethyl sulfoxide (DMSO)
   Other provided information: Dosing solutions were prepared the day of the assay. Test material storage conditions were not reported.

2. Indicator Cells: Primary rat hepatocytes were freshly isolated from the liver of a 275-g (preliminary cytotoxicity assay) or 335-g (DNA repair assay) male Tif.RAIf(SPF) rat. The source of the rat was not reported.

3. Control Substances: Williams' Medium E served as the negative control and DMSO at a final concentration of 1% was used as the solvent control. The positive control was dimethylnitrosamine (DMN) at 100 mM.

4. Medium: WME: Williams' Medium E; WME+: Williams' Medium E with 10% fetal bovine serum.

5. Test Compound Concentrations Used:

   (a) Cytotoxicity assay: Seven doses ranging from 15.625 to 1000 µg/mL were tested.

   (b) Repair assay: Four doses (0.25, 1.25, 6.25, and 31.25 µg/mL) were tested.

[1]Mitchell, A.D., Casciano, D.A., Meltz, M.L., Robinson, D.E., San, R.H.C., Williams, G.M., and Von Halle, E.S. (1983) Unscheduled DNA synthesis tests: A report of the U.S. Environmental Protection Agency Gene-Tox Program. Mutat. Res. 123:363-410.

B. STUDY DESIGN:

1. Cell Preparation:

(a) Perfusion technique: The method of hepatocyte isolation was not reported.

(b) Hepatocyte harvest/culture preparation: Recovered cells were cultivated in WME+.

2. UDS Assay:

(a) Treatment: Similar treatment methods were used for the preliminary cytotoxicity assay and the DNA repair assay. Cells ($4x10^6$ cells/well in WME+) were plated into multi-well culture dishes containing gelatinized coverslips and allowed to attach for 1.5-2 hours. Unattached cells were removed and viable cells were cultured overnight in WME+. In the cytotoxicity assay, the test material was added to duplicate cultures; treated hepatocytes were incubated for 5 hours, washed, stained with Trypan blue and fixed. Viability was determined by assessing dye exclusion in 100 cells.

The UDS assays were initiated by adding the selected test material doses, negative control (WME+), solvent control (DMSO), or positive control (DMN) to the prepared cultures, followed immediately by the addition of 4 µCi/mL [^{3}H] thymidine (TdR). Cultures were incubated for five hours, washed, fixed in acetic acid:ethanol (1:3), dried, mounted and prepared for autoradiography by an unspecified method. There was no indication that the slides were coded prior to analysis.

(b) Preparation of autoradiographs/grain development: Slides were exposed for 6 days, developed and stained with hematoxylin-eosin.

(c) Grain counting: The nuclear grains of 150 cells (50/coverslip) per treatment were counted. Background counts were determined by counting the grains in nuclear-sized area of a cell-free region of each coverslip. Net nuclear grain counts were not calculated, presumably because of the "negligibly low" background counts of ≤0.2.

3. Evaluation Criteria:

(a) Assay validity: No criteria for assay validity were reported.

(b) Positive response: The assay was considered positive if the test material caused a doubling in the number of grains per nucleus relative to the control.

4. Protocol: Not provided; however, the primary data were reported.

C. REPORTED RESULTS:

1. Cytotoxicity Assay: Seven doses of the test material ranging from 15.625 to 1000 µg/mL were evaluated. The values for the five intermediate doses were not reported. No cytotoxicity data were provided, but the study author reported that results of the cytotoxicity assay indicated that the highest "usable" concentration was 31.25 µg/mL. There is no indication within the report (except possibly from the grain counts) of any cytotoxicity at the highest tested dose.

2. UDS Assay: Triplicate cultures of hepatocytes exposed to 0.25–31.25 µg/mL were evaluated for UDS. As shown in Table 1, solvent-treated cultures had unacceptably high values for both mean nuclear grain count (3.25 grains/nucleus) and percentage of cells with ≥5 nuclear grains (24.0%). Under optimal conditions, net incorporation rates for solvent control cultures should generally be <1 grain/nucleus[2] and the percentage of cells in repair rarely exceeds 10%. Our reviewers assume that the presentation of gross nuclear counts without the proper adjustments for background [^{3}H] TdR incorporation accounted, at leat in part, for the high solvent control values. Additionally, since silver grains are not evenly distributed over the slides, counting the background grains in a cell-free portion of the slide is an unacceptable procedure. Instead, the grains in a nuclear-sized area of the cytoplasm adjacent to each nucleus should be counted and subtracted from the nuclear grain count.

   However, even if the accepted approach for determining net nuclear grains were applied to these data, the study would still be unacceptable because of the prolonged attachment period (overnight) and the short exposure time (5 hours). Our contention that the assay conditions were suboptimal is illustrated by the results with the positive control (100 mM DMN). Although 100 mM DMN induced a genotoxic response (Table 1), this concentration is at least three orders of magnitude higher than that necessary to obtain a positive result in the UDS assay under optimal conditions.[3] Barfknecht et al.[4] have demonstrated that an 18-hcur exposure period results in much greater sensitivity to DMN and other chemicals. Similarly, the U.S. Environmental Protection Agency Gene-Tox Program recommends an 18-hour exposure. We conclude, therefore, that the study is invalid and the test material results can not be assessed. We, nevertheless, noted that there was suggestive evidence of a cytotoxic response at 6.25 and 31.25 µg/mL CGA-169374 technical, as indicated by decreases in nuclear grain counts and percentages of cells with ≥5 nuclear grains.

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[2]Mitchell, A.D. et al. (1983). Mutat. Res. 123:363-410.

