A 01

CODING FORMS FOR SRC INDEXING

Microfiche No.		
OTS0560025		
New Doc ID	Old Doc ID	
88010000102S	8EHQ-0401-14890S	
Date Produced	Date Received	TSCA Section
03/20/01	04/02/01	8E
Submitting Organization		
CIBA SPECIALTY CHEMS CORP		
Contractor		
COVANCE LABS INC		
Document Title		
INITIAL SUBMISSION: LTR FROM CIBA SPEC CHEMS CORP TO USEPA, SUBM OF HEALTH EFFECTS STUDIES OF AGEFLEX AMA & FM4 (MIXT, []), & CGI 1397 [], W/ATTCHMTS & DATED 3/29/01 (SANITIZED)		
Chemical Category		
AGEFLEX AMA, AGEFLEX FM4, CGI 1397 (CONFIDENTIAL)		

A.03

Ciba Specialty Chemicals
North America

MR 46170

8EHQ-0401-14890

COMPANY SANITIZED

Ciba

March 29, 2001

Via Federal Express
Confidential

RECEIVED
01 APR -2 PM 3:37

Document Control Office (7407)
Room G99 East Tower
Attention: Section 8(e)
Office of Pollution Prevention and Toxics
Environmental Protection Agency
401 M Street, SW
Washington, DC 20460-0001

SANITIZED COPY

Subject: TSCA 8(e) Notices - a) CGI 1397; b) Ageflex AMA; c) Ageflex FM4

Dear Section 8(e) Coordinator:

This letter contains Confidential Business Information. Confidential Information Bracketed { }. The attached reports do not contain CBI.

In accordance with EPA's March 16, 1978 Policy Statement on Section 8(e) reporting under the Toxic Substances Control Act (TSCA), the EPA's June, 1991 TSCA Section 8(e) Reporting Guide, Ciba Specialty Chemicals Corporation wishes to bring to the attention of the Environmental Protection Agency results observed in three separate studies:

- An acute oral toxicity study was conducted on a commercial product Ageflex AMA which is a mixture of 2-Propenoic acid, 2-methyl-, propyl ester (CASRN 2210-28-8) and 2-Propenoic acid, 2-methyl-, 2-propenyl ester (CASRN 96-05-9). We are enclosing a copy of the study entitled, ***"Acute Oral Toxicity Study of Ageflex AMA in Rats"***. Male and female rats were dosed at 100, 400, 600 mg/kg of body weight. All animals treated at 100 mg/kg appeared normal and exhibited body weight gain throughout the study. Three males and two females treated at 400 mg/kg died within 6 days of treatment, whereas four females and four males treated at 600 mg/kg died within 3 days of treatment. Clinical signs of toxicity were observed in animals treated at 400 and 600 mg/kg. All surviving animals returned to a normal appearance by day 14 after treatment. The estimated oral LD50 values in rats were determined to be 400, 455, and 431 mg/kg for males, females, and the sexes combined, respectively.

- A dermal irritation/corrosion study was conducted on a commercial product Ageflex FM4, which chemically is 2-Propenoic acid, 2-methyl-, 2-[(1,1-dimethylethyl) amino] ethyl ester (CASRN 3775-90-4). We are enclosing a copy of the study entitled, ***"Primary Dermal Irritation/Corrosion Study of Ageflex FM4 in Rabbits"***. Albino rabbits were exposed to 3-minute, 1-hour, or 4-hour periods. No evidence of corrosion but very slight erythema and slight edema reactions were observed at the three test sites receiving 3-minute exposure. Corrosive reactions were observed at one site receiving the 1-hour exposure and at all three

2001 APR -5 AM 10:27

8EHQ-01-14490
88 0100001028

540 White Plains Road
Tarrytown, New York 10591

Tel. 914-785-4311
Fax 914-785-4147

test sites receiving the 4-hour exposure. Severe erythema and moderate to severe edema reactions were observed at all three 4-hour exposure sites.

