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

005841

MEMORANDUM

APR 15 1987

OFFICE OF PESTICIDES AND TOXIC SUBSTANC

SUBJECT: Triazole alanine: 4F3074; Action Code 232; Accession #265203-265209; Record #182804; Caswell #862B

TO: Lois Rossi, PM-21
Registration Division (TS-767C)

FROM: Alan C. Katz
Toxicologist, Review Section 3
Toxicology Branch/HED (TS-769C)
Alan C Katz 4/7/87

THROUGH: Marcia vanGemert, Ph.D.
Head, Review Section 3
Toxicology Branch (TS-769C)
M van Gemert 4/13/87
4/15/87

Action Requested:

Review toxicity data on triazole alanine (a major plant metabolite of TILT and other triazole fungicides).

Discussion/Conclusions:

A. "THS 2212 (Triazolylalanine) Subchronic Toxicity to Dogs on Oral Administration (13-Week Feeding Study)" Bayer Report No. 12562. April 28, 1986 (revised). Authors: E. von Keutz and P. Groning. Bayer A.G. Institute of Toxicology, Wuppertal-Elberfeld.

A Data Evaluation Report on this study was previously issued by this reviewer (Tox Branch Document No. 004469, 6/13/85). The study was classified "Core-Supplementary," pending submission of additional data. The additional data requested included: individual and summary tables for observations of clinical signs, as well as individual ophthalmologic observations; histopathologic evaluations for all animals, including certain tissues (i.e., liver, kidneys and all tissues showing possible toxicity based on findings in high dose animals) from the low and mid dose groups; and clarification with respect to whether all gross necropsy findings were included in the report.

The revised study report contained all additional requested data, including quality assurance statements and historical control data for the occurrence of pituitary and thyroid cysts.

No treatment-related changes were apparent with respect to clinical signs or ophthalmoscopic observations. The study pathologists confirmed that all gross necropsy findings were included in the study report; no treatment-related alterations were observed.

Histopathologic evaluation revealed the presence of thyroid and pituitary cysts as shown in Table 1. Historical control data for the incidence of these cysts in

15 studies in dogs are presented in Table 2. Although the study durations (between 2 weeks and 2 years) were cited for each of the historical control groups, the more pertinent specification of age of the animals was not included. Other aspects of the rationale for selection of the 15 studies cited as historical controls are likewise unclear; for example, there is no indication that these studies were performed in the same facility or within a reasonably proximate time period as the subject study, and no details are given pertaining to similarities in housing conditions, basal feed, source of animals, etc. Overall, however, the historical data provide a sufficient basis for concluding that pituitary and thyroid cysts are not rare in untreated beagle dogs. Thus, the incidence of thyroid cysts in the mid dose group (1 of 4 males and 1 of 4 females) and high dose group (1 of 4 males and 0 of 4 females) in the present study are not considered treatment-related. On the other hand, the incidence of pituitary cysts shows a possible dose-related trend in females (0, 1, 2, and 2 of 4 in control, low, mid and high dose groups, respectively). It is also noted that a greater severity grade (1-2, or slight-moderate) was assigned for pituitary cyst in a high dose animal compared to all the others (grade 1, or slight). The incidence of pituitary cysts (50%) in mid and high dose females exceeds that of all but 3 of the 15 historical control female groups. The major difficulty in interpreting the potential toxicological significance of this lesion appears to be attributable to the small group sizes. This reviewer finds that, as a whole, the evidence for a treatment-related effect on the pituitary is not compelling, and the distribution among treated groups likely occurred by chance. Other histopathologic alterations were found sporadically in control and treated groups, with no apparent relationship to treatment.

As noted in the initial DER for this study, mean body weight gain of high dose females was reduced compared to that of the control group. Although the reduced weight gain was not statistically significant, it is considered to be a treatment-related effect. On this basis, the NOEL for the study is 8000 ppm and the LEL is 20,000 ppm. Although it appears that the animals may have tolerated a somewhat higher dietary concentration, the classification of this study is upgraded to Core-Minimum.

