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CASWELL FILE

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

008292

MAR 18 1991

3/18/1991

MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

SUBJECT: **TRIAZOLYL ALANINE:** Submission of Supplementary Data in Response to Comments by the Toxicology Branch.

FROM: Jess Rowland, M.S, Toxicologist Jess Rowland 3/11/91
Section II, Toxicology Branch II (HFAS)
Health Effects Division (H7509C)

TO: S. Lewis/ M. Stone
Product Manager (21)
Registration Division

THRU: K.Clark Swentzel, Section Head K. Clark Swentzel 3/11/91
Section II, Toxicology Branch II (HFAS)
Health Effects Division (H7509C)
and
Marcia van Gemert, Ph.D., Chief Marcia van Gemert 3/13/91
Toxicology Branch II (HFAS)
Health Effects Division (H7509C)

STUDY IDENTIFICATION: **EPA I.D. No.: 056736 Record No.: 257905**
MRID No: 413268-00
HED Project No: 0-0478 Caswell No.: 862B.
Registrant: CIBA-GEIGY Corporation

ACTION REQUESTED: Review of submitted supplementary data to allow upgrading of toxicity studies.

SUMMARY:

1. **Point Mutation Test with Chinese hamster cells V79 with CGA-131013 Technical. MRID No. 413268-01.**

Based on the review of the additional data submitted by the registrant, this study (Study No.Ciba-Geigy 860258; 7/11/86; MRID No.265204) is **upgraded to acceptable** and satisfies Guideline requirements for genetic effects Category I, Gene Mutations.

2. **Micronucleus Test (Chinese Hamster) with CGA-131013 Technical. MRID No. 413268-02.**

Based on the review of the additional data submitted by the registrant, this study (Study No. Ciba-Geigy 860185; 7/11/86; MRID No. 265204) is **upgraded to acceptable** and satisfies Guideline requirements for genetic effects Category II, Structural Aberrations.

3. First Supplement to Triazole Alanine EPA Guidelines No. 83-4. MRID No. 413268-03.

The Two-Generation reproduction study in the rat with triazole alanine [Study No.CTL/P/1168; Accession Nos.265205,265206, 265207] **cannot be upgraded.** The study must be repeated to meet guideline requirements (83-4) for a two-generation reproduction study.

4. First Amendment to Triazole Alanine EPA Guidelines No. 83-4. MRID No. 423268-04.

This amendment provided data corrections to the original report (ICI Report No. CTL/P/1168, 8/19/86); these changes do not alter the interpretation of the study results.

REVIEW

The registrant, CIBA-GEIGY Corporation submitted comments/additional data in response to comments in review of triazole alanine data package; review dated April 15, 1987 (Memo: M. Van Gemert to L.Rossi).

1. Point Mutation Test with Chinese hamster cells V79 with CGA-131013 Technical. MRID No. 413268-01.

Based on the reevaluation of the Chinese hamster V79 mammalian cell gene mutation assays with triazole alanine and in consideration of the new information provided by the registrant, it was concluded that the test material was not mutagenic. The analytical data showed the compound to be stable under the conditions of use. Therefore, this study **(Study No.860258; MRID No. 265204)** is **upgraded to acceptable** and satisfies Guideline requirements for genetic effects Category I, Gene Mutations.

2. Micronucleus Test (Chinese Hamster) with CGA-131013 Technical. MRID No. 413268-02.

Based on the additional information provided by the registrant, which included the rationale for slide selection and submission of statistical data, it was concluded that the test material, triazole alanine (CGA-131013 technical) was not clastogenic in the in vivo micronucleus assay. The quality control measures used to select slides for analysis were adequately explained, justifiable, and in accordance with recommended procedures. Therefore, this study **(Study No.860185; MRID No.265204)** is **upgraded to acceptable** and satisfies Guideline requirements for genetic effects Category II, Structural Aberrations.