- A contact hypersensitivity maximization-test was conducted on an experimental photoacid generator, CGI 1397, which chemically is {[redacted]}. We are enclosing a copy of the study entitled, ***"Dermal Sensitization Study of CGI 1397 in Guinea Pigs - Maximization Test"***. Five of the 20 animals in the test group exhibited intense dermal reactions and 15 of 20 exhibited mild to moderate dermal reactions to the initial challenge application of the test material. A second challenge dose was conducted 19 days after the initial challenge application. All animals exhibited mild to intense dermal reactions to the second challenge application. Based on the number of animals that exhibited sensitization reactions, CGI 1397 is considered to be a skin sensitizer.

Based upon current EPA guidelines, it is felt these results warrant reporting under TSCA 8(e). A sanitized copy of this letter as well as a Confidential Business Information (CBI) Substantiation for CGI 1397 is also enclosed. The reports do not contain CBI, but an additional "sanitized" copy has been included for your distribution. Please call the undersigned if you have any questions concerning this submittal.

Respectfully,

Ciba Specialty Chemicals Corporation

Thomas Barber
Manager, Regulatory Compliance

RECEIVED

A 05..

Confidentiality Substantiation for CGI 1397

SANITIZED COPY

Substantiation Questions

1. Is your company asserting this confidential business information (CBI) claim on its own behalf? If the answer is no, please provide the company name, address, and telephone number of entity asserting claim.

   Yes

2. For what period do you assert your claim(s) of confidentiality? If the claim is to extend until a certain event or point in time, please indicate that event or time period. Explain why such information should remain confidential until such point.

   Confidential treatment is requested indefinitely.

3. Has the information that you are claiming as confidential been disclosed to any other governmental agency, or to this Agency at any other time? Identify the Agency to which the information was disclosed and provide the date and circumstances of the same. Was the disclosure accompanied by a claim of confidentiality? If yes, attach a copy of said document reflecting the confidentiality agreement.

   The confidential information concerning this product has been disclosed to the EPA under PMN P00-1189. An NOC has not yet been filed for this product.

4. Briefly describe any physical or procedural restrictions within your company relating to use and storage of the information you are claiming CBI.

   The precise chemical identity of this compound is known only to selected research, product safety, and product management in the US. The composition cards/data sheets are marked "Confidential" and treated accordingly. Such information files are kept in a secure location and are not available to other members of Ciba except on a need-to-know basis.

5. If anyone outside your company has access to any of the information claimed CBI, are they restricted by confidentiality agreement(s)? If so, explain the content of the agreement(s).

   No one outside of Ciba Specialty Chemicals in the United States has access to the information.

6. Does the information claimed as confidential appear or it referred to in any of the following:

   a. Advertising or promotional material for the chemical substance or the resulting end product;

      No

   b. Material Safety Data Sheets or other similar materials (such as technical data sheets) for the substance or resulting end product (Include copies of this information as it appears when accompanying the substance and/or product at the time of transfer or sale);

      No

   c. Professional or trade publications; or

      No

d. Any other media or publications available to the public or to your competitors.

No

If you answered yes to any of the above, indicate where the information appears, include copies, and explain why it should nonetheless be treated as confidential.

7. Has EPA, another federal agency, or court made any confidentiality determination regarding information associated with this substance? If so, provide copies of such determinations.

No

8. Describe the substantial harmful effects that would result to your competitive position if the CBI information is made available to the public? In your answer, explain the causal relationship between disclosure and any resulting substantial harmful effects. Consider in your answer such constraints as capital and marketing cost, specialized technical expertise or unusual processes, and your competitors' access to your customers. Address each piece of information claimed CBI separately.

This compound is being evaluated as a promising photoacid generator for the semiconductor industry. Ciba's competitors in the similar business could easily begin similar research endeavors were they to learn, via this 8(e) submission, the precise identity of this compound. Obviously, their work would be facilitated because they would not have to perform the extensive research screening which Ciba Specialty Chemicals did to arrive at this specific compound. It is quite conceivable that such a competitor could develop a similar product which would be as effective as any we may develop; it is possible to do so in a shorter period of time. This would clearly limit or defeat Ciba's efforts to develop a commercially viable product based upon the chemistry presented in the 8(e) notification. Ciba Specialty Chemicals would have expended the time and money for R&D, but the benefit of return would have gone to others.

