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Incoming

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

010588

SEPTEMBER 15, 1993

MEMORANDUM

OFFICE OF PESTICIDES AND TOXIC SUBSTANCES

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

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

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

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

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

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

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

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

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I. BACKGROUND

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

II. AGENCY'S REVIEW OF REGISTRANT'S RESPONSE

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Deficiencies: Lack of purity and stability data.

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

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

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

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

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

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

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

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

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

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

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

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

III. REVIEW OF THE NEW MUTAGENICITY STUDIES

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

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

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

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

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

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

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

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IV. CONCLUSIONS

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

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Reviewed by Steven L. Malish, Ph.D., Toxicologist S.L. Malish 9/14/93
Tox. Branch II, Section IV (H7509C)
Secondary Reviewer: Jess Rowland, M.S., Acting Section Head Jess Rowland 9/14/93
Tox. Branch II, Section IV (H7509C)

DATA EVALUATION REPORT

STUDY TYPE: 84-4 Unscheduled DNA Synthesis in Primary Rat Hepatocytes

MRID Number: 427100-12 DP Barcode: D189836

P.C. Code: 128847 Caswell No. not available

TEST MATERIAL: CGA-169374 tech.

SYNONYMS: Difenoconazole

STUDY NUMBER: 923124

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

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

TITLE OF REPORT: Tests for Other Genotoxic Effects Autoradiographic DNA Repair Test on Rat Hepatocytes

AUTHOR: TH. Hertner

REPORT ISSUED: 10/19/92

SUMMARY:

CGA-169374 tech. (92.2% a.i.) was considered to be negative in the unscheduled DNA synthesis assay in rat primary hepatocytes as measured by an autoradiographic method at concentrations up to 50.0 ug/ml.

CORE CLASSIFICATION: Acceptable; this study satisfies the guideline requirement [84-4] for Category III, Other Genotoxic Effects and is acceptable for regulatory purposes.

I. INTRODUCTION:

The basis of the Unscheduled DNA Synthesis (UDS) Assay uses the incorporation of a DNA precursor, tritiated thymidine into the nuclei of primary hepatocytes previously treated with the test compound. An increase in the tritiated thymidine into the nuclear material of the cells, as determined by an increase in silver grains over the cell nucleus, using autoradiography, indicates that the test compound induces UDS.

II. MATERIALS:

1. Test Material

Test Compound:	CGA-169374 Technical
Synonym:	Difenoconazole
Batch No:	P.807002
Purity:	91.8% to 92.2% a.i.
Description:	Solid
Storage:	Not available
Stability:	Stable

Solvent: DMSO at a final concentration of 1%.

2. Indicator Cells: Primary rat hepatocytes were obtained by the in situ infusion of the livers of male Tif: RAIf (SPF) rats (weighing 170 to 350 gm) obtained from Tierfarm, Sissein, Switzerland.

3. Medium: Williams' Medium E supplemented with 10% fetal bovine serum.

4. Control Materials:

Positive Control: 2-AAF (45 uM/l)

Negative Control: DMSO at a final concentration of 1%.

5. Test Compound Concentrations Used:

Preliminary Toxicity Tests:

Part I (Cytotoxicity}: 0.98. 1.96, 3.91, 7.82, 15.63. 31.25, 62.5, 125, 250, 500 and 1,000 ug/ml.

Part II (DNA synthesis): 0.46, 1.39, 4.17, 12.5, 25 and 50 ug/ml.

Initial UDS Assay: 0.46, 1.39, 4.17, 12.5, 25 and 50 ug/ml.

Repeat UDS Assay: same concentrations as above.

III. METHODS:

A. Study Design:

For the toxicity studies Part I (cytotoxicity) and Part II (DNA synthesis inhibition) and the UDS Assay, fresh rat hepatocytes were added, respectively, to various concentrations of the test article, positive control (hydroxyurea in Part II; 2-AAF in UDS Assay) and the negative (DMSO) control. One (1) hour after initiation of the test substances, ^{3}H-thymidine was added to the medium (Part II and the UDS Assay) and the samples incubated and then analysed for nuclear grains. All concentrations were measured in triplicate.

B. Analysis of Test Article Formulation:

The lowest concentration stock solution of CGA tech. was analyzed to confirm the concentrations and the stability of the test substance in the DMSO vehicle. The values found in the 2 samples analyzed were 104.3% and 86.4% of nominal. No tabular information was supplied.

C. Test Substance Preparation:

CGA tech. was dissolved in DMSO at room temperature. The final concentration of DMSO in the culture medium was 1%.

D. Cell System Preparation:

Liver Perfusion

Primary liver hepatocytes were obtained from normal adult male rats. The animals were dissected and the liver perfused *in situ* through the portal vein with calcium free Hanks' solution (BSS) and then with a 0.05% collagenase solution in BSS. The liver was removed from the animal and placed in a dish containing BSS and antibiotics.

Cell Isolation

After opening Glisson's capsule, the cells were dispensed by gently shaking in the solution, filtered and washed with BSS. Finally the cells were suspended in Williams' medium E and analyzed for viability by trypan-blue exclusion. The viability of the hepatocytes prepared by this method was >80%.

Hepatocyte Plating

Freshly prepared male rat hepatocytes were cultured in Williams' Medium E containing 10% fetal bovine serum, supplemented with antibiotics and incubated in a humidified atmosphere with 5% CO_2 at 37° C. Hepatocytes were used for the Toxicity Tests in Part I and II and the UDS Assay as noted below. For the Part I cytotoxicity

and the UDS Assay, a series of compartments in Multiplates containing gelatinized Thermanox coverslips were seeded with $4x10^5$ cells per compartment. The cells were allowed to attach to the coverslips during an attachment period of 1.5-2 hours. Unattached cells were then removed by washing with BSS and the cultures refed with culture medium.