[3]Barfknecht, T.R., Naismith, R.W., and Kornburst, D.J. (1987). Variations on the standard protocol design of the hepatocyte DNA repair assay. Cell. Biol. Toxicol. 3:193-209.

[4]Ibid.

[5]Mitchell, A.D. et al. (1983). Mutat. Res. 123:363-410.

TABLE 1. Results of the Unscheduled DNA Synthesis (UDS) Rat Hepatocyte Assay with CGA-169374 Technical

Treatment	Dose/mL	Nuclear Grains±S.D.[a]	Percent cells with ≥5 Nuclear Grains[b]
Negative Control			
Culture medium	--	1.81±1.31	5.3
Solvent Control			
Dimethyl sulfoxide	--	3.25±2.04	24.0
Positive Control			
Dimethylnitrosamine	100 mM	18.8±8.12	99.3
Test Material			
CGA-169374 technical	0.25 µg	3.26±1.87	26.0
	1.25 µg	3.31±2.09	27.3
	6.25 µg	2.07±1.77	10.0
	31.25 µg	0.79±0.96	1.3

[a]Means and standard deviations of the count of 150 cells; 50 cells from triplicate slides were analyzed.

[b]Calculated by our reviewers

D. REVIEWERS' DISCUSSION/CONCLUSIONS: The study is unacceptable and should be repeated using currently recommended methodology.[6] The following specific protocol changes should be made:

1-- To ensure maximum sensitivity, hepatocytes should be used immediately following the 1.5-2 hour attachment period, and cells should be exposed to the test material for 18-20 hours.

2-- Cytoplasmic grain counts should be determined by counting nuclear-sized areas adjacent to the nucleus, and net nuclear grain counts should be determined by subtracting the mean cytoplasmic grain count from the nuclear grain count.

3-- Cytotoxicity data should be presented to support the study author's statement that the highest usable concentration was attained.

4-- Slides should be coded prior to analysis to prevent bias.

It is noteworthy that the majority of issues raised in the review of this study were previously discussed with the sponsor (see summary of EPA/CIBA-GEIGY meeting conducted by Dr. Jane Harris, EPA Section Head, Toxicology Branch, on June 20, 1986). It would appear, therefore, that the sponsor is aware of EPA's position on the UDS assay but has elected to ignore the consensus opinion of the participants at this meeting.

E. QUALITY ASSURANCE MEASURES: Was the test performed under GLPs? Yes. (A quality assurance statement was signed and dated August 29, 1985.)

F. CBI APPENDIX: Appendix A, Materials and Methods, CBI pp. 5, 9-11.

CORE CLASSIFICATION: Unacceptable. The study does not satisfy Guideline requirements (§84.4) for genetic effects Category III, Other Mutagenic Mechanisms.

---

[6]Mitchell, A.D. et al. (1983). Mutat. Res. 123:363-410.

APPENDIX A

MATERIALS AND METHODS
CBI pp. 5, 9-11

RIN 1360-95

TOX REVIEW 009689 DIFENOCONAZOLE

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Pages 397 through 400 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.

____ Information about a pending registration action.

✓ FIFRA registration data.

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____ 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.

009689

DOC 930099

FINAL

DATA EVALUATION REPORT

DIFENOCONOZALE (CGA-169374)

Study Type: Mutagenicity: Unscheduled DNA Synthesis Assay in Human Fibroblasts

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 Reviewer Lynne T. Haber, Ph.D. Date 6/8/92

Independent Reviewer William McLellan, Ph.D. Date 6/8/92

QA/QC Manager Sharon Segal, Ph.D. Date 6/8/92

Contract Number: 68D10075
Work Assignment Number: 1-79
Clement Number: 91-267
Project Officer: James Scott

GUIDELINE SERIES 84: MUTAGENICITY
UDS

MUTAGENICITY STUDIES

Approved by:

EPA Reviewer: Karen Whitby, Ph.D.
Review Section II,
Toxicology Branch II/HED H7509C
Signature: [signature]
Date: 6/17/92

EPA Section Head: Clark Swentzel
Review Section II,
Toxicology Branch II/HED H7509C
Signature: [signature]
Date: 6/22/92

DATA EVALUATION REPORT

STUDY TYPE: Mutagenicity: In vitro unscheduled DNA synthesis assay in human fibroblasts.

EPA IDENTIFICATION Numbers:

Shaughnessy No.: 128847

MRID Number: 420900-26

TEST MATERIAL: CGA-169374 technical

SYNONYMS: Difenoconazole; (+)-cis-trans-1-{2-[4-(4-chlorophenoxy)-2-chlorophenyl]-4-methyl-1,3-dioxolan-2-yl-methyl}-1H-1,2,4-triazole; $C_{19}H_{17}Cl_2N_3O_3$

SPONSOR: Ciba-Geigy Corp., Greensboro, NC

STUDY NUMBER: 850568

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

TITLE OF REPORT: CGA-169374 Technical Test for Other Genotoxic Effects Autoradiographic DNA Repair Test on Human Fibroblasts

AUTHOR: E. Puri

REPORT ISSUED: August 30, 1985

CONCLUSIONS-EXECUTIVE SUMMARY: No conclusions can be reached from the unscheduled DNA synthesis (UDS) human fibroblast assay conducted with CGA-169374 technical at concentrations ranging from 0.08 to 10 µg/mL. While there was no evidence that the test material was genotoxic, there was also no evidence that a cytotoxic concentration was reached. The study should be conducted at concentrations including at least one cytotoxic dose both in the presence and absence of exogenous metabolic activation, as required by EPA

Guidelines. Slides should also be coded prior to analysis to eliminate bias, and cytotoxicity data should be included in the study report.