TABLE 1. Occurrence of Cysts in Thyroid and Pituitary

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	Control		3200 ppm		8000 ppm		20,000 ppm	
	M	F	M	F	M	F	M	F
Animals examined	4	4	4	4	4	4	4	4
Thyroid cysts	0	0	0	0	1	1	1	0
Pituitary cysts	1	0	0	1	0	2	0	2

TABLE 2. Historical Control Data (Thyroid and Pituitary Cysts)

Test Substance	Number of Controls		Study Duration	No. of Cysts: Pituitary		Thyroid	
	M	F		M	F	M	F
R1513	4	4	2 years	0	1	1	0
BAY k5552	4	4	1 year	0	1	1	0
BAY e9736	4	4	1 year	0	2	1	0
BAY Va 9391	4	4	1 year	0	0	3	1
BAY 71628	3	3	3 mos.	0	0	0	1
Bay g 6575	3	3	3 mos.	1	0	0	0
SLJ 0312	4	4	3 mos.	2	0	1	1
BAY Vi 9142	4	4	3 mos.	0	1	1	0
NTN 6867	4	4	3 mos.	1	0	0	0
NAK 1654	6	6	3 mos.	3	0	0	0
SIR 8514	4	4	3 mos.	1	0	1	1
BAY p6296	2	2	34 days	0	0	1	0
BAY f1936	2	2	4 weeks	1	1	0	0
BAY m2397	2	2	2 weeks	2	0	0	1
BAY k5552	2	2	2 weeks	0	1	1	0

B. "Triazolylalanine (THS 2212) Study for Subchronic Toxicity to Rats (Three-Month Feeding Study)" Bayer Report No. 12397. April 29, 1986 (revised). Authors: D. Maruhn and E. Bomhard. Bayer A.G. Institute of Toxicology, Wuppertal-Elberfeld (Histopathological report by G. Pappritz, Experimental Pathology Services A.G.).

A Data Evaluation Report on this study was previously issued by this reviewer (Tox Branch Document No. 005094, 5/9/86). Additional data were subsequently submitted and reviewed (Tox Branch Document No. 005352); the study was classified as "Core-Minimum."

In the present submission, additional data were included to address unresolved questions regarding homogeneity of the diet and Quality Assurance certification of the study. An appropriate Quality Assurance statement, signed 10/16/84 by H.P. Schulz, was provided for this study (identified as Study #T 9015049). The question of homogeneity remains unresolved. As noted in the memorandum from A. Katz to H. Jacoby, 7/24/86 (Tox Document No. 005352): "Homogeneity results are considered invalid, pending clarification. The data presented were generated in association with Study No. T 8015796; however, the subchronic rat study was identified as No. T 9015049. In order to evaluate the relevance of the homogeneity data, assurance must be provided tat the methods and materials used in both studies were identical with respect to diet preparation..." The additional data provided by the registrant does not appear to address this specific issue, other than to indicate that the same general SOP was followed for diet preparation for the subchronic study (#T 9015049) as was used for the rangefinding study (#T 8015796), i.e., no indication was found that specific parameters of diet blending critical to homogeneity (such as minimum mixing time, blender style and capacity, batch size, and methods of premix preparation if applicable) were the same for both of the studies.

The classification of this study as "Core-Minimum" is unchanged. No additional data are required.

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C. Point Mutation Test with Chinese Hamster Cells V79 with CGA-131013 Technical. CIBA-GEIGY Limited. Test No. 860258. July 11, 1986.

The DER for this study is attached. Triazole alanine appeared to be mutagenic in V79 Chinese hamster cells with metabolic activation (rat liver S9 microsomal mix) and nonmutagenic without activation. As noted in the DER, this reviewer questions the stability of the compound under the conditions of preparation used in this assay. Pending submission of the appropriate stability data, this experiment is considered acceptable.

D. Autoradiographic DNA Repair Test on Rat Hepatocytes with CGA-131013 Technical. CIBA-GEIGY Limited. Test No. 860184. July 11, 1986.

The DER for this study is attached. Under the conditions of this assay, triazole alanine did not cause a detectable increase in unscheduled DNA synthesis in Arochlor-1254-induced rat hepatocytes in vitro. The registrant should explain the rationale for enzyme induction in studies of this type. The study is considered marginally acceptable.

E. Micronucleus Test (Chinese Hamster) with CGA-131013 Technical. CIBA-GEIGY Limited. Test No. 860185. July 11, 1986.

Under the conditions of this study, no overt mutagenic effect was found; however, additional data are required, as noted in the attached DER. Pending submission and evaluation of the additional data, this study is considered incomplete and unacceptable.

F. Salmonella/Mammalian-Microsome Mutagenicity Test with CGA-131013 Technical. CIBA-GEIGY Limited. Test No. 860187. July 11, 1986.

The DER for this study is attached. In this Ames assay, using S. typhimurium strains TA 98, TA 100, TA 102, TA 1535 and TA 1537, each with and without metabolic activation, triazole alanine was found nonmutagenic.