E. Toxicity Tests:

1. Part I (Cytotoxicity)

A toxicity test was performed to determine the highest concentration of the test material to be used in the UDS Assay.

The medium in each test compartment was supplemented with various concentrations (0.98 to 1000 ug/ml) of the test material and the negative control. The treatment period with the test substances lasted for 16-18 hours.

After treatment, the medium was removed and the cells were washed 2 times with BBS and stained with Trypan Blue (0.2%) for 5 minutes. The cells were then washed and fixed. The percentage of unstained (viable) cells and the morphological quality of the cells were judged.

The highest concentration to be used in the UDS Assay was determined according to the following criteria:

* A sufficiently large number of cells must adhere to the coverslips.

* At least 24% of the cells must show viability upon examination by means of the vital-staining technique.

* A sufficient number of viable cells must be in good condition upon morphological examination.

2. Part II (DNA synthesis Inhibition)

Gelatinized tissue culture flasks were seeded with 3-4 x 10^6 cells. After the attachment period, the cultures were refed with fresh culture medium.

Rat heptocytes were treated with either the test substance, the positive control (10 mM/l hydroxyurea) or the DMSO negative control. Treatment conditions were similar to that described above in Part I (cytotoxicity) study. One (1) hour after addition of the test material, ^{3}H-thymidine was added to each culture.

After washing the cells, nuclei were isolated and washed. Half of the nuclear suspension was used for determination of radioactivity by liquid scintillation counting. In the other half of the

suspension, the DNA content was determined by measuring the fluorescence after staining with Hoechst Dye 33258. Relative DNA synthesis (in the critical range of concentrations tested in the UDS Assay) was calculated by dividing the ^{3}H dpm values by the corresponding DNA content.

F. UDS Assay (initial and repeat):

Fresh rat hepatocytes were added, respectively, to six (6) concentrations of the test article (0.46, 1.39, 4.17, 12.5, 25 and 50 ug/ml), positive control (2-AAF, 45 uM/l) and negative (DMSO) control. One (1) hour after addition of the test substances, ^{3}H-thymidine was added and the samples incubated for 16-18 hours.

Cell Fixation

At the end of the treatment period, the cells were washed 2 times with BSS. The nuclei were swollen by treatment with 1% sodium citrate and the cells fixed with ethanol:acetic acid. The coverslips were mounted on microscope slides and prepared for autoradiography.

Preparation of Autoradiograph/Grain Development:

The slides were coated with autoradiographic emulsion, Ilford K.5, stored in the dark in a refrigerator for 3 days, developed and fixed. The slides were then stained with hematoxylin and eosin.

Grain Counting

Slides were read blind using an automated colony counter attached to a light microscope. The grains in the nucleus of 3 slides (50 cells/slide) were counted in random areas of each dose group and from the positive and negative controls. The incorporation of radioactivity into the cytoplasm was counted in 3 cytoplasmic regions adjacent to the nucleus having an area equivalent to that of the nucleus.

The net values were calculated by subtracting the average grain count over the cytoplasm from the total grain count over the nuclei.

To make sure that not only effects on the majority of the cells within the treated hepatocyte population were recognized but also effects on particularly sensitive subpopulations, the percentage of nuclei which were in repair were calculated for each slide/group. Nuclei in repair were defined as those nuclei with grain counts exceeding the 90% percentile value of the grain count of the vehicle control. These percentages were calculated for both the mean gross counts and mean net counts of the negative control and the groups treated with CGA Tech.

Cells which were in the DNA synthesis phase showing >120 grains/nucleus were excluded from the determination. The background in the autoradiographies was determined in cell-free areas.

G. Assay Criteria:

1. Criteria for Positive Response in the UDS Assay:

* The mean gross number and mean net number of silver grains per nucleus in relation to the respective vehicle controls show a statistically significant difference at any concentration and the mean net value was at least 2.0.

* The percentage of nuclei in repair, with respect to the gross and net numbers of silver grains, show a statistically significant difference at any concentration as compared to the respective negative controls.

2. Criteria for a Negative Response in the UDS Assay:

* The mean gross number and the mean net number of silver grains per nucleus as well as the percentage of nuclei in repair, with respect to their gross and the net numbers of silver grains, in relation to the vehicle control were not statistically different at any concentrations and no concentration relationship was seen.

3. Assay Acceptance Criteria:

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

* The viability of the heptocytes collected from the perfusion process should exceed 70%.

* The gross labelling in the vehicle cultures should not exceed an average of eight total grains/nucleus.

* The net values in the vehicle control cultures should not exceed an average of 2 grains per nucleus.

* The positive control should result in a marked positive response.

* Grain count data for a given treatment must be obtained from at least 2 replicate cultures and at least 50 cells per culture.

* A minimum of 4 concentrations of the test substance and negative and positive controls should be analyzed for

nuclear grain counts.

* The highest analyzed concentration used in the assay should show signs of toxicity, as defined in the toxicity test, show test material insolubility or have a maximum concentration of at least 5 mg/ml.

H. Statistical Analysis:

The evaluated grain counts were subjected to a statistical analysis by comparing each treatment group to the respective vehicle control group by Dunnett's one-tailed t test. This test was applied to the mean gross values, the mean net values and after suitable transformation to the percentages of nuclei in repair, with respect to their gross as well as to their net values. The analyses were performed with the ANOVA Procedure (SAS software) using the Dunnettu option in the Means statement. All comparisons were performed on a level of significance (alpha) of 0.01.