STUDY CLASSIFICATION: Unacceptable. The study does not satisfy Guideline requirements (§84.4) for genetic effects Category III, Other Mutagenic Mechanisms.

A. MATERIALS:

1. Test Material: CGA-169374 technical

   Description: Not provided
   Lot number: Batch number P. 503001
   Purity: 94.5%
   Receipt date: Not reported
   Stability: Ensured by sponsor
   Contaminants: None listed
   Solvent used: Dimethyl sulfoxide (DMSO)
   Other provided information: Dosing solutions were prepared the day of the assay. Test material storage conditions were not reported.

2. Indicator Cells: Human fibroblasts, line CRL 1121, obtained from ATCC, Rockville, MD

3. Control Substances: Dulbecco's minimal essential medium served as the negative control and DMSO at a final concentration of 1% was used as the solvent control. The positive control was 4-Nitroquinoline-N-oxide (4NQO) at 5 µM.

4. Medium: MEM: Dulbecco's minimal essential medium; MEM+: MEM with 10% fetal bovine serum.

5. Test Compound Concentrations Used:

   (a) Cytotoxicity assay:

   - First test: Seven doses ranging from 15.625 to 1000 µg/mL were tested. A vehicle control was also tested.
   - Second test: Seven doses ranging from 0.625 to 40 µg/mL were tested. A vehicle control was also tested.

   (b) Repair assay: Four doses (0.08, 0.4, 2.0, and 10 µg/mL) were tested.

B. STUDY DESIGN:

1. UDS Assay:

(a) Treatment: Similar treatment methods were used for the preliminary cytotoxicity assay and the DNA repair assay. Cells ($3x10^4$ cells/well in MEM+) were seeded into multi-well culture dishes containing glass coverslips and cultured overnight. In the cytotoxicity assay, the test material was added to duplicate cultures, and cultures were incubated for five hours, washed, stained with Trypan blue and fixed. Viability was determined by assessing dye exclusion in 100 cells.

The UDS assays were initiated by adding the selected test material doses, negative control (MEM+), solvent control (DMSO) or positive control (4NQO) to each of four replicate cultures, followed immediately by the addition of 2 µCi/mL [^{3}H] thymidine (TdR). Cultures were incubated for five hours, washed, fixed in acetic acid:ethanol (1:3), dried, mounted and prepared for auto-radiography by an unspecified method. There was no indication that the slides were coded prior to analysis.

(b) Preparation of aut radiographs/grain development: Slides were exposed for 6 days, developed and stained with hematoxylin-eosin.

(c) Grain counting: The nuclear grains of 200 cells (50/coverslip) per treatment were counted. Background counts were determined by counting the grains in nuclear-sized areas of cell-free regions of each coverslip. Net nuclear grain counts were not calculated presumably because of the "negligibly low" background counts (≤0.2).

4. Evaluation Criteria:

(a) Assay validity: No criteria for assay validity were reported.

(b) Positive response: The assay was considered positive if the test material caused the number of grains per nucleus to more than double relative to the control.

5. Protocol: Not provided. However, the primary data were reported.

C. REPORTED RESULTS:

1. Cytotoxicity Assay: In the first test, seven doses of the test material ranging from 15.625 to 1000 µg/mL were evaluated. The study author reported that the test substance was toxic at all tested concentrations. Seven concentrations of CGA-169374 technical ranging from 0.625 to 40 µg/mL were evaluated in the second test. The five intermediate concentrations were not reported. No cytotoxicity data were provided, but the study author reported that results of the cytotoxicity assay indicated

that the highest "usable" concentration was 10 µg/mL. Further explanation was not provided.

2. UDS Assay: Four-fold replicate cultures of fibroblasts exposed to 0.08-10 µg/mL were evaluated for UDS. As shown in Table 1, there was no evidence that CGA-169374 technical was genotoxic at any tested concentration. However, there was also no indication that cytotoxic concentrations were reached, other than a statment on the printout of primary data that the cells were "not well" at the high dose (10 µg/mL). No further explanation was given, and the study author did not report any cytotoxic effect at any assayed test material concentration.

   Since the cytoplasmic background with fibroblasts is virtually zero, the method used for determining nuclear grain counts is acceptable. However, the EPA Guidelines require that the UDS assay with human fibroblasts be conducted in both the presence and absence of an exogenous metabolic system.

   We conclude, therefore, that the study is invalid and the test material results can not be assessed.

D. REVIEWERS' DISCUSSION/CONCLUSIONS: The study is unacceptable and should be repeated using currently recommended methodology.[1] The following specific protocol changes should be made:

   1-- Test material concentrations should be chosen so that cytotoxicity is observed at the high dose.

   2-- The experiment should be conducted in both the absence and presence of S9 activation.

   3-- Slides should be coded prior to analysis to prevent bias.

E. QUALITY ASSURANCE MEASURES: Was the test performed under GLPs? Yes. (A quality assurance statement was signed and dated August 29, 1985.)

F. CBI APPENDIX: Appendix A, Materials and Methods, CBI pp. 5, 9-11.

CORE CLASSIFICATION: Unacceptable. The study does not satisfy Guideline requirements (§84.4) for genetic effects Category III, Other Mutagenic Mechanisms.

---

[1]Mitchell, A.D., Casciano, D.A., Meltz, M.L., Robinson, D.E., San, R.H.C., Williams, G.M., and Von Halle, E.S. (1983) Unscheduled DNA synthesis tests: A report of the U.S. Environmental Protection Agency Gene-Tox Program. Mutat. Res. 123:363-410.

