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CORNING Hazleton

Volume ____ of ____ of Submission
CGA-329351 Technical

European Registration Dossier
Dossier File N°: 5.2.6 / 02
Ciba File N°: 329351/135

FINAL REPORT

Study Title:

Dermal Sensitization Study of CGA-329351 Technical in Guinea Pigs - Closed Patch Technique (EPA Guideline 81-6)

Data Requirement:

EPA Guideline 81-6

Author:

5.1.2.e Woo

Study Completion Date:

July 18, 1995

Performing Laboratory:

Corning Hazleton, Inc.
3301 Kinsman Boulevard
Madison, Wisconsin 53704

Laboratory Project Identification:

CHW 50400232

Volume 1 of 1 of Study

Sponsor:

Ciba Crop Protection
Ciba-Geigy Corporation
Greensboro, North Carolina

CHW 50400232

STATEMENT OF NO DATA CONFIDENTIALITY CLAIMS

Dermal Sensitization Study of CGA-329351 Technical
in Guinea Pigs - Closed Patch Technique
(EPA Guideline 81-6)

No claim of confidentiality is made for any information contained in this study on the basis of its falling within the scope of the Federal Insecticide, Fungicide, and Rodenticide Act §10 (d) (1) (A), (B), or (C).

Company Ciba-Geigy Corporation

Company Agent 5.1.2.e Woo Date 7/24/95

Senior Regulatory Manager
Title

5.1.2.e Woo
Signature

These data are the property of the Ciba-Geigy Corporation, and as such, are considered to be confidential for all purposes other than compliance with FIFRA §10. Submission of these data in compliance with FIFRA does not constitute a waiver of any right to confidentiality which may exist under any other statute or in any other country.

CHW 50400232

COMPLIANCE STATEMENT

Dermal Sensitization Study of CGA-329351 Technical
in Guinea Pigs - Closed Patch Technique
(EPA Guideline 81-6)

This study was conducted in accordance with the following Good Laboratory Practice Standards with the exception that analysis of the test and positive control material mixtures for concentration, homogeneity/solubility, and stability was not conducted:

Japan Ministry of Agriculture, Forestry and Fisheries
Notification No. 59 Nohsan 3850
Director-General of Agricultural Production Bureau, 10 August 1984

United States Environmental Protection Agency FIFRA:
Good Laboratory Practice Standards, 40 CFR 160

Organisation for Economic Cooperation and Development's Principles of Good Laboratory Practice, C(81)30(Final)

[redacted] DABT Date 7/18/95

Toxicology
Corning Hazleton, Inc.

[redacted] Date 7-18-95

Acute Studies
Corning Hazleton, Inc.

[redacted] Date 7/19/95

Ciba Crop Protection
Ciba-Geigy Corporation

CHW 50400232

QUALITY ASSURANCE STATEMENT

This report has been reviewed by the Quality Assurance Unit of Hazleton Wisconsin, Inc., in accordance with the Environmental Protection Agency (EPA) Good Laboratory Practice Standards, 40 CFR 160.35 (b) (6) (7), the Organisation for Economic Cooperation and Development (OECD) Principles of Good Laboratory Practice, C(81)30(Final), and the Japanese Ministry of Agriculture, Forestry, and Fisheries (MAFF) Good Laboratory Practice Standards for Toxicology Studies on Agricultural Chemicals, 59 NohSan No. 3850. The following inspections were conducted and findings reported to the Study Director and management. Written status reports of inspections and findings are issued to Hazleton management monthly according to standard operating procedures.