I. Regulatory Compliance:

A quality assurance statement and statement of compliance with the Good Laboratory Practice standards were signed and dated.

A statement of No Confidentiality Claims was signed and dated.

III. RESULTS:

A. Preliminary Toxicity Studies:

In the toxicity study Part I (cytotoxicity), concentrations of 62.5 ug/ml and higher provided toxic as evidenced by no viable cells on the slide. The 31.35 ug/ml concentration was the highest concentration yielding a sufficient number of adhered viable cells (31%) which were of suitable quality for scoring. The lower concentrations had a viability between 47 and 83% while the negative control showed a viability of 80%. Based on these results, 50 ug/ml was selected as the highest concentration used in the UDS Assay. Precipitation of the CGA tech. in the culture medium was observed at concentrations greater than 15.63 ug/ml.

In the toxicity study Part II (DNA synthesis inhibition), 50 ug/ml presented a nearly complete inhibition (93.3%) of DNA synthesis versus the negative control. At all other concentrations, DNA synthesis was between 36% and 53% of the negative control. Precipitation in the culture medium was observed at concentrations >25.0 ug/ml.

B. UDS Assays:

1. Gross Nuclear Grain Counts:

In the initial UDS Assay with CGA tech. at the 50 ug/ml, cytotoxicity was seen as evident by the fact that only 45/150 cells could be scored for nuclear grains. At 25 ug/ml a high number of dead cells indicating cytotoxicity was seen.

Concentrations of 0.046, 1.39, 4.17, 12.5, 25 and 50 ug/ml resulted in mean gross nuclear grain counts, respectively, of 2.45, 2.49, 2.62, 2.76, 2.44 and 1.96 mean grains/nucleus versus the negative control value of 2.05. No statistical significance was seen. The percentage of nuclei in repair, however, at 12.5 ug/ml revealed statistical significance ($p<0.01$) in comparison to the negative control. Positive controls had a mean net nuclear grain count/nucleus of 11.47 (Table 1).

In the repeat of the UDS Assay, treatment with CGA technical at concentrations of 0.046, 1.39, 4.17, 12.5, 25 and 50 ug/ml resulted in mean gross nuclear grain counts, respectively, of 2.71, 2.53, 2.91, 2.49, 2.23 and 1.95 versus the negative control value of 1.92. Statistical significance ($p<0.01$) was seen at 4.17 ug/ml. Positive controls had a mean net nuclear grain count/nucleus of 12.48 (Table 1).

Table 1

Summary of Gross Nuclear Grain Counts[1]

Treatment	Conc.	Assay	
		Initial	Repeat
		Gross Grains/Nucleus Mean±S.D.	
Negative Control DMSO	1%	2.05±1.15	1.92±1.27
Positive Control 2-AAF	45 uM/l	11.47±3.36	12.49±3.69
CGA tech.	50 ug/ml	1.96±1.02	1.95±1.20
CGA tech.	25 ug/ml	2.44±1.32	2.23±1.18
CGA tech.	12.5 ug/ml	2.76±1.41*	2.49±1.32
CGA tech.	4.17 ug/ml	2.62±1.44	2.91±1.55**
CGA tech.	1.39 ug/ml	2.49±1.27	2.53±1.43
CGA tech.	0.46 ug/ml	2.45±1.35	2.71±1.64

[1]Adapted from report, p. 34 and 36.
* The absolute value presented was not statistically significant but the percentage increase compared to the control showed a statistical significant ($p<0.01$) increase.
** $p<0.01$

2. Net Nuclear Grain Counts:

In the initial UDS Assay, treatment with CGA tech. at concentrations of 0.046, 1.39, 4.17, 12.5, 25 and 50 ug/ml resulted in mean net nuclear grain counts, respectively, of 0.69, 1.07, 0.94, 1.21, 1.01 and 0.84 versus the negative control value of 0.69. Statistical significance ($p<0.01$) occurred at 12.5 ug/ml. Positive controls had a net nuclear grain count/nucleus of 9.17 (Table 2).

In the repeat of the UDS Assay, treatment with CGA tech. at concentrations of 0.046, 1.39, 4.17, 12.5, 25 and 50 ug/ml resulted in mean net nuclear grain counts, respectively, of 0.92, 0.63, 1.08, 1.17, 0.65 and 0.70 versus the control value of 0.40. Statistical significance ($p<0.01$) occurred at 4.17 and 12.5 ug/ml. The positive control had a mean net nuclear grain count of 9.26 (Table 2).

Table 2

Summary of Net Nuclear Grain Counts[1]

Treatment	Conc.	Assay	
		Initial	Repeat
		Net Grains/Nucleus Mean±S.D.	
Negative Control DMSO	1%	0.69±1.27	0.40±1.46
Positive Control 2-AAF	45 uM/l	9.17±3.36	9.26±4.08
CGA tech.	50 ug/ml	0.84±1.35	0.70±0.81
CGA tech.	25 ug/ml	1.01±1.49	0.65±1.32
CGA tech.	12.5 ug/ml	1.21±1.73**	1.17±1.47**
CGA tech.	4.17 ug/ml	0.94±1.67	1.08±1.75**
CGA tech.	1.39 ug/ml	1.07±1.45	0.63±1.65
CGA tech.	0.46 ug/ml	0.69±1.58	0.92±1.68

[1]Adapted from report, p. 35 and 37.
** $p<0.01$

IV. DISCUSSION:

In the preliminary toxicity study (Part I), concentrations of 62.5 ug/ml and higher provided toxic as evidenced by a lack of viable cells on the slide. Moreover, a nearly complete inhibition of DNA synthesis inhibition (Part II) was measured at this concentration but not at the lower concentra+ ons. In the UDS Assay, the 50 ug/ml concentration exerted some toxicity toxicity as evidenced by only a few cells that could be scored.