9. Has the substance been patented in the US or elsewhere? Is the patent for the substance currently pending?

The 8(e) substance holds a world patent WO99/01429 dated January 14, 1999 and US Patent Number 6,004,724, dated December 21, 1999.

10. Is this substance/product commercially available and if so, for how long has it been available on the commercial market?

This substance is not commercially available.

a. If on the commercial market, are your competitors aware that the substance is commercially available in the US?

N/A

b. If not already commercially available, describe what stage of research and development (R&D) the substance is in, and estimate how soon a market will be established.

This product is in the early stage of applications R&D; a commercial market has not yet been established in the US.

c. What is the substance used for and what type of product(s) does it appear in?

This product is being evaluated as a photoacid generator for resists in semiconductor and display manufacturing.

11. Describe whether a competitor could employ reverse engineering to identically recreate the substance.

    Based upon the chemical structure, a competitor could very well employ reverse engineering to recreate the compound.

12. Do you assert that disclosure of this information you are claiming CBI would reveal:

    a. confidential processes used in manufacturing the substance;

       No

    b. if a mixture, the actual portions of the substance in the mixture, or;

       N/A

    c. information unrelated to the effects of the substance on human health or the environment?

       No

    If your answer to any of the above questions is yes, explain how such information would be revealed.

13. Provide the Chemical Abstract Service Registry Number for the product, if known. Is your company applying for a CAS number now or in the near future? If you have applied for a CAS number, include a copy of the contract with CAS.

    The CASRN for this product is { ■ }

14. Is the substance or any information claimed CBI the subject of FIFRA regulation or reporting? If so, explain.

    No

A.08.

Final Report

Primary Dermal Irritation/Corrosion Study of AGEFLEX FM4 in Rabbits

SANITIZED COPY

PREPARED FOR:
Ciba Specialty Chemicals Corporation

COVANCE STUDY NUMBER:
00706545

ISSUE DATE:
08 December 2000

Covance Laboratories Inc.
P.O. Box 7545
Madison, WI 53707-7545
Deliveries: 3301 Kinsman Blvd., Madison, WI 53704
Tel: 608/241-4471 Fax: 608/241-7227

A-09

COVANCE
THE DEVELOPMENT SERVICES COMPANY

Sponsor:

Ciba Specialty Chemicals Corporation
Tarrytown, New York

FINAL REPORT

Study Title:

Primary Dermal Irritation/Corrosion Study of AGEFLEX FM4 in Rabbits
(EPA/OECD Guidelines)

Author:

Steven M. Glaza

Study Completion Date:

08 December 2000

Testing Facility:

Covance Laboratories Inc.
3301 Kinsman Boulevard
Madison, WI 53704

Laboratory Study Identification:

Covance 00706545

COMPLIANCE STATEMENT

Primary Dermal Irritation/Corrosion Study of AGEFLEX FM4 in Rabbits
(EPA/OECD Guidelines)

This study was conducted in accordance with the following Good Laboratory Practice Standards:

United States Environmental Protection Agency TSCA:
Good Laboratory Practice Standards, 40 CFR 792

Organisation for Economic Co-operation and Development Principles of Good Laboratory Practice, ENV/MC/CHEM(98)17

Steven M. Glaza
Study Director
Covance Laboratories Inc.

08 DEC 2000
Date

Richard Bokowski
Sponsor's Representative

24 March 2001
Date

QUALITY ASSURANCE STATEMENT

This report has been reviewed by the Quality Assurance Unit of Covance Laboratories Inc., in accordance with the Environmental Protection Agency (EPA) Good Laboratory Practice Standards, 40 CFR 792 and the Organisation for Economic Cooperation and Development (OECD) Principles of Good Laboratory Practice, ENV/MC/CHEM(98)17. The following inspections were conducted and findings reported to the study director and study director management.