Inspection Dates From	To	Phase	Date Reported to Study Director	Date to Management
04/07/95	04/07/95	Protocol Review	04/07/95	05/10/95
04/11/95	04/11/95	Dose Preparation	04/11/95	05/10/95
06/27/95	06/28/95	Data/Report Review	06/28/95	07/10/95

5.1.2.e Woo

7-18-95

Representative, Quality Assurance Unit   Date

STUDY IDENTIFICATION

Dermal Sensitization Study of CGA-329351 Technical
in Guinea Pigs - Closed Patch Technique
(EPA Guideline 81-6)

Test Material	CGA-329351 Technical, FL-950307 (Batch Code 501004) (Active Ingredient - 96.6%)
Sponsor	Ciba Crop Protection Ciba-Geigy Corporation 410 Swing Road P.O. Box 18300 Greensboro, NC 27419
Sponsor's Representative	[redacted] Ciba Crop Protection Ciba-Geigy Corporation 410 Swing Road P.O. Box 18300 Greensboro, NC 27419 (910) [redacted]
Study Director	[redacted] Corning Hazleton, Inc. P.O. Box 7545 Madison, WI 53707-7545 (608) [redacted]
Study Location	Corning Hazleton, Inc. 3301 Kinsman Boulevard Madison, WI 53704
Study Timetable	
Study Initiation Date	April 7, 1995
Experimental (In-life) Start Date	April 11, 1995
In-life End Date	May 20, 1995
Experimental Termination Date	May 20, 1995
Study Completion Date	July 18, 1995

CHW 50400232

KEY PERSONNEL

Toxicology

5.1.2.e Woo PhD, DABT
Director

Acute Studies

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Study Director
Manager

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Study Coordinator

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In-life Supervisor

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Report Supervisor

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Laboratory Animal Medicine

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Supervisor

CONTENTS

SUMMARY

The delayed contact hypersensitivity potential of CGA-329351 Technical was evaluated in albino guinea pigs. The test material, when applied undiluted during the induction and challenge phases, did not elicit any dermal irritation at challenge. Based on these results, this test material is not considered to be a dermal sensitizer in guinea pigs.

OBJECTIVE

The objective of this study was to assess the delayed contact hypersensitivity potential of a test material in guinea pigs.[1,2,3]

All procedures used in this study were in compliance with the Animal Welfare Act Regulations. In the opinion of the Sponsor and study director, the study did not unnecessarily duplicate any previous work. The dose levels, method, frequency, and duration of administration utilized in this study were chosen based on the requirements of the regulatory test guidelines.

TEST AND POSITIVE CONTROL MATERIALS

Test Material Identification

The test material was identified as CGA-329351 Technical, FL-950307 (Batch Code 501004) and described as a viscous, brown liquid.

Test Material Purity and Stability

The Sponsor assumes responsibility for purity and stability determinations (including under test conditions). A Sponsor-supplied certificate of analysis is retained in the study file. The percent active ingredient was reported to be 96.6%. Analysis of the test material mixtures for concentration, homogeneity/solubility, and stability was not conducted or requested by the Sponsor.

Positive Control Material Identification

The positive control material was identified as 2,4-dinitrochlorobenzene (Sigma Chemical Company, St. Louis, Missouri; Lot 41H08112) and described as a yellow, crystalline powder.

Positive Control Material Purity and Stability

The certificate of analysis, obtained from the supplier, documents the purity of the positive control material as 98.4%. The stability of this reagent grade material was the responsibility of Corning Hazleton (CHW) and was deemed to be adequate for the conduct of this study. The acceptable stability of the

positive control material mixtures under conditions of the test has been determined by CHW in a previous study. Analysis of the positive control material mixtures for concentration, homogeneity/solubility, and stability was not done.

Storage and Retention

The test and positive control materials were stored at room temperature. A reserve sample of the test and positive control materials was taken and will be retained in a freezer set to maintain a temperature of -20°C ±10° for 10 years in accordance with CHW Standard Operating Procedure (SOP). Any unused test material will be discarded after issuance of the final report according to CHW SOP. Any unused positive control material will be retained for future tests until the indicated expiration date is reached.

Safety Precautions

The test and positive control material handling procedures were according to CHW SOPs and policies.

TEST SYSTEM

Test Animal

Young adult albino guinea pigs of the Crl:(HA)BR strain were procured from Charles River Laboratories, Inc., Portage, Michigan on March 13, 1995 for the irritation screening study and on March 27, 1995 for the definitive study.