No tabular analytical data was included in the report. The lack of this data would not compromise this study because toxicity was seen at the higher dosage range in the preliminary toxicity studies and the UDS Assay.

Using the criteria for a valid test and the evaluation criteria for test performance, the test article was found to be negative in the UDS Assay at dose levels of from 0.46 to 50 ug/ml. Toxicity was seen at concentrations of 50 ug/ml and higher.

V. CONCLUSIONS:

CGA-169374 tech. (92.2% a.i.) was considered to be negative in the unscheduled DNA synthesis Assay in rat primary hepatocytes as measured by an autoradiographic method at concentrations of up to 50 ug/ml.

VI. CORE CLASSIFICATION: Acceptable; this study satisfies the guideline requirement [84-4] for Category III, Other Genotoxic Effects and is acceptable for regulatory purposes.

010588

FINAL

DATA EVALUATION REPORT

CGA 169374

Study Type: Metabolism

Prepared for:

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

Prepared by:

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

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

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

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

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

GUIDELINE SERIES 85-1: Metabolism

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

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

DATA EVALUATION REPORT

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

EPA IDENTIFICATION NUMBERS

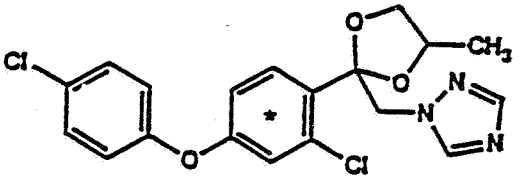
Tox. Chem. Number:

P.C. Code:

MRID Numbers: 427100-14; 427100-13

TEST MATERIAL: CGA 169374

CHEMICAL STRUCTURE:



*[^{14}C] labelling position

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

SPONSOR: Ciba-Geigy Corporation, Greensboro, NC

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

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

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

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

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

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

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

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

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

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

A. MATERIALS (Report 1)

Unlabeled Test Compound

Lot Number: 255/02
Purity: Not reported
Contaminants: Not reported

Radiolabeled Test Compound

Contaminants: None reported
Description: Not reported
Radiochemical purity: >97%
Stability: At least 1 week at 4°C
Specific activity: 30.8 μCi/mg
Batch number: CL-XVI-89

Test Animals

Species: Rat
Strain: Sprague-Dawley
Source: Charles River Japan, Inc.
Age: 7-8 Weeks
Weight: Males--209-256 g; Females--168-235 g

For materials used in MRID Study No. 427100-13, see Appendix I.

B. STUDY DESIGN

Animal husbandry (Report 1): Animals were acclimated for more than a week. Food (MF, Oriental Yeast Co., Ltd) and water were available ad libitum throughout the study except overnight prior to dosing until 4 hours postdosing. The temperature was maintained at 23°C ± 2°C and humidity was kept at 55% ± 10%. The study author did not indicate what type of light/dark cycle was maintained.

Group arrangement: In Report 1, the following groups were used.

Studies	Dose (mg/kg)	No. of Animals Males	Females
Single low-dose with Hi Sil 233	0.5	3	3
Single high-dose with Hi Sil 233	300.0	3	3
Single high-dose without Hi Sil 233	300.0	3	3

In Report 2, three females received a single dose of 300 mg/kg of [phenyl-^{14}C] CGA 169374.

Dose Rationale: Doses were based on results of previously conducted studies (MRID Nos. 420900-28/29/30/31) and a preliminary experiment (one male rat/group) to determine sampling times for blood concentrations of the test compound.

Dosing Suspensions: In Report 1, doses were administered via gavage at a volume of 5 mL. The low-dose suspension was prepared by first removing the toluene under nitrogen gas, then dissolving the test material in a solution containing 1.0 g Hi Sil 233 and 250 mL ethanol. This solution was then mixed with 1% carboxymethyl cellulose (CMA).

The high-dose suspension containing Hi Sil 233 was prepared by dissolving unlabeled test material in acetone then adding labeled test material and Hi Sil 233. The acetone was then removed under nitrogen gas. This solution was then mixed with 1% CMA.

The high-dose suspension not containing Hi Sil 233 was prepared by first dissolving both labeled and unlabeled test material in toluene and then removing the toluene under nitrogen gas. Petroleum ether was added and then removed with nitrogen until tne mixture was crystallized. The crystal was then mixed with 1% CMA and crushed.

For dose preparation in Report 2, see Appendix I.

Sample Collection and Preparation: In Report 1, blood samples were collected at a volume of 100 µL at 30 minutes and 2, 4, 6, 8, 12, 24, 36, 48, 60, 72, 96, 120, 144, and 168 hours after administration of the test material. Bile samples were collected at 1, 2, 4, 6, 8, 24, and 48 hours; urine samples were collected at 4, 8, 24, and 48 hours; and fecal samples were collected at 24 and 48 hours after administration of the test material. At 48 hours, animals were sacrificed and the GI contents were collected. For sample collection in Report 2, see Appendix I.

Blood: Samples were dissolved in 2 mL of tissue solubilizer (SOLUENE-350, Packard) and decolorized with 0.4 mL of benzene saturated with benzoyl peroxide. Concentration of radioactivity was determined after the addition of 13 mL of scintillator (ECONOFLUOR, Du Pont NEN Research Products) to each sample.

Bile: Samples were diluted with water to 100 mL. Radioactivity was determined by liquid scintillation counting (LSC) (ATOMLIGHT, Du Pont NEN Research Products).