G. Triazole Alanine: Two-Generation Reproduction Study in the Rat. ICI. Report No. CTL/P/1168 (Revised). August 19, 1986. Authors: G. Milburn, R. Birtley, I. Pate, K. Hollis, S. Moreland. Imperial Chemical Industries PLC, Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK. Accession Nos. 265205 through 265207.

The DER for this study is attached. The study report is considered as Supplementary data. The Core-classification may be upgraded following submission of missing data by the Registrant, as cited in the DER.

H. "Potential of Triazole Alanine to Inhibit Protein Biosynthesis in Microorganisms," September 19, 1986. Authors: B. Thede, E. Padgett and G. Marco. Report No. ABR-86057. Agricultural Division, CIBA-GEIGY Corp., Greensboro, NC. Accession No. 265208.

The effect of triazole alanine on protein synthesis in microorganisms was tested using E. coli, S. cerevisiae and A. flavus. Due primarily to deficiencies cited in the attached DER, the study is considered unacceptable.

I. "Balance Study of ^{14}C-Triazole Alanine in Orally Dosed Rats," March 24, 1986. Authors: K. Lai and B. Simoneaux. Report No. ABR-86023. Agricultural Division, CIBA-GEIGY Corp., Greensboro, NC. Accession No. 265209.

This study is classified as Core-Minimum. The DER is attached. Ring-labelled ^{14}C-triazole alanine appeared to be rapidly absorbed and eliminated following oral administration at doses up to and including 994 mg/kg.

J. "The Metabolism of Triazole Alanine in the Rat," June 3, 1986. Authors: K. Lai and B. Simoneaux. Report No. ABR-86041. Agricultural Division, CIBA-GEIGY Corp., Greensboro, NC. Accession No. 265209.

This study is classified as Core-Minimum. The DER is attached. The major urinary metabolites during the 24-hour period following oral administration of ^{14}C-triazole alanine appeared to be: (1) unchanged triazole alanine and (2) N-acetyl triazole alanine.

DATA EVALUATION REPORT

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A. Study Type: Mutagenicity: Point Mutation Test in Mammalian Cells

B. Compound: Triazole alanine; CGA 131 013 technical (Batch no. TLB 1207 6. Lieferung) 97.4% a.i.

C. Study Report Citation: Dollenmeier, P. (July 11, 1986). "Point Mutation Test with Chinese Hamster Cells V79" Test No. 860258

Testing Facility: Ciba-Geigy Limited
Basle, Switzerland

Sponsor: Agricultural Division
Ciba-Geigy Corporation

D. Reviewed by: Alan C. Katz, M.S., D.A.B.T.
Toxicologist
Toxicology Branch
Hazard Evaluation Division (TS-769C)

Alan C. Katz (Signature)
1/6/87 (Date)

E. Secondary Review by: Irving Mauer, Ph.D.
Geneticist
Toxicology Branch (TS-769C)

Irving Mauer (Signature)
01-08-87 (Date)

F. Procedures:

See Appendix for details, as excerpted from the study report. A single cytotoxicity test and duplicate mutagenicity assays were performed with and without metabolic activation (rat liver S9 microsomal mix). Ethylmethanesulphonate (300 nl/ml, without activation) and N-nitroso-dimethylamine (1 ul/ml, with activation) were used as positive controls. Two negative controls (medium only) were included with each assay. The duplicate mutagenicity assays were performed "independently" and simultaneously, by different technicians.

G. Results:

The results of the cytotoxicity and mutagenicity tests with CGA 131 013 are summarized in Tables 1 through 3, as excerpted from the study report.

The highest concentration of CGA 131 013 used in the mutagenicity test was 10 mg/ml. This selection was reportedly based on prior solubility determinations. In the cytotoxicity test, viability of the cells exposed to 10 mg/ml CGA 131 013 was 66-67% relative to negative controls, with or without the S9 mix.

Data for both positive controls clearly demonstrated the ability of the experiments to detect their mutagenicity.

In the tests without metabolic activation, mutations were not increased in CGA 131 013-treated plates relative to negative controls. With activation, however, there was a slight increase in mutagenic frequency in the plates at the 10 mg/ml concentration.

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H. Discussion:

Note, p. 7 of study report: "Four grams of CGA 131 013 technical were suspended in 309.6 ml bidistilled water at room temperature and autoclaved for 20 minutes at 120°C." Data are required to demonstrate the stability of CGA 131 013 in water at 120°C.