Housing

After receipt, the animals were acclimated for a period of at least 7 days. During acclimation and throughout the study, the animals were individually housed in screen-bottom stainless steel cages. Environmental controls for the animal room were set to maintain a temperature of 19° to 25°C, a relative humidity of 50% ±20%, and a 12-hour light/12-hour dark lighting cycle. In cases where variations from these conditions existed, they were documented and considered to have had no adverse effect on the study outcome.

Animal Diet

The animals were provided continuous access to Certified Guinea Pig Diet #5026, PMI Feeds, Inc., and water. The feed is routinely analyzed by the manufacturer for nutritional components and environmental contaminants. Samples of the water are periodically analyzed by CHW. There were no known contaminants in the feed or water at levels that would have interfered with or affected the results of the study.

Group Assignments

Twenty-eight healthy, acclimated male albino guinea pigs, identified by animal number and corresponding ear tag and weighing from 405 to 549 g, were selected and divided into four groups consisting of an irritation screening group of four animals, a test group of 10 animals, a naive control group of 10 animals, and a positive control group of four animals.

Justification for Species Selection

Historically, the albino guinea pig has been the animal of choice for skin sensitization studies.

PROCEDURES

Irritation Screening Study

An irritation screening study using four animals was conducted to determine the irritation threshold of the test material. The test material was administered undiluted and at concentrations of 25%, 50%, and 75% w/v in 80% ethanol in deionized water with each animal receiving two different concentrations of the test material. The appropriate test material concentrations, in the amount of 0.4 mL, were applied to adhesive patches (Hill Top Chamber®, 25-mm diameter). The patches were then placed on two shaved sites (one on the right and one on the left dorsal quadrants) on each animal, covered with an overlapping strip of dental dam, and overwrapped with Elastoplast® tape. The patches remained in place for 6 hours after which they were removed and the sites washed with lukewarm tap water and patted dry with a disposable paper towel. The application sites were observed for dermal reactions at approximately 24 and 48 hours after test material application.

Definitive Study

Based on the results of the irritation screening study, the test material was administered undiluted for the induction phase and for the challenge application. All test and positive control material mixtures used in the irritation screening or definitive phases of the study were stored at room temperature until administered.

Induction Phase. On the day of test material application, the hair was removed from the backs of each animal in the test and positive control groups with electric clippers. The undiluted test material was applied to each animal in the test group by placing 0.4 mL on an adhesive patch (Hill Top Chamber®, 25-mm diameter) and placing the patch on the induction site along the dorsal anterior left quadrant. The patch was covered with dental dam and overwrapped with Elastoplast® tape. The dressing remained in place for a period of approximately 6 hours after which it was removed and the induction site was washed with lukewarm tap water and patted dry with a disposable paper towel. The positive control material, 0.3% w/v

2,4-dinitrochlorobenzene (DNCB) in 80% v/v ethanol in deionized water, was administered as a 0.4 mL dose to the positive control animals in the same manner used for the test material. The animals in the test and positive control groups received one application per week for 3 weeks for a total of three applications. Due to the strong irritation present in the induction site of the positive control animals, the third induction dose for these animals was applied to an induction site slightly posterior to the initial site. The naive control animals were not treated during this phase of the study.

Challenge Phase. Two weeks following the administration of the third induction dose, a challenge dose of 0.4 mL of test material was administered along the dorsal anterior right quadrant of the test group animals in the same manner as during the induction phase of the study. At this time the 10 naive (previously untreated) control animals were also treated in the same manner with a challenge application of the test material. The positive control material was administered at a concentration of 0.1% w/v in acetone. The method used for the positive control group was the same as that of the test group.

Reason for Route of Administration

Historically, the dermal route has been the route of choice for determining delayed contact hypersensitization.

Observations

On the day of the 24-hour examination following the irritation screening and challenge applications, the application sites of the respective animals were depilated by applying Neet® depilatory. After approximately 20 minutes the depilatory was washed from the application sites.

The respective application sites were examined and scored for dermal reactions according to the Buehler[4] scoring scale at approximately 24 and 48 hours following the irritation screening, induction, and challenge applications.