Urine: Samples were prepared in the same manner as bile samples.

Feces: Samples were homogenized in 300 mL of 50% methanol. Radioactivity was determined for 1 mL of each sample of the homogenate by LSC.

Gastrointestinal contents: Samples from males only were prepared in the same manner as fecal samples.

Carcass: Samples were solubilized by heat in 0.5 M sodium hydroxide containing toluene in a ratio of 10:1. Following homogenization in 900 mL of water, radioactivity was counted.

Whole body autoradiography: Samples (2, 24, and 168 hours postdosing) of 0.5 mL aliquots were prepared from one male given an oral dose of 0.5 mg/kg. Following hair removal and filling of the nasal and anal cavities with 5% CMA, the carcass was frozen in a dry ice-acetone mixture at -70°C. Tail and limbs were surgically removed and the carcass was then embedded in 5% CMA and cut at 35 μm at -25°C. The slices were covered with a protective film (4 μm, Dia Foil) and contacted with X-ray films (MARG $_3$H type, Konica). Films were exposed at 4°C for 35 days and consequently developed.

Entero-hepatic circulation: Samples from males only were prepared by cannulating the bile ducts and injecting bile (collected from previously dosed males) into the duodenum at a dose of 24.5μg/ 28.0 kBq/8 mL bile/kg. Samples of urine, feces, and bile from the cannulated animals were prepared as described and taken at the same times as previously stated.

Tissues: Animals were sacrificed by bleeding from the abdominal aorta at 2, 24, and 168 hours after administration of 0.5 mg/kg and 4, 48, and 168 hours after administration of 300 mg/kg. Tissue samples were weighed, solubilized with 2 mL of tissue solubilizer (SOLUENE-350), and if necessary, decolorized with 0.4 mL of benzene saturated with benzoyl peroxide. Radioactivity was determined by LSC. Bone samples were combusted with an automatic sample oxidizer (ASC-113, Aloka) and radioactivity was then determined.

Metabolic Analysis: In Report 2, metabolite analyses were conducted on urine samples of females dosed with 300 mg/kg [^{14}C]-phenyl] CGA 169374 (high dose). Metabolites were isolated by high performance liquid chromatography (HPLC) and thin-layer chromatography (TLC) and then identified by using mass spectrometry (MS). (For studies regarding fecal metabolites, see Appendix I.)

Materials and methods were the same as those stated in Appendix I with the following exceptions: (1) Day-1 urine samples from three high-dose females were pooled and filtered through an acrodisc nylon filter to remove particulates; (2) HPLC determinations were conducted with Perkin-Elmer Model 410 solvent delivery system, Perkin-Elmer LC 95 UV detector at 281 nm, and IN/US β-RAM Model 1A LC Radiodetector Foxy Model 200 fraction collector; and (3) 20x20 cm precoated 250 micron silica gel F-254 plates (Merck) were used for TLC determinations.

Compliance:

- Signed Statements of No Data Confidentiality Claims, dated February 18, 1993 (Report 1) and March 8, 1993 (Report 2) were provided.

- Signed Statements of Compliance with EPA GLPs were not provided. Report 2 was considered to be a part of the original study and therefore, covered by its GLP Statement. Report 1 was not conducted in compliance with EPA GLPs. However, a statement, signed and dated February 22, 1993, was submitted stating that "...the study in this volume has been conducted in accordance with good and acceptable scientific practices." It was conducted according to the Japanese MAFF GLPs.

- A signed Statement of Quality Assurance, dated February 25, 1993, was only provided for Report 2.

C. RESULTS

Elimination and Recovery (Report 1)

Mean total recovery of radioactivity (urine, feces, bile, GI content, and carcass) after 48 hours in 0.5- and 300- mg/kg males, was 97.3% and 96.5%, respectively, and in females was 97.3% and 95.4%, respectively (Table 1). Mean recovery of radioactivity after 48 hours in males at 0.5 and 300 mg/kg were in urine 13.9% and 1.0%, respectively; in feces, 3.9% and 17.1%, respectively; in bile, 73.3% and 55.6%, respectively; in the GI content, 1.9% and 20.0%, respectively; and in the remaining carcass, 4.3% and 2.8%, respectively. In females, recoveries at the same doses 48 hours postdosing were 8.9% and 1.2% in urine, respectively; 1.8% and 22.0% in feces, respectively; 76.4% and 38.6% in bile, respectively; 7.4% and 31.8% for the GI content, respectively; and 2.8% and 1.8% in the remaining carcass, respectively.

At the low dose, slight (but probably not biologically relevant) sex-related differences were observed in the rate of excretion from the bile (Table 1). By 24 hours postdosing, females had excreted 72.5% of the radioactivity as compared to 59.8% in males. By 48 hours, cumulative excretion in the bile was similar in both sexes. At the high dose, a slight sex-related difference was observed only at 48 hours with males excreting 17% more radioactivity via the bile than females and both sexes excreting 20% to 30% less compared to the low dose. Dose-dependent differences were also noted in the excretion of radioactivity via the GI content; 18% and 25% more radioactivity was excreted in males and females, respectively, at 300 mg/kg when compared to the low dose (Table 1).

In bile-cannulated males following an intraduodenal injection, percent radioactivity recovered via the bile was 3.3%, 24.0%, 58.2%, 75.9%, and 79.6% at 2, 4, 8, 24, and 48 hours, respectively. After 48 hours,

urinary and fecal excretions were 4.1% and 13.7%, respectively. The GI content and carcass did not contain radioactivity.

Estimation of eliminated radioactivity via expired air was not conducted in this study.