3. First Supplement to Triazole Alanine EPA Guidelines No. 83-4. MRID No. 413268-03.

This supplement contained additional data on the Two-Generation Reproduction Study in the Rat **[Study No.CTL/P/1168; Accession Nos.265205,265206, 265207]**. This study was classified **as supplementary** due to the following deficiencies: 1) dose levels employed (0, 500, 200, 10,000 ppm) may not be sufficiently high to demonstrate parental toxicity; 2) statistical analysis of reproductive parameters was not included; 3) tables of males and females mated were not included; 4) food consumption during pregnancy, lactation, and weaning for parents was not reported; and 5) historical control data for the observed abnormalities were not provided.

In this submission, the registrant provided data on maternal parents of the Fo generation in relation to the pairings used in mating, food consumption and body weight data and statistical analyses, and historical control data for the observed abnormalities. These data satisfy deficiencies Nos.3, 4, and 5 listed above.

The submission, however, **did not** address deficiencies No. 1 and 2 listed above, namely, a rationale was not given for dose selection and there was no discussion on the lack of parental toxicity in the submitted study, and in addition, statistical analyses of reproductive parameters were not conducted. Consequently, this study **cannot be upgraded.** This study must be repeated to meet guideline requirements (83-4). Toxicology branch will consider the study to be adequately performed if the highest dose tested is 1 g/kg of body weight. This upper limit dose is equivalent to dietary concentration of approximately 20,000 ppm.

4. First Amendment to Triazole Alanine EPA Guidelines No. 83-4.

This amendment provided data corrections to the original report (ICI Report No. CTL/P/1168, 8/19/86); these changes did not alter the interpretation of the study results.

Tox. Chem. No. 862 B File Last Updated _____ Current Date 3/5/91

Triazole alanine

Study/Lab/Study #/Date	Material	EPA Accession No.	Results: LD50, LC50, PIS, NOEL, LEL	Tox. Cat.	Core-Grade/ Doc. #
Reproduction-2 generation Species: Rat ICI Central Tox Lab CTL/P/1186; 8/19/86	Triazole alanine 97.8%	265205 265206 265207 413268-03	Additional information not satisfactory; study cannot be be upgraded--study must be repeated at higher dose levels		Supplementary
Mutagenicity-Point mutation Species:Ch.ham.V79 cells Ciba-Geigy 860258	Triazole alanine;CGA 131013 Tech 97.4% a.i	265204 413268-01	Additional information--Study upgraded to acceptable		Acceptable
Mutagenicity-Micronucleus Species:Ch.ham.bone marrow Ciba-Geigy 860185	Triazole alanine;CGA 131013 Tech 97.4% a.i	265204 413268-02	Additional information--Study upgraded to acceptable		Acceptable.

EPA No.: 68D80056
DYNAMAC No.: 345-B
TASK No.: 3-45B
March 7, 1991

DATA EVALUATION RECORD

TRIAZOLE ALANINE

Mutagenicity--Response to CIBA-GEIGY's Comments Regarding the EPA Review of the in vivo Micronucleus Assay in Chinese Hamsters

APPROVED BY:

Robert J. Weir, Ph.D.
Program Manager
Dynamac Corporation

Signature: Robert J. Weir

Date: 3/6/91

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EPA No.: 68D80056
DYNAMAC No.: 345-B
TASK No.: 3-45B
March 7, 1991

DATA EVALUATION RECORD

TRIAZOLE ALANINE

Mutagenicity--Response to CIBA-GEIGY's Comments Regarding the EPA Review of the in vivo Micronucleus Assay in Chinese Hamsters

REVIEWED BY:

Nancy E. McCarroll, B.S.
Principal Reviewer
Dynamac Corporation

Signature: Nancy E. McCarroll
Date: 3-7-91

I. Cecil Felkner, Ph.D.
Independent Reviewer
Dynamac Corporation

Signature: Ira Cecil Felkner
Date: 3-7-91

APPROVED BY:

Nicolas P. Hajjar, Ph.D.
Department Manager
Dynamac Corporation

Signature: William L McLellan for
Date: 3-7-91

Jess Rowland, M.S.
EPA Reviewer, Section II
Toxicology Branch II
(H-7509C)

Signature: Jess Rowland
Date: 3-11-91

K. Clark Swentzel
EPA Section Head, Section II
Toxicology Branch II
(H-7509C)

Signature: K. Clark Swentzel
Date: 3/11/91

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DATA EVALUATION RECORD

Tox. Chem. No.:
EPA File Symbol:

STUDY TYPE: Triazole alanine.