Buehler Sensitization Scoring Scale

No reaction	0
Very faint erythema, usually nonconfluent	0.5
Faint erythema, usually confluent	1.0
Moderate erythema	2.0
Strong erythema, with or without edema	3.0

Clinical observations were conducted daily throughout the study. Body weights on the irritation screening animals were determined only on the day of treatment. Body weights on the definitive animals were taken on Day 1, at weekly intervals throughout the study, and at termination of the in-life phase.

Termination

At termination of the in-life phase, all animals were designated to be euthanized and discarded.

Evaluation of Challenge Responses

Determination of sensitization was based on the dermal reactions to the challenge dose. Grades of 1 or greater in the test animals may indicate evidence of sensitization, provided grades of less than I are seen in the naive control animals.

Statistical Analyses

No statistical analyses were required by the protocol.

Location of Raw Data, Records, and Final Report

The raw data, records, and an original signed copy of the final report will be retained in the archives of CHW in accordance with CHW SOP.

RESULTS/DISCUSSION

IRRITATION SCREENING STUDY

Individual body weights and dermal reactions are in Table 1. A very faint erythema reaction was observed in one animal treated with the 50% w/v mixture of test material in 80% ethanol in deionized water. No other dermal irritation was observed. All animals appeared normal during this phase of the study.

DEFINITIVE STUDY

Clinical Observations and Body Weights

Individual body weights are in Table 2 and individual clinical signs are in Table 3. All animals appeared normal throughout the study. There was no meaningful effect on body weight gain.

Dermal Reactions to CGA-329351 Technical

Individual dermal reactions for the test and naive control animals are presented in Table 4. No dermal reactions were observed in the animals in the test group when administered the undiluted test material during the induction or challenge phases of the study. None of the naive control animals reacted to the challenge application of the test material.

Dermal Reactions to the Positive Control (DNCB)

Individual dermal reactions for the positive control animals are presented in Table 4. The positive control animals were considered to have been sensitized because of the moderate dermal reactions they exhibited to the 0.1% w/v concentration of DNCB in acetone at challenge.

Historical data compiled at CHW have indicated that a 0.1% w/v concentration of DNCB in acetone produces only minimal irritation, if any at all, in previously untreated animals when treated in the same manner as for the challenge application.

CONCLUSION

Based on the results obtained, this test material, CGA-329351 Technical, is not considered to be a dermal sensitizer in guinea pigs when tested by the closed patch technique.

SIGNATURE

5.1.2.e Woo

Date 7-18-95

Study Director
Acute Studies

REFERENCES

1. Hitch, R. K., "Dermal Sensitization Study," *Pesticide Assessment Guidelines, Subdivision F, Hazard Evaluation: Human and Domestic Animals*, U.S. Environmental Protection Agency Office of Pesticide and Toxic Substances, Series 81-6, pp. 59-62 (November 1982).

2. "Dermal Sensitization Study," *Guidance on Toxicology Study Data for Application of Agricultural Chemical Registration*, Ministry of Agriculture Forestry and Fisheries, 59 Nohsan No. 4200, January 28, 1985.

3. "Skin Sensitisation," *Organisation for Economic Cooperation and Development's Guidelines for Testing of Chemicals*, Section 406 (adopted May 12, 1981).

4. Buehler, E. V. and Ritz, H. L., "Planning, Conduct, and Interpretation of Guinea Pig Sensitization Patch Tests," *Current Concepts in Cutaneous Toxicity*, p. 28 (1980).

Table 1

Individual Body Weights and Dermal Reactions -
Irritation Screening Study

Animal Number	Body Weight (g)	Dermal Reactions: Concentration (% w/v in 80% ethanol alcohol in deionized water) 25 Hour 24	25 Hour 48	50 Hour 24	50 Hour 48	75 Hour 24	75 Hour 48	100 Hour 24	100 Hour 48
E36642	549	0.0	0.0	*	*	*	*	0.0	0.0
E36643	513	*	*	0.0	0.0	0.0	0.0	*	*
E36644	536	0.0	0.0	*	*	0.0	0.0	*	*
E36645	546	*	*	0.0	0.5	*	*	0.0	0.0

* Concentration not tested on this animal.