As the data in Table 3 show, the normalized mutant frequency found in the negative controls of the replicate test (experiment no. 860258/1) with microsomal activation exceeded those of the CGA 131 013 treated groups at concentrations up to and including 8 mg/ml; therefore, the calculated mutant frequency factor of 1.4 may be artificially low. At 10 mg/ml, the normalized mutant frequency was more than five times that found at the lowest (0.5 mg/ml) concentration. Data presented in the study report indicate that the negative control mean mutant frequency value in this test was well within the range of historical control values.

I. Conclusions/Classification:

Under the conditions of this assay, CGA 131 013 technical was non-mutagenic in V79 Chinese hamster cells without metabolic activation. Results of the experiments with activation suggest that the compound possesses weak mutagenic potential.

This study is classified as conditionally acceptable, pending submission and evaluation of data required to establish the stability of the test compound under the conditions applied in this assay (i.e., preparation of suspension in bidistilled water, autoclaved for 20 minutes at 120°C).

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Pages 10 through 12 are not included.

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_____ Identity of product inert ingredients.

_____ Identity of product impurities.

_____ Description of the product manufacturing process.

_____ Description of quality control procedures.

_____ Identity of the source of product ingredients.

_____ Sales or other commercial/financial information.

_____ A draft product label.

_____ The product confidential statement of formula.

_____ Information about a pending registration action.

✓ FIFRA registration data.

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DATA EVALUATION REPORT

005841

A. Study Type: Mutagenicity: Micronucleus Test (Chinese hamster)

B. Compound: Triazole alanine; CGA 131 013 technical (Batch no. TLB 1207 6. Lieferung) 97.4% a.i.

C. Study Report Citation: Strasser, P. (July 11, 1986)
"Micronucleus Test (Chinese hamster)"
Test No. 860185

Testing Facility: Ciba-Geigy Limited
Basle, Switzerland

Sponsor: Agricultural Division
Ciba-Geigy Corporation

D. Reviewed by: Alan C. Katz, M.S., D.A.B.T.
Toxicologist
Toxicology Branch
Hazard Evaluation Division (TS-769C)

Alan C Katz
(Signature)
3/10/87
(Date)

E. Secondary Review by: Irving Mauer, Ph.D.
Geneticist
Toxicology Branch (TS-769C)

Irv Mauer
(Signature)
03-11-87
(Date)

F. Procedures:

See Appendix for details, as excerpted from the study report.

Route of Administration: Oral (gavage)

Animals: Chinese hamsters
males- 4-9 weeks old, 22-36 g
females- 6-10 weeks old, 18-28 g

Diet: NAFAG No. 924

Water: Tap, ad libitum

Environmental: 22-23°C
55-78% humidity
12-hour on/off light cycle

Caging: Individual
Type: not specified

Study Design:

a) A preliminary (tolerance) test was performed to determine the appropriate dose level for the mutagenicity test. Two hamsters/sex/group were given single doses of 200, 1000 or 5000 mg/kg CGA 131 013.

Based on the results of the tolerance test, the limit dose of 5000 mg/kg was used in the mutagenicity assay.

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b) The mutagenicity test was performed according to the following schedule:

Treatment	Number of Animals Sacrificed			
	Total	16 hrs	24 hrs	48 hrs
CGA 131 013 Technical, 5000 mg/kg	24/sex	8/sex	8/sex	8/sex
Negative control (vehicle)	24/sex	8/sex	8/sex	8/sex
Positive control (ENDOXAN®, 64 mg/kg)	8/sex	---	8/sex	---

Slide preparation:

Bone marrow smears were prepared and stained with May-Grunwald and Giemsa solutions.

Observations:

Slides were "coded" prior to analysis. The coded slides were evaluated for quality of staining and, on this basis, the slides of 5 animals/sex/group showing "the best differentiation between mature and polychromatic erythrocytes are selected for later scoring"; an exception in this assay, however, was that the slides of only 4 CGA 131 013-treated males at 24 hours were found acceptable, and the slides of 6 females (rather than 5) of the same treatment group and sacrifice interval were scored.

One thousand polychromatic erythrocytes per animal were scored for the incidence of micronuclei. In addition, the polychromatic:normochromatic ratio was determined on the basis of a total count of 1000 erythroctes for each animal.

Statistics:

The chi-square test for statistical significance was reportedly applied.