STUDY TYPE: Mutagenicity--Response to CIBA-GEIGY's comments regarding the EPA review of the *in vivo* micronucleus assay in Chinese hamsters.

MRID NUMBER: 413268-02.

SYNONYM/CAS NUMBER: CGA-131013 technical.

SPONSOR: CIBA-GEIGY Corp., Greensboro, NC.

TESTING FACILITY: CIBA-GEIGY Ltd., Basle, Switzerland.

TITLE OF REPORT: Micronucleus Test (Chinese Hamster) with CGA-131013 Technical.

AUTHOR: Strasser, P.

STUDY NUMBER: 860185.

REPORT ISSUED: July 9, 1987.

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CONCLUSIONS - Executive Summary:

In response to an EPA toxicology review of an in vivo Chinese hamster micronucleus assay conducted with triazole alanine, the requested information was submitted by the performing laboratory. Based on this new information, which included the rationale for slide selection and submission of statistical data, we conclude that 5000 mg/kg CGA-131013 administered once by oral gavage did not increase the frequency of micronuclei in polychromatic erythrocytes harvested from male and female Chinese hamsters sacrificed 16, 24, or 48 hours postexposure. The quality control measures used to select slides for analysis were adequately explained, justifiable, and in accordance with recommended procedures. The submitted statistical data support the conclusion that CGA-131013 was not clastogenic in this in vivo micronucleus assay. We assess, therefore, that the study satisfies Guideline requirements for genetic effects Category II, Structural Aberrations.

Study Classification: The study is upgraded to acceptable.

A. BACKGROUND:

An EPA toxicology review of an in vivo Chinese hamster micronucleus assay conducted with triazole alanine (CGA-131013 technical; Batch No. TLB 1207 6 Lieferung; 97.4% active ingredient)[1] was completed on March 11, 1987 (see Appendix A).

EPA reviewers deferred drawing definitive conclusions and classified the study as incomplete and unacceptable based on the lack of information on the method used to select slides for scoring (i.e., the "best" slides from five animals/sex/group) and the lack of a statistical assessment of the data. In response to EPA's request for additional information, the performing laboratory submitted the requested information (see Appendix B).

B. REVIEWERS' ASSESSMENT:

Based on the submission of new information, the following conclusions have been drawn:

1. Slide Selection Procedures: The study author stated that "A quality evaluation of the prepared slides is routinely performed on all slides before scoring." The quality control procedures included screening slides to determine

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[1]Strasser, P. Micronucleus Test (Chinese hamster) Test No. 860185. (Unpublished study prepared by CIBA-GEIGY Ltd., Basle, Switzerland, for CIBA-GEIGY Corp., Greensboro, NC; dated July 11, 1986). Accession No. 265204.

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whether cells had perfect morphology and whether the staining of cells was vigorous. Schmid (1975)[2] was cited to support the study author's position that good technical quality of the slides is a prerequisite for analysis of micronuclei induction in erythrocytes. We concur with the study author's position and conclude that the quality control procedures for slide selection were justified, based on the recommended approach, and did not introduce bias into the scoring of cells.

2. Statistical Assessment: Statistical data to support the study author's statement that polychromatic erythrocytes with micronuclei in the treatment and control groups were analyzed at $p < 0.05$ by X^2 test and submitted for review (Appendix C). These data agree with the conclusion that there were no statistically significant differences between the CGA-131013-treated groups and the negative controls at any of the test intervals.