Tissue Distribution (Report 1)

Maximum concentrations of radioactivity in tissues were observed after 2 hours for the low-dose group and 4 hours for the high-dose group (Table 2). In the low dose group at 2 hours postdosing, the major accumulation was found in the liver; 14.96% of the recovered radioactivity was detected in males, while 8.29% was detected in females. Skin, blood, skeletal muscle, kidney, and fat constituted ≈1%-6% of recovered radioactivity, while the remaining tissues accounted for <1%.

In the high-dose group at 4 hours postdosing, the major accumulation was found in skeletal muscle and skin; 4.81% and 4.94% of the recovered radioactivity was found in skeletal muscle of males and females, respectively, while 3.77% and 8.38% was found in skin of males and females, respectively. Liver and fat constituted 2%-7% of recovered radioactivity, while the remaining tissues accounted for <1%.

Whole body autoradiography (at 0.5 mg/kg in males only) at 2 hours postdosing, demonstrated the highest content of radioactivity in the GI content, intestinal content, and bile in bile ducts, followed by liver, kidney, and adrenals. At 24 hours, the highest content was found in intestinal content, bile in bile ducts, urine in the bladder, and gastric content, followed by kidney and liver. At 168 hours, no radioactivity was detected in any tissue.

Pharmacokinetics (Report 1)

Slight sex-related differences were noted regarding Tmax, Cmax, and the rate of elimination of radioactivity at both dose levels. Blood concentrations peaked earlier in females than in males at 0.5 mg/kg, and the concentrations in females were 52% and 63% of the concentrations in males at 0.5 and 300 mg/kg, respectively. In addition, the rate of elimination was faster in females than males during the first 12 hours then became slightly slower in females than males from 24 hours to 168 hours at 0.5 mg/kg. No sex-related differences for elimination rate were noted at 300 mg/kg. Detailed results are presented in the text below and in Table 3.

In low-dose animals, the highest radioactive concentration in blood was found after 2 hours in males (0.327 ppm equiv.) and after 30 minutes in females (0.169 ppm equiv.; Table 3). Mean half-lives in males and females from Tmax to 12 hours, were 6.2 and 4.4 hours, respectively; from 24 hours to 168 hours, they were 2.3 and 3.7 days, respectively.

In high-dose animals, the highest radioactive concentration in blood was found after 4 hours in both males and females (47.89 and 30.02 ppm

equiv., respectively; Table 3). Mean half-lives in males and females from 24 hours to 72 hours, were 22 and 24 hours, respectively; from 96 hours to 168 hours, they were 3.8 and 3.4 days, respectively. No major differences were noted in results between high doses with Hi Sil 233 and those without Hi Sil 233.

Metabolism (Report 2)

Isolation and identification of major metabolites from day-1 urines of 300-mg/kg females showed three peaks, 1, 2, and 3, accounting for 6%, 3.9%, and 2.0%, respectively, of the administered dose. These peaks were identified as free CGA 205375 and HO-CGA 205375, sulfate conjugates of these compounds, and hydroxy acetic acid. The proposed metabolic pathway of CGA-169374 is presented in Figure 1. Detailed results are reported below.

Peak 1 was further isolated into components 1AS, 1A2, 1BS, and 1B2. The data indicated that 1AS was a sulfate conjugate of HO-CGA-205375 and accounted for 2% of the administered dose. 1A2 (1.6% of the dose) was further isolated into 1A2-1 (0.5% of the dose) and 1A2-2 (1.0% of the dose), which were proposed to be sulfate conjugates isomeric with 1AS. 1BS and 1B2 were proposed to be isomers of HO-CGA 205375 and accounted for 1.7% of the administered dose.

Peak 2 was further isolated into components 2A (3.1% of the dose) and 2B (0.3% of the dose). 2A was further isolated into CGA 205375 (0.2% of the dose) and sulfate conjugate of CGA 205375 (2.8% of the dose). 2B was not identified.

Peak 3 was further isolated into components 3A (0.2% of the dose) and 3B (1.8% of the dose). 3A was not identified. The data indicated that 3B was the hydroxyacetic acid metabolite of HO-CGA 205375.

D. REVIEWERS' DISCUSSION/CONCLUSIONS

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

suggests that saturation is reached at the higher dose level resulting in an increase of unabsorbed test material.

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

Following the administration of 300 mg/kg [^{14}C-phenyl] CGA 169374, 3 major urinary metabolites were isolated and further identified as sulfate conjugates (and their isomers) of HO-CGA 205375, isomers of HO-CGA 205375, and the hydroxyacetic metabolite of HO-CGA 205375. With one exception (hydroxyacetic acid), these metabolites have either been previously identified or are conjugates of known metabolites. The major urinary metabolites of CGA 169374 have been identified and no single unknown metabolite accounted for >1.1% of the dose.

Based on the above results, these studies have adequately addressed the EPA comments made on previously submitted metabolism studies (MRID Nos. 420900-28/29/30/31).

RIN 1360-95

TOX REVIEW 10588 DIFENOCONAZOLE

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____ 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.

APPENDIX I

SOURCE: DER 1-79, MRID Nos. 420900-28/29/30/31

FINAL

DATA EVALUATION REPORT

CGA-169374

Study Type: Metabolism

Prepared for:

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

Prepared by:

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

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

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

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

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

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

Signature: Karen E. Whitby, Ph.D.
Date: 9/16/93

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

Signature: _____
Date: _____

DATA EVALUATION REPORT

STUDY TYPE: Metabolism

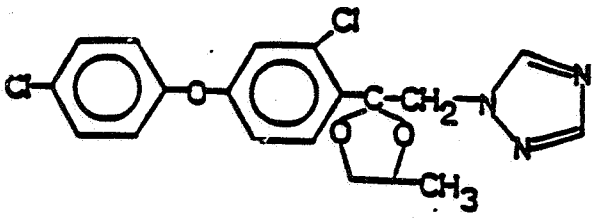
EPA IDENTIFICATION NUMBER:

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]

#10588

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

010588

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.