Inspection Dates From	Inspection Dates To	Phase	Date Reported to Study Director and Study Director Management
09/07/00	09/07/00	Protocol Review	09/07/00
09/13/00	09/13/00	Test Article Administration	09/13/00
10/03/00	10/03/00	Protocol Amendment Review	10/03/00
11/27/00	11/27/00	Data Review	11/27/00
11/27/00	11/27/00	Report Review	11/27/00

Representative, Quality Assurance Unit

08 Dec 2000
Date

STUDY IDENTIFICATION

Primary Dermal Irritation/Corrosion Study of AGEFLEX FM4 in Rabbits
(EPA/OECD Guidelines)

Test Material	AGEFLEX FM4
Sponsor	Ciba Specialty Chemicals Corporation 540 White Plains Road Tarrytown, NY 10591
Sponsor's Representative	Richard Balcomb Ciba Specialty Chemicals Corporation Telephone No.: 914.785.4496 Facsimile No.: 914.785.4147
Study Director	Steven M. Glaza Covance Laboratories Inc. Telephone No.: 608.241.7292 Facsimile No.: 608.242.7936
Testing Facility	Covance Laboratories Inc. 3301 Kinsman Boulevard Madison, WI 53704
Study Timetable	
Study Initiation Date	08 September 2000
Experimental Start Date	12 September 2000 (animal selection) 13 September 2000 (initiation of treatment)
Experimental Termination Date	07 October 2000

KEY PERSONNEL

Toxicology

Steven M. Glaza
Study Director

Rose M. Bridge
Administrative Supervisor

Toxicology Operations

Jeffrey B. Hicks
In-life Supervisor

Quality Assurance

Nancy M. Centanni
Manager

Laboratory Animal Medicine

Donna J. Clemons, DVM
Diplomate, ACLAM
Associate Director

CONTENTS

COMPLIANCE STATEMENT 2

QUALITY ASSURANCE STATEMENT 3

STUDY IDENTIFICATION 4

KEY PERSONNEL 5

SUMMARY 8

OBJECTIVE 8

TEST MATERIAL 8
- Identification 8
- Purity and Stability 9
- Storage and Retention 9

TEST SYSTEM 9
- Test Animal 9
- Housing 9
- Animal Diet 9
- Animal Selection and Preparation of Exposure Area 10
- Justification for Species Selection 10

PROCEDURES 10
- Preparation of Test Material 10
- Treatment 10
- Reason for Route of Administration 10
- Observations 11
- Termination 11
- Statistical Evaluation 11
- Location of Raw Data, Records, and Final Report 11

RESULTS/DISCUSSION 11

SIGNATURE 12

REFERENCES 13

TABLE

Primary Dermal Irritation Scoring Scale (Draize Technique) 14
1 Individual Dermal Irritation Scores - 3-Minute Exposure Period 15
2 Individual Dermal Irritation Scores - 1-Hour Exposure Period 16
3 Average Primary Dermal Irritation Scores 17
4 Individual Body Weights (g) 17

APPENDIX 18
Protocol Deviation 18

SUMMARY

The primary dermal irritation/corrosion potential of AGEFLEX FM4 was evaluated when applied to the skin of rabbits under 3-minute, 1-hour, and 4-hour semioccluded conditions. Evidence of corrosion was not observed at any of the three test sites receiving the 3-minute exposure. Reactions indicative of corrosion were observed at the one test site receiving the 1-hour exposure and at all three test sites receiving the 4-hour exposure. Based on these results, this test material is considered to be corrosive to the skin of rabbits under 1-hour and 4-hour semioccluded conditions.

OBJECTIVE

The objective of this study was to assess the relative level of primary skin irritation/corrosion of a test material on rabbits under semioccluded conditions.[1,2,3]

All procedures used in this study were in compliance with the Animal Welfare Act, the Guide for the Care and Use of Laboratory Animals, and the Office of Laboratory Animal Welfare. In the opinion of the Sponsor and study director, the study did not unnecessarily duplicate any previous work. All procedural times presented in this report fall within the acceptable ranges as specified in Covance standard operating procedure (SOP).

TEST MATERIAL

Identification

The test material was identified as AGEFLEX FM4, Lot No. 0019400047, and described as a clear colorless liquid.

Purity and Stability
Information concerning the test material's purity and stability (including under test conditions) was provided by the sponsor and is retained in the study file.

Storage and Retention
The test material was stored at room temperature. Any unused test material will be discarded after issuance of the final report according to Covance SOP.

TEST SYSTEM

Test Animal
Adult albino rabbits of the Hra:(NZW)SPF strain were procured from Covance Research Products Inc., Kalamazoo, Michigan, on August 16 and September 13, 2000.