Based on the new information, we concluded that the study should be upgraded and classified as acceptable.

C. CBI APPENDIX: Appendix A, Data Evaluation Report; Appendix B, CIBA-GEIGY's Response to EPA Comments, CBI pp. 6 and 7; Appendix C, Statistical Analysis Summary, CBI p. 8.

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[2]Schmid, W. The micronucleus test. Mutat. Res. 31(1975): 9-15.

APPENDIX A

Data Evaluation Report

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.

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.

005841

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.

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Page_____ is not included in this copy.

Pages 14 through 17 are not included.

The material not included contains the following type of information:

_____ Identity of product inert ingredients.

_____ Identity of product impurities.

_____ Description of the product manufacturing process.

_____ Description of quality control procedures.

_____ Identity of the source of product ingredients.

_____ Sales or other commercial/financial information.

_____ A draft product label.

_____ The product confidential statement of formula.

_____ Information about a pending registration action.

✓ FIFRA registration data.

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

_____ The document is not responsive to the request.

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

APPENDIX B

CIBA-GEIGY's Response to EPA Comments
CBI pp. 6 and 7

Page_____ is not included in this copy.

Pages 19 through 20 are not included.

The material not included contains the following type of information:

_____ Identity of product inert ingredients.

_____ Identity of product impurities.

_____ Description of the product manufacturing process.

_____ Description of quality control procedures.

_____ Identity of the source of product ingredients.

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_____ The product confidential statement of formula.

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✓ 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 C

Statistical Analysis Summary
(CBI p. 8)

Page 22 is not included in this copy.

Pages _____ through _____ are not included.

The material not included contains the following type of information:

_____ Identity of product inert ingredients.

_____ Identity of product impurities.

_____ Description of the product manufacturing process.

_____ Description of quality control procedures.

_____ Identity of the source of product ingredients.

_____ Sales or other commercial/financial information.

_____ A draft product label.

_____ The product confidential statement of formula.

_____ Information about a pending registration action.

✓ FIFRA registration data.

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

_____ The document is not responsive to the request.

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

EPA No.: 68D80056
DYNAMAC No.: 345-A
TASK No.: 3-45A
March 7, 1991

DATA EVALUATION RECORD

TRIAZOLE ALANINE

Mutagenicity--Response to CIBA-GEIGY's Comments Regarding the EPA Review of the Mammalian Cells in Culture Gene Mutation Assay in Chinese Hamster V79 Cells

APPROVED BY:

Robert J. Weir, Ph.D.
Program Manager
Dynamac Corporation

Signature: Robert J. Weir

Date: 3/6/91

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EPA No.: 68D80056
DYNAMAC No.: 345-A
TASK No.: 3-45A
March 7, 1991

DATA EVALUATION RECORD

TRIAZOLE ALANINE

Mutagenicity--Response to CIBA-GEIGY's Comments Regarding the EPA Review of the Mammalian Cells in Culture Gene Mutation Assay in Chinese Hamster V79 Cells

REVIEWED BY:

Nancy E. McCarroll, B.S.
Principal Reviewer
Dynamac Corporation

Signature: Nancy E. McCarroll
Date: 3-7-91

I. Cecil Felkner, Ph.D.
Independent Reviewer
Dynamac Corporation

Signature: I. Cecil Felkner
Date: 3-7-91

APPROVED BY:

Nicolas P. Hajjar, Ph.D.
Department Manager
Dynamac Corporation

Signature: William L. McLellan for
Date: 3-7-91

Jess Rowland, M.S.
EPA Reviewer, Section II
Toxicology Branch II
(H-7509C)

Signature: Jess Rowland
Date: 3/11/91

K. Clark Swentzel
EPA Section Head, Section II
Toxicology Branch II
(H-7509C)

Signature: K. Clark Swentzel
Date: 3/11/91

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DATA EVALUATION RECORD

Tox. Chem. No.:
EPA File Symbol:

CHEMICAL: Triazole alanine.