RIN 1360-95

TOX REVIEW 10588 DIFENOCONAZOLE

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Reviewed by Steven L. Malish, Ph.D., Toxicologist S.L. Malish 9/14/93
Tox. Branch II, Section IV (H7509C)
Secondary Reviewer: Jess Rowland, M.S., Acting Section Head
Tox. Branch II, Section IV (H7509C) 9/14/93

DATA EVALUATION REPORT 010588

STUDY TYPE: 84-2(b) In Vivo Mammalian Bone Marrow Cytogenetic Test: Chromosomal Analysis

MRID Number: 427100-11 DP Barcode: D189836

P.C. Code: 128847 Caswell No. not available

TEST MATERIAL: CGA-169374 Technical

SYNONYMS: Difenoconazole

STUDY NUMBER: 911041

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

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

TITLE OF REPORT: Structural Chromosomal Aberration Test Micronucleus Test, Mouse

AUTHOR: Dr. B. Ogorek

REPORT ISSUED: June 24, 1992

SUMMARY:

No increase in micronucleated polychromatic erythrocytes occurred with CGA-169374 (91.2% a.i.), Technical administered up to 1,600 mg/kg to mice.

CORE CLASSIFICATION: Acceptable; this study satisfies the guideline requirement [84-2] for Category II, Structural Chromosomal Aberrations and is acceptable for regulatory purposes.

I. INTRODUCTION:

This study assess the potential of the compound to induce both chromosomal breaking substances (clastogens) and aneuploidy inducing substance (aneugens). These effects are manifested by the formation of micronuclei in bone marrow polychromatic erythrocytes in vivo.

II. MATERIALS and METHODS:

A. Test Substance:

Test Compound:	CGA-169374, Technical
Chemical:	N/A
Batch No:	P.807002
Purity:	91.8% a.i.
Description:	Solid
Storage:	Not available
Stability:	Stable

Solvent: Arachis Oil (10 ml/kg)

B. Controls:

1. Negative Control: Arachis Oil (10 ml/kg)

2. Positive Control: Cyclophosphamide (CPA) [10 ml/kg]

Solvent: NA

C. Test Animals:

Species:	Mice
Strain:	Tif: MAGf (SPF)
Sex:	Male and Female
Age:	Not available
Weight:	females 23-36 gm; males 25-40 gm at initiation
Source:	Ciba-Geigy Animal Farm, Sissein, Switzerland
Acclimatized:	≥1 days
Caging:	Groups of 2/cage
Feed:	NAFAG No. 890 ad libitum
Water:	tap water ad libitum

D. Study Design:

1. Range Finding Test

The range-finding test was performed in 2 phases. The first phase was consisted of 1 animal/sex with each receiving a dose of either 2,000, 500 and 125 mg/kg. In the second phase, 1 animal/sex received a dose of 2,000 mg/kg. The animals were observed for 3 days (length of the micronucleus test plus 1 day).

2. Micronucleus Study

a. Part 1

One hundred and twelve (112) animals were divided into 7 groups of 8 animals/sex and dosed by oral gavage at 10 ml/kg. Three (3) test groups received 1,600 mg/kg of the CGA technical. The 3 groups in the negative contr group received a single dose of arachis oil while the positive control received 64 mg/kg of cyclophosphamide (CPA). Animals in the test group and the corresponding negative control group were terminated either at 16, 24 or 48 hours after dosing. The CPA positive control was terminated at 24 hours after dosing (Table 1).

b. Part 2

Eighty (80) animals divided into 5 groups of 8 animals/sex. Dosing was by oral gavage at 10 ml/kg. Four (4) groups received either 0, 400, 800 or 1,600 mg/kg of the CGA technical. The negative control received a arachis oil while the positive control received 64 mg/kg of CPA. All animals were terminated at 24 hours after dosing (Table 1).

Table 1

Experimental Design

Treatment	Sac.Time	Treated Males	Treated Females
Part 1			
CGA tech. 1600 mg/kg	16	8	8
CGA tech. 1600 mg/kg	24	8	8
CGA tech. 1600 mg/kg	48	8	8
Negative Control (10 ml/kg)	16	8	8
Negative Control (10 ml/kg)	24	8	8
Negative Control (10 ml/kg)	48	8	8
CPA Positive Control (64 mg/kg)	24	8	8
Part 2			
CGA tech. 400 mg/kg	24	8	8
CGA tech. 800 mg/kg	24	8	8
CGA tech. 1600 mg/kg	24	8	8
Negative Control (10ml/kg)	24	8	8
CPA Positive Control (64 mg/kg)	24	8	8

2. Dosing

Arachis Oil was found to be the best suited vehicle, yielding a 2,000 mg/kg suspension of the test article, CGA, Tech. All test substances, CGA, Tech. and the negative and positive controls were administered at a volume of 10 ml/kg by oral gavage.

3. Dosing Solution Analysis:

An assay was developed to analyze the test material in arachis oil. The reader is referred to the original report for details of the assay procedure. Two (2) samples of the 40 mg/ml and 60 mg/ml dosing solutions were analyzed. The results indicate that the solutions were within -15% to 21% of the nominal concentrations (Table 2).