TABLE 1. Results of the Unscheduled DNA Synthesis (UDS) Human Fibroblast Assay with CGA-169374 Technical

Treatment	Dose/mL	Nuclear Grains±S.D.[a]	Percent cells with ≥5 Nuclear Grains[b]
Negative Control			
Culture medium	--	1.08±1.05	0.5
Solvent Control			
Dimethyl sulfoxide	--	0.83±0.90	0.0
Positive Control			
4-Nitroquinoline-N-oxide	5 µM	38.9±13.29	100.0
Test Material			
CGA-169374 technical	0.08 µg	1.38±1.14	1.0
	0.4 µg	1.27±0.98	0.5
	2.0 µg	1.09±0.96	1.0
	10 µg	1.36±1.31	3.0

[a]Means and standard errors of the count of 200 cells; 50 cells from each of four slides were analyzed.

[b]Calculated by our reviewers

APPENDIX A

MATERIALS AND METHODS
CBI pp. 5, 9-11

RIN 1360-95

TOX REVIEW 009689 DIFENOCONAZOLE

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Pages 408 through 411 are not included.

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____ Description of quality control procedures.

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____ 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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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.

009689

DOC930099

FINAL

DATA EVALUATION REPORT

DIFENOCONOZALE (CGA-169374)

Study Type: Mutagenicity: Unscheduled DNA Synthesis Assay in Human Fibroblasts

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 Reviewer Lynne T. Haber, Ph.D. Date 6/8/92

Independent Reviewer William McLellan, Ph.D. Date 6/8/92

QA/QC Manager Sharon Segal, Ph.D. Date 6/8/92

Contract Number: 68D10075
Work Assignment Number: 1-79
Clement Number: 91-267
Project Officer: James Scott

C0412

GUIDELINE SERIES 84: MUTAGENICITY
UDS

MUTAGENICITY STUDIES

Approved by:

EPA Reviewer: Karen Whitby, Ph.D. Signature:
Review Section II,
Toxicology Branch II/HED H7509C Date: 6/17/92
EPA Section Head: Clark Swentzel Signature:
Review Section II,
Toxicology Branch II/HED H7509C Date: 6/22/92

DATA EVALUATION REPORT

STUDY TYPE: Mutagenicity: In vitro unscheduled DNA synthesis assay in human fibroblasts.

EPA IDENTIFICATION Numbers:

Shaughnessy No.: 128847

MRID Number: 420900-26

TEST MATERIAL: CGA-169374 technical

SYNONYMS: Difenoconozale; (+)-cis-trans-1-{2-[4-(4-chlorophenoxy)-2-chlorophenyl]-4-methyl-1,3-dioxolan-2-yl-methyl)-1H-1.2,4-triazole; $C_{19}H_{17}Cl_2N_3O_3$

SPONSOR: Ciba-Geigy Corp., Greensboro, NC

STUDY NUMBER: 850568

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

TITLE OF REPORT: CGA-169374 Technical Test for Other Genotoxic Effects Autoradiographic DNA Repair Test on Human Fibroblasts

AUTHOR: E. Puri

REPORT ISSUED: August 30, 1985

CONCLUSIONS-EXECUTIVE SUMMARY: No conclusions can be reached from the unscheduled DNA synthesis (UDS) human fibroblast assay conducted with CGA-169374 technical at concentrations ranging from 0.08 to 10 µg/mL. While there was no evidence that the test material was genotoxic, there was also no evidence that a cytotoxic concentration was reached. The study should be conducted at concentrations including at least one cytotoxic dose both in the presence and absence of exogenous metabolic activation, as required by EPA

Guidelines. Slides should also be coded prior to analysis to eliminate bias, and cytotoxicity data should be included in the study report.

STUDY CLASSIFICATION: Unacceptable. The study does not satisfy Guideline requirements (§84.4) for genetic effects Category III, Other Mutagenic Mechanisms.

A. MATERIALS:

1. Test Material: CGA-169374 technical

   Description: Not provided
   Lot number: Batch number P. 503001
   Purity: 94.5%
   Receipt date: Not reported
   Stability: Ensured by sponsor
   Contaminants: None listed
   Solvent used: Dimethyl sulfoxide (DMSO)
   Other provided information: Dosing solutions were prepared the day of the assay. Test material storage conditions were not reported.

2. Indicator Cells: Human fibroblasts, line CRL 1121, obtained from ATCC, Rockville, MD

3. Control Substances: Dulbecco's minimal essential medium served as the negative control and DMSO at a final concentration of 1% was used as the solvent control. The positive control was 4-Nitroquinoline-N-oxide (4NQO) at 5 µM.

4. Medium: MEM: Dulbecco's minimal essential medium; MEM+: MEM with 10% fetal bovine serum.

5. Test Compound Concentrations Used:

   (a) Cytotoxicity assay:

   - First test: Seven doses ranging from 15.625 to 1000 µg/mL were tested. A vehicle control was also tested.
   - Second test: Seven doses ranging from 0.625 to 40 µg/mL were tested. A vehicle control was also tested

   (b) Repair assay: Four doses (0.08, 0.4, 2.0, and 10 µg/mL) were tested.

B. STUDY DESIGN:

1. UDS Assay:

(a) Treatment: Similar treatment methods were used for the preliminary cytotoxicity assay and the DNA repair assay. Cells ($3x10^4$ cells/well in MEM+) were seeded into multi-well culture dishes containing glass coverslips and cultured overnight. In the cytotoxicity assay, the test material was added to duplicate cultures, and cultures were incubated for five hours, washed, stained with Trypan blue and fixed. Viability was determined by assessing dye exclusion in 100 cells.

The UDS assays were initiated by adding the selected test material doses, negative control (MEM+), solvent control (DMSO) or positive control (4NQO) to each of four replicate cultures, followed immediately by the addition of 2 µCi/mL [^{3}H] thymidine (TdR). Cultures were incubated for five hours, washed, fixed in acetic acid:ethanol (1:3), dried, mounted and prepared for auto-radiography by an unspecified method. There was no indication that the slides were coded prior to analysis.