G. Results/Discussion:

Individual data are presented in Tables 1 through 4, as excerpted from the study report. Although it was reported that there were no statistically significant differences between the CGA 131 013-treated group and the negative controls at any of the intervals tested with respect to the incidence of micronuclei, no statistical data were provided. Overall, values reported for the CGA 131 013 groups appeared to be within a range comparable to that of the concurrent negative controls, while the incidence of micronuclei in the positive controls was clearly demonstrated to be in a much higher range; nevertheless, the data are considered incomplete, pending submission of statistical data.

This reviewer is concerned that the method used in selection of slides for scoring may introduce the possibility of bias; it is therefore recommended that all slides of sufficient quality (rather than the 5 "best" per sex) be scored.

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H. Conclusion/Classification:

Although no obvious mutagenic effect was found, definitive conclusions based on the results of this study will be deferred until the appropriate statistical data are received. In the interim, this study is considered incomplete and unacceptable.

Tox. Review 005841

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✓ FIFRA registration data.

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APPENDIX

Procedures

(Excerpted from Study Report)

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DATA EVALUATION REPORT

005841

A. Study Type: Mutagenicity: DNA Repair (Rat Hepatocytes)

B. Compound: Triazole alanine; CGA 131 013 technical (Batch no. TLB 1207 6. Lieferun
97.4% a.i.

C. Study Report Citation: Puri, E. (July 11, 1986)
"Autoradiographic DNA Repair Test on Rat Hepatocytes"
Test No. 860184

Testing Facility: Ciba-Geigy Limited
Basle, Switzerland

Sponsor: Agricultural Division
Ciba-Geigy Corporation

D. Reviewed by: Alan C. Katz, M.S., D.A.B.T.
Toxicologist
Toxicology Branch
Hazard Evaluation Division (TS-769C)

(Signature)
3/5/87
(Date)

E. Secondary Review by: Irving Mauer, Ph.D.
Geneticist
Toxicology Branch (TS-769C)

(Signature)
03-06-87
(Date)

F. Procedures:

See Appendix for details, as excerpted from the study report. CGA 131 013 was tested for induction of unscheduled DNA synthesis in rat hepatocytes in vitro at concentrations of 0.08, 0.4, 2 and 10 mg/ml. The hepatocytes were isolated from a male rat (Tif.RAIf) following induction with Arochlor 1254. The culture medium served as a negative (vehicle) control; 4-aminobiphenyl (50 uM was used as a positive control. ^{3}H-thymidine was added to the medium (8uCi per 2 ml).

An electronic counter was used to count the nuclear and cytoplasmic silver grains. One hundred fifty cells (50 cells per slide x 3 slides) in each group were scored. Cytoplasmic incorporation of radioactivity was evaluated by counting the silver grains in 3 nuclear-equivalent areas adjacent to the nuclei in each of 50 cells per slide. (NOTE: Grain counts for each individual nucleus-equivalent of cytoplasm were not reported).

G. Results/Discussion:

The results of the cytotoxicity and UDS assay with CGA 131 013 are summarize in Tables 1 through 4, as excerpted from the study report.

The highest concentration of CGA 131 013 used in the mutagenicity test was 10 mg/ml. This selection was reportedly based on the solubility limit rather than cytotoxicity. In the cytotoxicity test, viability of the cells exposed to 10 mg/ml CGA 131 013 was comparable to that of the negative controls.

As shown in the data tables, the CGA 131 013 groups were comparable to the negative controls at all concentrations tested with respect to nuclear and cytoplasmic grain counts, i.e., there was no evidence of a compound-related increase in DNA repair.

Data for the positive control appeared to validate the sensitivity of the UDS assay. However, the rationale for conducting the assay following Arochlor induction is subject to question, i.e., assurance should be provided that induction does not, in this case, enhance de-activation. Since the assay was not replicated with non-induced hepatocytes, the registrant should provide justification for use of an enzyme inducer when using primary cultures as in this experiment.

GLP compliance and Quality Assurance statements were included in the study report.

H. Conclusions/Classification:

Under the conditions of this assay, CGA 131 013 technical at concentrations up to and including 10 mg/ml did not cause a detectable increase in unscheduled DNA synthesis in Arochlor 1254-induced rat hepatocytes in vitro.

This study is classified as marginally acceptable.

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Pages 36 through 44 are not included.

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✓ FIFRA registration data.