Table 2

Concentration Analysis[1]

Nominal Concentration (mg/ml)	Determined Concentration (mg/ml)	% of Nominal Concentration
40[2]	49.9/46.6	124.8/116.5
160[3]	130.7/144.5	81.7/90.3

[1]Adapted from original report, p. 38.
[2]Solution used for the 400 mg/kg dose level.
[3]Solution used for the 1,600 mg/kg dose level.

4. In Vivo Observations

All animals were observed for morbidity and mortality and pharmacological and toxicological effects.

5. Body Weight

Body weights of each animal were recorded prior to test substance administration to allow adjustments of the dosing volume.

6. Necropsy

Animals were killed at the appropriate intervals by cervical dislocation.

7. Preparation of Bone Marrow and Slides

The shafts of both femurs were removed and the marrow harvested with fetal calf serum and prepared on slides. The slides were stained with May-Grunwald/Giemsa, mounted and then scanned under a light microscope for micronuclei.

8. Criteria for Scoring Micronuclei

Micronuclei are uniform, darkly stained, more or less round bodies in the cytoplasm of erythrocytes. Inclusions which are reflective, improperly shaped or stained or which are not in the focal plain of the cell were judged to be artifacts and not scored as micronuclei. Cells containing more than 1 micronuclei were only counted once.

Prior to analysis, the slides were coded. The slides of 5 animals/sex/dose showing good differentiation between mature and polychromatic erythrocytes (PCEs) were scored. From each animal, the ratio of polychromatic to normochromatic erythrocytes was determined and 5000 polychromatic erythrocytes were scored for micronuclei.

9. Assay Evaluation Criteria

The results of the experiment was evaluated with respect to the mean number PCEs with micronuclei. The groups were compared by treatment, sampling time and sex of the animals.

10. Criteria for Negative Effect:

The test substance was considered to be negative in the test system if there was no statistically significant differences between the mean number of micronucleated PCEs in the groups treated with the test substance and that of the respective negative control and if the mean number of micronucleated PCEs did not exceed the range accepted for the negative control (<0.20%).

11. Assay Acceptance Criteria

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

* The high dose level should be the maximum tolerated dose causing no deaths in a group of 4 animals in the range - finding test

* The quality of the slides must show a clear differentiation between PCEs and Normochromatic erythrocytes (NCEs).

* At least 5 animals/sex/dose per dose and the control group should be evaluated.

* The positive control should fulfil the criteria for an active substance.

12. Statistics

Differences were assessed by the Chi-Squared test ($p<0.05$). If there was no significant differences between animals of either sex, the data from males and females were pooled.

13. Regulatory Compliance

A quality assurance statement and a statement of compliance with Good Laboratory Practice Standards were signed and dated.

A statement of No Data Confidentiality Claims was signed and dated.

III. RESULTS:

A. Range-Finding:

In part 1, all animals at all dosage levels (2,000, 500 and 125 mg/kg) survived the test material. When the test was repeated at 2,000 ppm (part 2), 1/2 animals survived (Table 3). The authors choose 1,600 mg/kg as the highest tolerated dose to be used in the Micronucleus Study.

Table 3

Results of Range-Finding Study

Dosage (mg/kg)	No. of Animals	Mortality (Dead/Total)
Part 1		
2000	2	0/2
500	2	0/2
125	2	0/2
Part 2		
2000	2	1/2

B. Micronucleus Study:

1. Morality:

No mortality was seen throughout the study.

2. Observations:

In both the range-finding and main study, clinical signs of toxicity at 1,600 mg/kg included poor general condition, laterocumbency, ataxia and piloerection. Similar but less pronounced effects were seen at the 800 and 400 mg/kg dosage levels in the main study.

3. Cytogenetic Analysis:

Part 1

In mice treated with CGA technical at 1,600 mg/kg, bone marrow cells showed no significant increase in the incidence of chromosomal aberrations in at any time period versus the vehicle control at 16, 24 and 48 hours. The mean percentage of micronucleated PCEs was 0.04% (16 hr), 0.04% (24 hr) and 0.02% (48 hr) versus 0.02% in the control (Table 2 Appended).

In mice treated with the positive control CPA, a statistically significant ($P<0.05$) increase of 1.34% occurred in the incidence of micronucleated PCEs versus the controls [0.02%](Table 2 Appended).

Part 2

In mice treated with CGA technical at 400, 800 and 1,600 mg/kg, bone marrow cells showed no significant increase in the incidence of micronucleated PCEs at the 24 hour time period versus the vehicle control. The mean percentage of micronucleated PCEs was 0.01% (400 mg/kg), 0.01% (800 mg/kg) and 0.02% (1,600 mg/kg) versus 0.01% in the negative control (Table 7 Appended).

In mice treated with the positve control CPA, a statistically significant ($p<0.05$) increase in micronucleated PCEs of 1.11% occurred in the incidence of micronucleated PCEs versus the controls [0.01%] (Table 7 Appended) .

IV. DISCUSSION:

The study meets the Assay Acceptance Criteria and the Criteria for a Negative Effect. All results from CGA tech. treated animals were within the latest historical control range (1990) of from 0.00% to 0.12% micronucleated PCEs while the results from the positive CPA control animals ranged from 0.22% to 1.98% micronucleated PCEs.

V. CONCLUSION:

No increase in micronucleated polychromatic erythrocytes occurred with CGA-169374 technical (91.2%) administered up to 1,600 mg/kg to mice.

VI. CORE CLASSIFICATION:

Acceptable; this study satisfies the guideline requirement [84-2] for Category II, Structural Chromosomal Aberrations and is acceptable for regulatory purposes.

RIN 1360-95

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