(b) Preparation of autoradiographs/grain development: Slides were exposed for 6 days, developed and stained with hematoxylin-eosin.

(c) Grain counting: The nuclear grains of 200 cells (50/coverslip) per treatment were counted. Background counts were determined by counting the grains in nuclear-sized areas of cell-free regions of each coverslip. Net nuclear grain counts were not calculated presumably because of the "negligibly low" background counts (≤0.2).

4. Evaluation Criteria:

(a) Assay validity: No criteria for assay validity were reported.

(b) Positive response: The assay was considered positive if the test material caused the number of grains per nucleus to more than double relative to the control.

Protocol Not provided. However, the primary data were reported.

REPORTED RESULTS

Cytotoxicity Assay In the first test, seven doses of the test material ranging from 15.625 to 1000 µg/mL were evaluated. The study author reported that the test substance was toxic at all tested concentrations. Seven concentrations of CGA-169374 technical ranging from 7.813 to 40 µg/mL were evaluated in the second test. The five intermediate concentrations were not reported. No cytotoxicity data were provided but the study author reported that results of the cytotoxicity assay indicated

that the highest "usable" concentration was 10 µg/mL. Further explanation was not provided.

2. UDS Assay: Four-fold replicate cultures of fibroblasts exposed to 0.08-10 µg/mL were evaluated for UDS. As shown in Table 1, there was no evidence that CGA-169374 technical was genotoxic at any tested concentration. However, there was also no indication that cytotoxic concentrations were reached, other than a statment on the printout of primary data that the cells were "not well" at the high dose (10 µg/mL). No further explanation was given, and the study author did not report any cytotoxic effect at any assayed test material concentration.

   Since the cytoplasmic background with fibroblasts is virtually zero, the method used for determining nuclear grain counts is acceptable. However, the EPA Guidelines require that the UDS assay with human fibroblasts be conducted in both the presence and absence of an exogenous metabolic system.

   We conclude, therefore, that the study is invalid and the test material results can not be assessed.

D. REVIEWERS' DISCUSSION/CONCLUSIONS: The study is unacceptable and should be repeated using currently recommended methodology.[1] The following specific protocol changes should be made:

   1-- Test material concentrations should be chosen so that cytotoxicity is observed at the high dose.

   2-- The experiment should be conducted in both the absence and presence of S9 activation.

   3-- Slides should be coded prior to analysis to prevent bias.

E. QUALITY ASSURANCE MEASURES: Was the test performed under GLPs? Yes. (A quality assurance statement was signed and dated August 29, 1985.)

F. CBI APPENDIX: Appendix A, Materials and Methods, CBI pp. 5, 9-11.

CORE CLASSIFICATION: Unacceptable. The study does not satisfy Guideline requirements (§84.4) for genetic effects Category III, Other Mutagenic Mechanisms.

---

[1] Mitchell, A.D., Casciano, D.A., Meltz, M.L., Robinson, D.E., San, R.H.C., Williams, G.M., and Von Halle, E.S. (1983) Unscheduled DNA synthesis tests: A report of the U.S. Environmental Protection Agency Gene-Tox Program. Mutat. Res. 123: 363-410.

TABLE 1. Results of the Unscheduled DNA Synthesis (UDS) Human Fibroblast Assay with CGA-169374 Technical

Treatment	Dose/mL	Nuclear Grains±S.D.[a]	Percent cells with ≥5 Nuclear Grains[b]
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Culture medium	--	1.08±1.05	0.5
Solvent Control			
Dimethyl sulfoxide	--	0.83±0.90	0.0
Positive Control			
4-Nitroquinoline-N-oxide	5 µM	38.9±13.29	100.0
Test Material			
CGA-169374 technical	0.08 µg	1.38±1.14	1.0
	0.4 µg	1.27±0.98	0.5
	2.0 µg	1.09±0.96	1.0
	10 µg	1.36±1.31	3.0

[a]Means and standard errors of the count of 200 cells; 50 cells from each of four slides were analyzed.

[b]Calculated by our reviewers

APPENDIX A

MATERIALS AND METHODS
CBI pp. 5, 9-11

RIN 1360-95

TOX REVIEW - 009689 DIFENOCONAZOLE

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____ Sales or other commercial/financial information.

____ A draft product label.

____ The product confidential statement of formula.

____ Information about a pending registration action.

✓ FIFRA registration data.

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

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

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 M 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

009689

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

Signature:
Date: 7/28/92

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

Signature: K. Clark Swentzel
Date: 8/3/92

DATA EVALUATION REPORT

STUDY TYPE: Metabolism

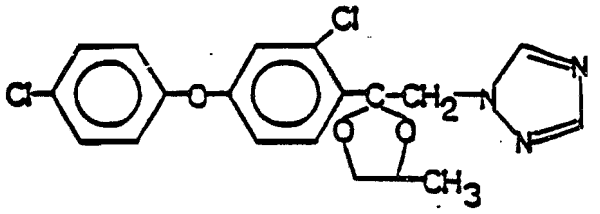
EPA IDENTIFICATION NUMBER:

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]

00424

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.

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.

A. MATERIALS

1. Test Substance

The unlabeled test material (lot numbers S85-0812-1 and B-04336) administered in the diet for the repeated-dosing study had a chemical purity of 94.5%.