STUDY TYPE: Mutagenicity--Response to CIBA-GEIGY's comments regarding the EPA review of the mammalian cells in culture gene mutation assay in Chinese hamster V79 cells

MRID NUMBER: 413268-01.

SYNONYM/CAS NUMBER: CGA-131013 technical.

SPONSOR: CIBA-GEIGY Corp., Greensboro, NC.

TESTING FACILITY: CIBA-GEIGY Ltd., Basle Switzerland.

TITLE OF REPORT: Point Mutation Test with Chinese Hamster Cells V79 with CGA-131013 Technical.

AUTHOR: Dollenmeier, P.

STUDY NUMBER: 860258.

REPORT ISSUED: September 9, 1987.

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CONCLUSIONS - Executive Summary:

In response to an EPA toxicology review of the in vitro Chinese hamster V79 cell gene mutation assays conducted with triazole alanine, the requested information was submitted by the performing laboratory.

Based on the reevaluation of the Chinese hamster V79 mammalian cell gene mutation assays with triazole alanine (500-10,000 μg/mL +/-S9) and in consideration of the new information provided by the performing laboratory, we assess that the test material was not mutagenic. We further assess that the analytic data furnished by the reporting laboratory support the study author's claim that triazole alanine was stable under the conditions of use. We conclude, therefore, that the study satisfies Guideline requirements for genetic effects Category I, Gene Mutations.

Study Classification: The study is upgraded to acceptable.

A. BACKGROUND:

An EPA toxicology review of two independently performed in vitro Chinese hamster V79 cell gene mutation assays with triazole alanine (CGA-131013 technical; Batch No. TLB 1207 6 Lieferung; 97.4% active ingredient)[1] was completed on January 8, 1987 (see Appendix A).

EPA reviewers had concluded that the results of experiments conducted in the presence of S9 activation suggest that CGA-131013 technical possessed weak mutagenic potential. EPA reviewers also concluded that, owing to the method used to prepare the test material stock solution (i.e., 4 g of the test material suspended in 309.6 mL of double-distilled water at room temperature and autoclaved for 20 minutes at 120°C), data were required to demonstrate that CGA-131013 was stable in water at 120°C. Based on these considerations, EPA reviewers classified the study "conditionally acceptable" pending the submission and evaluation of test material stability data. Comments were received from the performing laboratory regarding the EPA review of this study (See Appendix B).

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[1]Dollenmeier, P. Point Mutation Test with Chinese Hamster Cells V79. (Unpublished Study No. 860258 prepared by CIBA-GEIGY Ltd., Basle, Switzerland, for CIBA-GEIGY Corp., Greensboro, NC; dated July 11, 1989.) Accession No. 860258.

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B. REVIEWERS' ASSESSMENT:

A reevaluation of the study was conducted in light of new information provided by the performing laboratory. Our response to CIBA-GEIGY comments is as follows:

1. Test Material Stability: The study author stated that samples of the stock solutions prepared for the initial and repeat mutation assays were frozen at -20°C and held for further use. In response to the EPA review, analytical determinations were performed on both samples. The results indicated that the samples contained 95.6 and 95.0% of the nominal concentration (see Appendix C). Based on these results, our reviewers conclude that triazole alanine was stable under the conditions of use.

2. Weak Positive Response with S9 Activation: Although the initial EPA assessment of the study stated that CGA 131013 technical in the presence of S9 activation "may be weakly mutagenic," no definitive conclusions were reached. The EPA reviewers' concerns were related to the reproducible increases in the mutation frequency (MF) at the highest S9-activated level (10,000 µg/mL). However, several factors have prompted us to change our position. As shown in Table 1, there was an approximately threefold difference in the spontaneous MF for the negative control cultures used in the initial and repeat test. This finding is consistent with the variations in the frequency of spontaneous mutation to 6-thioguanine resistance (TG^R) seen in many laboratories. This acknowledged background MF variability illustrates the difficulty in interpreting data from V79 cell gene mutation assays and serves as the basis for the EPA Gene-Tox Program's definition of a positive response in this test system:

"A compound is classified as mutagenic in V79 cells only if it induces a mutation frequency that is at least 3 times higher than the spontaneous mutant frequency reported for that specific experiment. Compounds that do not meet this criterion will be classified as non-mutagenic in V79 cells."[2]

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[2]Bradley, M. O.; Bhuygan, B.; Francis, M. C.; Langenbach, R.; Peterson, A.; and Huberman, E. Mutagenesis by chemical agents in V79 Chinese hamster cells: A review and analysis of the literature. A report of the Gene-Tox Program. Mutat. Res. 87(1981): 81-142.

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TABLE 1. Representative Results from the S9-Activated Chinese Hamster V79 Gene Mutation Assays with CGA 131013 Technical

Substance	Dose/mL	Mean Mutant Colonies/ Dish ± S.D.	Mean Survivors/Dish ± S.D.	Mutation Frequency x 10^{-6}[a]	Fold Increase[b]
Solvent Control					
Distilled water	--	0.22 ± 0.55[c] (0.3)[e]	71.83 ± 9.15 (67.9)[e]	3.1 (4.6)[e]	--
		0.39 ± 0.61	64.00 ± 4.60	6.1	
	--	0.89 ± 0.76[d] (0.7)[e]	48.17 ± 4.49 (47.3)[e]	18.5 (14.7)[e]	--
		0.50 ± 0.71	46.50 ± 6.02	10.8	
Test Material	10,000 µg[f]	0.50 ± 0.51[c]	46.00 ± 6.72	10.9	2.4
	10,000 µg[f]	0.56 ± 0.92[d]	26.33 ± 16.27	21.1	1.4

[a]Mutation Frequency (MF) = $\frac{\text{Mean Mutant Colonies}}{\text{Mean Survivors}} \times 1000.$

[b]Fold Increase = $\frac{\text{MF of Test Dose}}{\text{MF of Solvent Control}}.$

[c]Results from the initial assay.

[d]Results from the repeat assay.

[e]Average results in ( ) were calculated by our reviewers.

[f]Results for lower doses (500, 1000, 2000, 4000, 6000, and 8000 µg/mL) did not indicate a mutagenic response.

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Based on this criterion, the ≈2.4- and 1.4-fold increases in the MF seen at 10,000 μg/mL CGA-131013 +S9 in the initial and repeat assays, respectively, do not constitute sufficient evidence of a positive mutagenic response. In addition, there was no indication of a dose-related effect. Similarly, the mean number of TG^R mutants at 10,000 μg/mL were not appreciably higher than the spontaneous rate in the first assay and were less than the spontaneous rate in the repeat assay. We conclude, therefore, that CGA-131013 was not mutagenic in this mammalian cell assay.

C. CBI APPENDIX: Appendix A, Data Evaluation Report; Appendix B, CIBA-GEIGY's Response to EPA Comments, CBI pp. 6, 7, and 10; Appendix C, Analysis Data, CBI p. 12.

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APPENDIX A

Data Evaluation Report

DATA EVALUATION REPORT

005841

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)

(Signature)
1/6/87
(Date)

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

(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). Ethylmethane-sulphonate (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).

Page_____ is not included in this copy.

Pages 33 through 35 are not included.

The material not included contains the following type of information:

_____ Identity of product inert ingredients.

_____ Identity of product impurities.

_____ Description of the product manufacturing process.

_____ Description of quality control procedures.

_____ Identity of the source of product ingredients.

_____ Sales or other commercial/financial information.

_____ A draft product label.

_____ The product confidential statement of formula.

_____ Information about a pending registration action.

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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 B

CIBA-GEIGY's Response to EPA Comments
CBI pp. 6, 7, and 10

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APPENDIX C

Analysis Data
(CBI p. 12)

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