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DATA EVALUATION REPORT

005841

A. Study Type: Mutagenicity: Ames-Type Assay

B. Compound: CGA 131013 technical (triazole alanine)
Batch no. TLB 1207 6.Lief
Purity: 97.4%
Vehicle: dimethyl sulfoxide

C. Study Report Citation:

Deparade, E. (7/11/86): Salmonella/Mammalian-Microsome Mutagenicity Test. Test No. 860187. Ciba-Geigy Limited, Basle, Switzerland

D. Reviewed by: Alan C. Katz, M.S., D.A.B.T.
Toxicologist
Toxicology Branch
Hazard Evaluation Division (TS-769C)

Alan C Katz (Signature)
3/11/87 (Date)

E. Secondary Review by: Irving Mauer, Ph.D.
Geneticist
Toxicology Branch (TS-769C)

(Signature)
03-11-87 (Date)

F. Procedures:

A description of materials and methods, as excerpted from the study report, is presented in the Appendix. S. typhimurium strains TA 98, TA 100, TA 102, TA 1535 and TA 1537 were used. The test compound was assayed in replicate experiments at concentrations of 0.20, 0.78, 3.13, 12.50 and 50.00 mg/ml (i.e., ranging from 20 to 5000 ug/plate) in DMSO using cultures with and without activation with rat liver S9 mixture. Positive controls, as listed in the Appendix, were used according to the bacterial strains tested. Counts were made using 3 Petri dishes per strain per concentration for each experiment. A preliminary cytotoxicity test was performed using test substance concentrations ranging from 0.8 ug/ml to 50 mg/ml.

G. Results:

Cytotoxicity data were not provided. A limiting concentration of 50 mg/ml (i.e., 5000 ug/plate) was used. At levels of 0.78 mg/ml and above, a precipitate formed in soft agar.

At all concentrations and in all 5 strains in the replicate experiments, with and without metabolic activation, mean revertant colony counts were comparable to respective negative control values. Counts for all positive controls were well above those of negative controls, adequately establishing the relative sensitivity of the assay in all cases. These data are shown in the attached Tables.

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H. Conclusion/Classification:

Under the conditions of this assay, CGA 131013 technical did not demonstrate mutagenic activity, with or without metabolic activation. This experiment is classified acceptable.

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

005841

Reviewed by : Whang Phang, Ph.D. Whang Phang 3/30/87
Section III, Toxicology Branch (TS-769c)
Secondary Reviewer: Marcia van Gemert, Ph.D. Marcia van Gemert 3/30/87
Section III, Toxicology Branch (TS-769c)

DATA EVALUATION REPORT

STUDY TYPE: Triazole alanine: Protein synthesis study with microorganism. TOX. CHEM. NO.:

ACCESSION NO.: 265203-265209 PROJECT NO.: 7-0138

TEST MATERIAL: Triazole alanine, a plant metabolite of propiconazole. Purity of the test agent was not specified.

SPONSOR: CIBA-GEIGY Corp.

TESTING FACILITY: Biochemistry Department, Agricultural Division, CIBA-GEIGY Corporation, Greensboro, NC.

CITATION: Thede, B., Padgett, E., and Marco, G. (1986). Potential of Triazole to Inhibition Protein Biosynthesis in Microorganisms. Report No.: ABR-86057. CIBA-GEIGY Corp. Greensboro, NC. Sept. 19, 1986.

CONCLUSION: The protein synthesis inhibitory potential of triazole alanine was tested in E. coli, S. cerevisiae, and A. flavus in the presence of ^{14}C-L-phenylalanine or ^{14}C-L-alanine. The results are too inconsistent to show whether the test agent has the protein synthesis inhibitory effects or not. In addition the report has many deficiences which include lack of individual experimental data, no explanation for various incubation times used, and not enough tested doses of triazole alanine.

It should be noted that additional studies in protein synthesis would not be required at this time.

Classification: Unacceptable

Materials and Methods:

A series of studies were carried out to test the potential of triazole alanine, a plant metabolite of propiconazole, in inhibiting protein synthesis in three micro-organisms. Escherichia coli (bacteria), Sac- charomyces cerevisiae (yeast), and Aspergillus flavus (fungi) were used in the studies. The details of the experimental procedures were presented in the Attachment A.

Experiments were conducted to construct growth curves which were used to estimate the period of maximum protein synthesis for these organisms. In addition, preliminary studies were also carried out to determine the appropriate concentrations of the test agents to be used. From these studies it was determined that 100 ppm of triazole alanine (TA) and of two positive controls could be utilized. The two positive controls were chloramphenicol (CA) and 5-methyltryptophan (MT).