CGA-169374, labeled in the phenyl ring (phenyl-^{14}C-CGA-169374) (lot numbers CL-IX-1 and CL-IX-7), had specific activities of 48.6 µCi/mg (low dose) and 0.2 µCi/mg (high dose), with a radiochemical purity of 98.6%. Figure 1 in MRID 420900-28 depicts the inner phenyl ring of CGA-169374 as being uniformly ^{14}C-labeled.

CGA-169374, labeled in the triazole ring (triazole-^{14}C-CGA-169374) (lot numbers CL-IX-5, CL-IX-31, CL-IX-8, and CL-IX-32A), had specific activities of 19.4-19.7 µCi/mg (low dose) and 0.2 µCi/mg (high dose), with a radiochemical purity of 98.1%.

2. Test Animals

Male and female Sprague-Dawley Crl:CD BR rats were obtained from Charles River Laboratories, Portage, MI. Rats were administered a single dose of 0.5 or 300 mg/kg CGA-169374, labeled at the phenyl or triazole ring. For the repeated-dosing study, rats received unlabeled CGA-169374 by gavage for 2 weeks followed by a single labeled dose the next day. The body weights of the rats ranged from 177 to 295 kg prior to dosing.

B. METHODS

1. Acclimation

Animals were acclimatized for at least 12 days before the administration of the test material. Rats were placed individually in Nalgene® metabolism chambers 2 days prior to exposure. The diet, Rodent Chow® 5002 (Purina Mills, Inc., St. Louis, MO), and tap water, were provided ad libitum throughout the study. No contaminants in the food and water were known to interfere with the study.

The dosing of animals was conducted at WIL Research Laboratories, Inc., Ashland, OH. The laboratory also purchased rats, prepared dosing suspensions according to CIBA-GEIGY procedures and radioassayed the dosing suspension.

2. Dosing Solutions

The dosing solutions described below refer to CGA-16937 labeled either at the phenyl or triazole rings. The radiolabeled 0.5 and 300 mg/kg dosing suspensions were prepared with 1% aqueous sodium carboxymethylcellulose (CMC) (Hercules, Inc., Wilmington, DE) and an equal concentration of Hi Sil 233 silica gel (Pittsburgh Plate and Glass, Pittsburgh, PA). The suspensions for the low-dose group also contained 2.5% ethanol. The dosing solutions were sonicated. The specific activities of the low-dose and high-dose radioactive solutions

were not provided. In the repeated-dosing study the unlabeled CGA-169374 was suspended with Hi Sil 233, 2.5% ethanol, and 1% aqueous CMC and were stable throughout the 14-day dosing period. To determine the amount of test material administered to the animal, the syringe was weighed prior to and after dosing.

Groups of rats (5/sex) were given a single oral dose of 0.5 or 300 mg/kg [^{14}C]CGA-169374, or were given a 2-week daily gavage dosing of 0.5 mg/kg/day unlabeled CGA-169374 followed by a single gavage administration of 0.5 mg/kg [^{14}C]CGA-169374 on day 15. The control group consisted of 3 male and 3 female rats. All animals were sacrificed at 7 days postexposure following the administration of the labeled CGA-169374.

3. Sample Collection

The urine and feces were collected, over dry ice, from animals at the following intervals: 0-4, 4-8, 8-12, 12-24, 24-48, 48-72, 72-96, 96-120, 120-144, and 144-168 hours after exposure to the [^{14}C]-labeled dose of CGA-169374. Feces was homogenized in water and combusted in manual and automatic Harvey Oxidizers. Cages were washed with methanol and water at postexposure day 7, and cage washings were collected for analysis. Following sacrifice by carbon dioxide at day 7 postexposure, major tissues were removed, weighed, homogenized, if necessary, and combusted. The feces, urine, and tissue samples, and cage washings were counted using a Beckman model 3801 liquid scintillation counter (LSC). The radioactivity in the carcass was not counted because it contained blood, muscle, and fat radioactivity that was already accounted for. Expired $^{14}CO_2$ was trapped with Oxifluor-CO_2 (National Diagnostic). Methods for statistical analyses were limited to means and standard deviations.

4. Metabolite Analysis

Metabolite analysis was conducted for the urine and feces samples collected on postexposure day 2 or 3 from one male and one female rat from each of the three oral-dose groups with phenyl and triazole labeling. Samples from these collection periods were chosen because elimination of radioactivity was high and metabolites were expected to be similar, although relative distribution may vary. The primary focus of the metabolite analysis was on fecal metabolites since most of the radioactivity was eliminated by this excretory route. The feces samples were extracted with acetonitrile. In addition, pooled day-2 and day-3 feces from 4 high-dose phenyl-labeled females were extracted for bulk metabolite isolation. Liver samples from high-dose, phenyl-labeled males were combined, homogenized, and extracted for metabolite identification. The liver was examined for metabolites because it contained comparable phenyl-label distribution to other tissues.

Thin-layer chromatography (TLC) was performed in two solvent development systems: (1) 1:1:1 toluene/chloroform/ethanol and (2) 70:20:4:2 chloroform/methanol/formic acid/ water. Radioactive bands were detected using a Berthold model LB292 Beta Camera. Standards were visualized with ultraviolet light or in iodine chambers. Radioactive components

were extracted, placed in chloroform, and centrifuged. High performance liquid chromatography (HPLC) was performed on a Perkin Elmer model 410 solvent delivery system or a Spectra Physics SP 8700 solvent delivery system. Mass spectrometry was performed on a VG 70-250 SQ double focusing mass spectrometer. Nuclear magnetic resonance (NMR) was performed on unknown radioactive metabolites in which samples were dissolved in deuterochloroform or perdeuteromethanol. Gas chromatography (GC) experiments were conducted using a HP-5890 gas chromatograph.

5 Protocols

The methods followed the study protocol.