Comment: All animals appeared normal throughout the irritation screening study.

Table 2

Individual Body Weights (g) - Definitive Study

Animal Number	Study Day 1	8	15	22	29	31
Test Group						
E36795	497	545	592	636	689	689
E36796	478	524	561	610	654	656
E36797	503	556	618	661	694	704
E36798	515	551	627	664	720	720
E36799	518	553	598	635	703	701†
E36800	533	598	637	699	754	751†
E36801	459	479	512	559	561	575
E36802	499	532	562	600	609	606†
E36803	482	499	557	581	638	638
E36804	438	480	533	587	640	637†
Naive Control Group						
E36805	542	597	669	731	787	789
E36806	512	571	630	684	723	714†
E36807	509	581	635	674	771	769†
E36808	440	471	516	563	595	585†
E36809	480	520	576	603	657	649†
E36810	499	539	604	654	711	709†
E36811	497	522	583	632	661	662
E36812	547	599	674	733	760	765
E36813	405	458	510	547	586	594
E36814	517	592	665	737	813	806†
Positive Control Group						
E36815	452	477	523	563	611	612
E36816	505	572	623	693	762	753†
E36817	528	588	634	655	712	720
E36818	497	523	577	643	672	695

† Denotes weight loss from previous weighing.

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Table 3

Individual Clinical Signs - Definitive Study

Animal Number	Observation	Day 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17-31
	Test Group																	
E36795	Appeared normal	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E36796	Appeared normal	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E36797	Appeared normal	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E36798	Appeared normal	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E36799	Appeared normal	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E36800	Appeared normal	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E36801	Appeared normal	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E36802	Appeared normal	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E36803	Appeared normal	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E36804	Appeared normal	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
	Naive Control Group																	
E36805	Appeared normal	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E36806	Appeared normal	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E36807	Appeared normal	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E36808	Appeared normal	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E36809	Appeared normal	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E36810	Appeared normal	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E36811	Appeared normal	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E36812	Appeared normal	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E36813	Appeared normal	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E36814	Appeared normal	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓

✓ Condition existed.

Table 3 (Continued)

Individual Clinical Signs - Definitive Study

Animal Number	Observation	Day 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17-31
		Positive Control Group																
E36815	Appeared normal	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E36816	Appeared normal	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E36817	Appeared normal	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E36818	Appeared normal	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓

✓ Condition existed.

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Table 4

Individual Dermal Reactions - Definitive Study

Animal Number	Induction Phase						Challenge Phase	
	Dose #1		Dose #2		Dose #3†			
	Hour		Hour		Hour		Hour	
	24	48	24	48	24	48	24	48
Test Group								
E36795	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
E36796	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
E36797	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
E36798	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
E36799	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
E36800	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
E36801	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
E36802	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
E36803	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
E36804	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Naive Control Group								
E36805	*	*	*	*	*	*	0.0	0.0
E36806	*	*	*	*	*	*	0.0	0.0
E36807	*	*	*	*	*	*	0.0	0.0
E36808	*	*	*	*	*	*	0.0	0.0
E36809	*	*	*	*	*	*	0.0	0.0
E36810	*	*	*	*	*	*	0.0	0.0
E36811	*	*	*	*	*	*	0.0	0.0
E36812	*	*	*	*	*	*	0.0	0.0
E36813	*	*	*	*	*	*	0.0	0.0
E36814	*	*	*	*	*	*	0.0	0.0
Positive Control Group								
E36815	1.0	2.0	3.0[n]	3.0[n]	2.0	2.0	2.0	2.0
E36816	1.0	2.0	3.0[n]	3.0[n]	2.0	2.0	2.0	2.0
E36817	2.0	2.0	3.0[a]	3.0[a]	2.0	2.0	2.0	2.0
E36818	2.0	2.0	3.0[n]	3.0[n]	2.0	2.0	2.0	2.0

* Animals not treated during the induction phase of the study.
a Subcutaneous hemorrhaging.
n Possible necrotic area.
† Due to the presence of strong irritation, the third induction dose for the positive control animals was moved to a site just posterior to the initial induction site.