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For the assays of amino acids incorporation and protein synthesis, 1 uCi of ^{14}C-L-phenylalanine and ^{14}C-L-alanine were added to the incubation. In these studies, radioactivity of incubation filtrate, trichloroacetic acid (TCA) soluble fraction, TCA insoluble fraction, and non-extractable protein fraction was measured. The amount of protein was measured in TCA soluble fraction, TCA insoluble fraction, and the incubation filtrate.

RESULTS and DISCUSSION:

The results of the growth curves indicate that E. coli had reached maximum growth rate at approximately 1 hr (Figure 1). The maximum growth rate for A. flavus and S. cerevisiae was difficult to determine based upon the experimental results (figure 2 and Table I). The growth rate of A. flavus was measured by the cell dry weight because these organisms are filamentous.

Table II shows the absorbance measurements of protein (750 nm) in 24 hr. incubation studies with varying concentrations of the test agent and the two positive controls. The positive controls, MT and CA, at 100 ppm were shown to inhibit protein synthesis in E. coli. In addition, MT also inhibited the protein synthesis of A. flavus. However, the test agent, TA, produced only marginal inhibition on the E. coli and no effect on the other two organisms relative to the two positive controls. The results of this study indicate that concentrations higher than 100 ppm of TA could be used in the subsequent studies.

In the protein synthesis studies with longer incubation periods (Table III), CA strongly inhibited the protein synthesis of E. coli in the presence of ^{14}C-L-phenylalanine or ^{14}C-L-alanine with an incubation period of 3 hrs. or more. For A. flavus, CA only inhibited the protein synthesis in the presence of ^{14}CL-alanine.

The positive control, MT, consistently inhibited the protein synthesis of S. cerevisiae in the presence of both radioactive amino acids. In comparison, the test agent inhibited the protein synthesis of E. coli and A. flavus in the presence of ^{14}C-L-alanine.

The effects of TA on radiolabeled phenylalanine and alanine incorporation are shown in Table IV. In E. coli, TA inhibited the incorporations of both radiolabeled phenylalanine and alanine.

The results are equivocal and are not appropriate to be used for determining the inhibitory potential of TA on the protein synthesis in the three microorganisms tested. The reasons are the following:

1). In the studies with 1 hr. of incubation period, the results indicate that TA inhibited protein in E. coli, A. flavus, and S. cerevisiae in the presence of both radiolabeled phenylalanine and alanine (Table V). In addition, the growth curve of E. coli indicates that maximum period of protein synthesis was approximate 1 hr. of incubation.

2). The studies have many deficiencies: a). no rationale was given concerning the various incubation times used in the final protein synthesis

studies (Table III); b). in testing the inhibitory effects of a compound on protein synthesis several concentrations of the test agent should be used; c). samples of thin layer chromatographic results should be presented to substantiate the summary data.

3). The registrant has not submitted any individual assay results to the agency for the reviewer to validate the data presented in the report.

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Pages 63 through 77 are not included.

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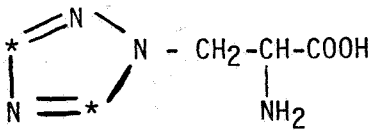
DATA EVALUATION REPORT

005841

A. Study Type: Metabolism

B. Compound: ^{14}C-triazole alanine; CL-V-94; CGA-131013



Radioactive purity: >99%

Vehicle: PEG (specifications not given)

C. Study Report Citation: Lai, K. and Simoneau, B. (6/3/86) "The Metabolism of Triazole Alanine in the Rat." CIBA-GEIGY Corp., Agricultural Division, Greensboro, NC. Report No. ABR-86041

D. Reviewed by: Alan C. Katz, M.S., D.A.B.T.
Toxicologist
Toxicology Branch
Hazard Evaluation Division (TS-769C)

Alan C Katz (Signature)
4/6/87 (Date)

E. Secondary Review by: Marcia van Gemert, Ph.D.
Head, Section 3
Toxicology Branch (TS-769C)

Marcia van Gemert (Signature)
4/13/87 (Date)

F. Introduction:

The purpose of this experiment was to determine the major urinary metabolites accounting for the radioactivity which was recovered at levels of >80% in urine in the balance study.

G. Procedures:

Animals: Sprague-Dawley rats, approximately 200 grams each; 6 per sex.

Source of animals: not specified.

This experiment was a continuation of the balance study, #ABR-86023. Treatment of animals was described in the Data Evaluation Report for that study. Additional details are provided in the Appendix of this report. TLC separations, using various solvent systems as described in the Appendix, were analyzed with a spark chamber or by radioautography.