C. REPORTED RESULTS

1. Elimination and Recovery

In the phenyl-^{14}C-CGA-169374 study, the recoveries of radioactivity in the feces were 81.38-86.72%, 78.06-78.95%, and 85.36-94.61% of the administered dose in the single low-dose, repeated low-dose, and single high-dose groups, respectively. For these groups, 12.93-17.19%, 19.01-19.25%, and 8.48-14.70% of the dose was recovered in the urine, respectively. The radioactivities in cage washings were negligible; 0.12-0.99% of the administered dose. Therefore, the mean total recoveries of radioactivity ranged from 97.94% to 104.31% of the administered dose after 7 days (Table 1).

In the triazole-^{14}C-CGA-169374 study, the radioactivities in the feces were 81.46-85.68%, 78.33-82.59%, and 87.83-88.51% of the administered dose in the single low-dose, repeated low-dose, and single high-dose groups, respectively. In the urine, the recoveries were 19.68-21.86%, 16.61-20.42%, and 10.71-11.50% of the dose, respectively. The radioactivities in cage washings were 0.00-0.53% of the administered dose. The mean 7-day recoveries ranged from 98.83% to 107.75% of the administered dose (Table 1).

No results were presented for elimination of radioactivity via expired air, although the methods section indicated that expired CO_2 was measured. It is expected that this is probably not a major route of elimination since CGA-169374 and its metabolites are probably not very volatile.

2. Tissue Distribution

For all oral dosing groups, the mean radioactivities in the tissues were very small or negligible. At 7 days postexposure, the total radioactivity in tissues after phenyl labeling was 0.0-0.84% of the dose. The highest amount of radioactivity was found in the carcass (0.84% of the dose; 2.7 ppm), followed by fat (0.42%; 0.89 ppm) and plasma (0.23%; 15.6 ppm) in the high-dose males. Radioactivities in the tissues after triazole labeling were 0.0-0.2% of the administered dose. The highest amount of radioactivity was in the liver (0.02% of the dose; 0.9 ppm) in the high-dose males. Radioactivity in other tissues after

triazole labeling was below the levels of quantification or detection.

In the phenyl-labeling study, the limit of detection (LOD) was <0.002, except for high-dose brain, which was ≤0.024 ppm, and the limit of quantitation (LOQ) was ≤0.004 ppm. In the triazole-labeling study, the LOD was ≤0.003 ppm in the low-dose groups and ≤0.280 ppm in the high-dose groups. The LOQ was ≤0.007 ppm in the low-dose groups and ≤0.817 ppm in the high-dose group. The limits varied depending on tissues, specific activity, aliquot size, and background values.

The authors compared radioactivities in tissues of low- and high-dose groups dosed with phenyl-[^{14}C] label. The ratios for high dose/low dose with phenyl label were about 700 for lung, liver, kidney, and carcass which is proportional to dose level. However, the ratio for fat was about 1000. The ratios for plasma was 474 and 579 for females and males, respectively. These lower ratios indicate possible protein binding of phenyl metabolites and binding sites were probably becoming saturated at the high dose.

3. Pharmacokinetics

Mean half-lives for excretion (hours required to eliminate 50% of total radioactivity excreted) were estimated from time versus percent dose excreted charts (CBI Figure 2-13, pp. 33-44) for the phenyl- and triazole-labeling studies. From the phenyl-labeling study, the low-dose groups had a mean half-life of 19-24 hours and the high-dose group had a mean half-life of 37-48 hours. From the triazole-label study, the low-dose groups had a mean half-life of 19-21 hours and the high-dose group had a mean half-life of 33 hours.

4. Metabolism

Overall recoveries from fecal extractions appeared to be very high for phenyl- and triazole-labeling studies (92-133% recovery except for a 79% recovery for the phenyl-labeled repeated-dose male). There were 3 major radioactive components detected in the feces. They were designated fecal metabolites A, B, and C (Table 2). Metabolite C was detected only in the high-dose animals labeled at triazole and phenyl rings. Metabolite C was isolated by HPLC and identified as CGA-205375 by TLC; this compound represented 6.66-24.19% of the administered dose.

HPLC analysis indicated that metabolite A represented the highest amount of radioactivity in the feces (18.43-71.77% of administered dose). Metabolite B represented ≤20.32% of the administered dose. No standards matched metabolites A and B, therefore, mass spectrometry was conducted using the feces of high-dose phenyl-labeled females. Metabolites A and B were identified as hydroxy-CGA-205375 and hydroxy-CGA-169374, respectively. The EI fragmentation indicated that the hydroxy substitution was at the diphenyl ether portion of the molecule since the oxygen is retained when the triazole moieties are lost. However, the specific substitution on the phenyl ring could not be determined. The investigators assumed that the parent compound has diastereomers because it has two chiral centers, and were able to separate the two metabolites, A and B, each into a pair of isomers (i.e., A1, A2, B1, B2)

by HPLC, but were indistinguishable by mass spectrometry. NMR analyses of the pure isomers indicated that all the hydroxy metabolites were substituted on the outer phenyl ring, probably at the ortho position to chlorine. Synthetic standards were then prepared based on the information obtained up to this point. However, chromatographic comparison found that hydroxylation of metabolites A2, B1, and B2 did not occur at the ortho or meta position to the chlorine on the outer ring. A potential explanation for the inconsistent results was explained by an NIH shift mechanism in which there is a chloride shift from the para to meta positions (Figure 2). This was confirmed by preparing 3 standards matching the structures of metabolites B1, B2, and A2. As shown in Figure 1, metabolites A1 and A2 were identified as ortho hydroxy-CGA-205375 (chloride retention) and 3-chloro,4-hydroxy CGA-205375 (chloride shift). Metabolites B1 and B2 were identified as diastereomers of the chloride shift substitution with 3-chloro,4-hydroxy substitution on the outer ring.

In the urine, no metabolite reached 10% of the administered dose. Free triazole, CGA-71019, was identified only in the urine of triazole-labeled groups by HPLC. This metabolite represented between 21% to 70% of the total radioactive residues in the urine. The phenyl-labeled urines had less polar metabolites and more complex distribution, however, no specific metabolites were identified because of the low relative abundance of individual metabolites.

CGA-189138 was detected in the liver of phenyl-labeled high-dose group by TLC, HPLC, and mass spectrometry. This metabolite is highly lipophilic, which probably explains its deposition in tissues. The recovery of the radioactivity in the liver extraction was 91%.

The proposed metabolic pathway of CGA-16937 is shown in Figure 1.

D. STUDY AUTHORS' CONCLUSIONS/QUALITY ASSURANCE MEASURES

The authors concluded that CGA-169374 is eliminated primarily in the feces of rats, probably due to the poor absorption of high molecular weight of CGA-169374 (MW 402) or/and biliary excretion of high molecular-weight metabolites A and B (i.e., approximately 366 and 422, respectively). The half-lives were approximately 20-22 hours for the low-dose rats and 33-48 hours for the high-dose rats. There were no major sex- or dose-related differences in the rate and route of elimination of CGA-169374. Seven days following single and repeated oral dosing of CGA-169374, the distribution of radioactivity in the rat tissues was negligible. Tissue distribution was different for the two radiolabels. More radioactivity was found in the tissues of rats dosed with phenyl label than the triazole label. The bridge between the phenyl and triazole rings must be susceptible to metabolic cleavage. The higher amount of radioactivity in the fat following phenyl labeling suggest that the phenyl-labeled metabolites must be nonpolar. The lower ratio of the high dose/low dose radioactivity in plasma indicates that phenyl-labeled metabolites may be binding to protein sites and that binding sites are probably becoming saturated at the high dose.

The metabolism of CGA-169374 indicated that there were three major metabolites in the feces. Metabolites A (hydroxy-CGA-205375) and B (hydroxyl-CGA-169374) are hydroxy-substituted metabolites, each have a pair of isomers. The site of hydroxylation is at the outer ring and involves the rearrangement of chloride by the NIH shift mechanism. Metabolite C, CGA-205375, is found only in the high-dose group, which probably indicates that the hydroxylation sites are saturated for conversion to metabolite A. Since metabolite B (Figure 1) was not detected, reduction of this ketone to metabolite B occurs rapidly. The urinary metabolite pattern was more complex than that of the feces because of greater variability of radioactive components in the urine among the groups. CGA-71019, free triazole, identified in the urine of triazole-labeled group and its byproduct, CGA-189138, identified in the liver of the phenyl-labeled group, indicated that there is a cleavage of the alkyl bridge between the ring systems.

Quality assurance statements and statements of compliance with Good Laboratory Practices for the study were signed on August 3, 1990 (Report 1) and March 12, 1991 (Report 2).

E. CONCLUSIONS BASED ON REVIEWERS' DISCUSSION AND INTERPRETATION OF DATA

The study adequately described the distribution and excretion of [^{14}C]CGA-169374 in rats following oral exposure. The data indicate that labeled CGA-169374 is absorbed to an undetermined extent from the gastrointestinal tract and eliminated primarily in the feces for all dosing groups. The authors speculated that biliary excretion appears to be a major excretion route as indicated by the high molecular weights of metabolites A and B. The low levels of radioactivity in tissues, as well as the rapid elimination, at 7 days postexposure, demonstrate that bioaccumulation and retention of CGA-169374 and/or its metabolites are low in rats.

It is noted that based on the data in this study it is not possible to determine what fraction of the oral dose has been absorbed after dosing. Although it is possible to speculate that some fraction of the radioactivity in feces results from absorbed material that has been excreted in bile, it is only possible to affirm that at least 8.5-12.9% of the dose (lowest urinary excretion values) is absorbed; the upper value of absorption (maybe up to 90-100%) is undefined. Additional determination of the extent of biliary excretion is required to estimate this upper level.

The metabolism of CGA-169374 appears to be extensive following oral dosing since metabolites A, B, and C represented most of the recovered radioactivity (at least 60% for most groups). The identification of metabolite A and B isomers was thorough. As the authors concluded, the dose-related occurrence of metabolite C is probably due to saturation of hydroxylation sites. The only urinary metabolite identified was free triazole in the urine of triazole-labeled groups. The method used to extract the radioactive components was appropriate because recovery of radioactivity was approximately 91%, however, only about 68% was identified (i.e., metabolites A, B, C). It is noted that metabolites A-C, identified in feces, may not necessarily reflect the results of

biotransformation in the rat and may reflect the results of the action of the gut flora. Further isolation and identification of other radioactive components in the feces and urine, using a larger amount of samples (i.e., pooled samples), would provide more information on pathways involved in CGA-169374 metabolism.

In summary, the study demonstrates that there are dose-related differences in metabolism and distribution. Saturation of metabolic pathway and, possibly poor absorption, seems to occur at the high dose of 300 mg/kg CGA-169374, as indicated by what might be increased fecal elimination and the appearance of metabolite C in the high-dose feces. There appears to be no sex-related differences in the distribution, metabolism, and elimination of CGA-169374. Two different labeling positions were used probably to ensure that the major metabolites were identified. Although the authors never explicitly mention which of the phenyl groups is being labeled. Figure 1 in MRID 42090028 depicts the inner phenyl ring of CGA-169374 as being uniformly ^{14}C-labeled.

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