H. Results:

Both the spark chamber and radioautographic observations provided evidence of only two radioactive zones. TLC results, presented in Table I (excerpted from the study report), show that the migration rates of the 2 urine moieties correlate

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G. Results (Cont'd):

with those of triazole alanine and N-acetyl triazole alanine standards in each of the 5 solvent systems used in this experiment. The analysis of the urinary metabolites separated with the Methanol (70): Pyridine (9.6): Formic acid (0.4): Water (20) solvent system showed that triazole alanine was excreted primarily unchanged, representing 82 to 93% of the 0-24 hour urinary radioactivity (as shown in Table II, also from the study report); N-acetyl triazole alanine was presumed to be the other major urinary moiety, accounting for 13 to 30% of the 0-24 hour radioactivity. The significance of an apparent decrease in the percentage of N-acetylation of the test material at the higher dose level is questionable.

H. Conclusions:

Under the conditions of this study, unaltered triazole alanine and the N-acetyl metabolite were the major urinary metabolites found during the initial 24 hours after dosing.

I. Classification:

Minimum

APPENDIX

Procedures

(Excerpted from Study Report)

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Pages 81 through 83 are not included.

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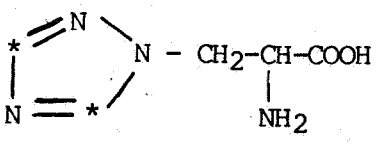
DATA EVALUATION REPORT

005841

A. Study Type: Metabolism

B. Compound: ^{14}C-triazole alanine; CL-V-94; CGA-131013



Radioactive purity: >99%

Vehicle: PEG (specifications not given)

C. Study Report Citation: Lai, K. and Simoneau, B. (3/24/86) Balance Study of ^{14}C-Triazole Alanine in Orally Dosed Rats. CIBA-GEIGY Corp., Agricultural Division, Greensboro, NC. Report No. ABR-86023

D. Reviewed by: Alan C. Katz, M.S., D.A.B.T.
Toxicologist
Toxicology Branch
Hazard Evaluation Division (TS-769C)

Alan C. Katz (Signature)
4/1/87 (Date)

E. Secondary Review by: Marcia van Gemert, Ph.D.
Head, Section 3
Toxicology Branch (TS-769C)

Marcia van Gemert (Signature)
4/13/87 (Date)

F. Procedures:

Animals: Sprague-Dawley rats, approximately 200 grams each; 6 per sex.

Source of animals: not specified.

Three groups of rats, 2/sex/group, were given ^{14}C-ring labelled triazole alanine by gavage at doses of 0.56, 54.4 or 993.7 mg/kg in a single dose by gavage. The rats were housed individually in stainless steel metabolism cages and were given food and water ad libitum. Urine and feces were sampled daily. The animals were killed 7 days after dosing, and tissue samples were taken (i.e., blood, heart, lungs, spleen, kidneys, liver, brain, muscle, fat and gonads). The radioassays (urine, feces and tissue) were performed using a Beta Tracor Model 6895 counter. Additional details are provided in the Appendix.

G. Results:

Total recoveries in excreta and tissues for low, mid and high dose levels averaged 101.2%, 90.7% and 92.1%, respectively. There did not appear to be any substantial sex-related differences with respect to excretion patterns. The major route of excretion for all dose levels was in urine, averaging between 81-82% for males and 84-85% for females. Absorption and excretion were rapid;

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G. Results (Cont'd):

24 hours after dosing, elimination of radioactivity in urine and feces ranged between 76-82% for males and 71-91% for females. Seven days after dosing, mean total tissue residue levels ranged between 0.1-1.0% in males and 0.1-1.4% in females.

Levels of detection of residues in tissue were 0.004 ppm, 0.40 ppm and 8.5 ppm for the low, mid and high dose animals, respectively. Seven days after administration, the total radioactivity levels in tissues were generally below the quantitation limits, with the following exceptions: 1 low dose male, 0.026 ppm in fat; 1 mid dose male, 0.97 ppm in plasma; and 1 high dose female, 11.12 ppm in fat and 65.49 ppm in spleen.

H. Conclusions:

Although only 2 animals/sex/group were used in this experiment, the data appeared to be sufficient to demonstrate rapid absorption and elimination of radioactivity following administration of single doses of ring-labelled ^{14}C-triazole alanine at levels up to and including 993.7 mg/kg.

I. Classification:

Minimum

APPENDIX

Procedures

(Excerpted from Study Report)

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