Housing
After receipt, the animals were acclimated for a period of at least 5 days. During acclimation and throughout the study, the animals were individually housed in suspended, stainless steel cages with the exception of one female (F05641) that was found out of the cage at the time of unwrapping. Environmental controls for the animal room were set to maintain a temperature of 16 to 22°C, a relative humidity of 30 to 70%, and a 12-hour light/12-hour dark 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 access to water *ad libitum* and a measured amount of high-fiber rabbit diet (#5326, Purina Mills, Inc.). The diet is routinely analyzed by the manufacturer for nutritional components and environmental contaminants. Samples of the water are periodically analyzed. There were no known contaminants in the diet or water at levels that would be expected to interfere with or affect the results of the study.

Animal Selection and Preparation of Exposure Area
One male and 5 female healthy, acclimated rabbits, weighing from 2,416 to 2,673 g and approximately 16 to 18 weeks of age, were utilized in this study. The animals were selected at random and identified by animal number and corresponding ear tag. On the day before treatment, the back and/or flanks of each animal were clipped free of hair to obtain the appropriate number of unblemished skin sites.

Justification for Species Selection
Historically, the New Zealand White albino rabbit has been the animal of choice for evaluating the effect of chemicals on the skin.

PROCEDURES

Preparation of Test Material
The test material was administered as received. Due to the insolubility of the test material in water, the pH was not determined.

Treatment
Initially, three rabbits received the undiluted test material applied to a single intact site on each rabbit's back under 4-hour semioccluded conditions. Due to the immediate corrosive responses observed in these animals, an additional single rabbit was treated with the test material in a similar manner under 3-minute and 1-hour semioccluded conditions. Based on the results from this one animal, two additional animals were treated with the test material under 3-minute semioccluded conditions.

On each rabbit, the area(s) of application was covered with an 8-ply 2.5-cm x 2.5-cm gauze patch secured with paper tape, loosely overwrapped with Saran Wrap®, and secured with Elastoplast® tape to provide a semiocclusive dressing. The wrappings applied to each test site were independent of the other sites, as applicable.

Reason for Route of Administration
Historically, the dermal route has been the route of choice based on the method of Draize.

Observations

At the end of the 3-minute, 1-hour, or 4-hour exposure periods, the appropriate patch from each animal was removed and the treated area was examined for evidence of corrosion. After the initial corrosion examination, any residual test material was removed using liquid Ivory® soap mixed with water, rinsed with water, and dried with disposable paper towels to prevent any further test material exposure. The test material was removed from the test sites as thoroughly as possible without irritating the skin. In the one animal receiving the 3-minute and 1-hour exposures, care was taken at the time of the first patch removal not to interfere with the semiocclusive overwrap of the remaining patch. Sixty minutes after patch removal for the animals receiving the 3-minute and/or 1-hour exposures, the appropriate application site was examined for erythema and edema reactions according to the Draize[4] technique (recorded as the 3-minute or 1-hour score). Subsequent examinations for these animals were made at 24, 48, 72, and 96 hours. The untreated skin of each animal was used for comparison.

Animals were weighed before test material administration.

Termination

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

Statistical Evaluation

No statistical evaluations 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 Covance in accordance with Covance SOP.

RESULTS/DISCUSSION

Individual dermal irritation scores for the 3-minute and 1-hour exposure periods are in Tables 1 and 2, respectively. Average primary dermal irritation scores and individual body weights are in Tables 3 and 4, respectively.

The primary dermal irritation/corrosion potential of AGEFLEX FM4 was evaluated when applied to the skin of rabbits under 3-minute, 1-hour, and 4-hour semioccluded conditions. No evidence of corrosion was observed at the three test sites receiving the 3-minute exposure. Very slight erythema and slight edema reactions were observed at the 3-minute exposure sites. Reactions indicative of corrosion (i.e. necrotic appearing areas or skin ulceration) were observed at the one test site receiving the 1-hour exposure and at all three test sites receiving the 4-hour exposure. At the time of patch/test material removal for the animals receiving the 4-hour exposure, the test site of one animal was ulcerated. In addition, severe erythema and moderate to severe edema reactions along with necrotic appearing areas were observed at all three 4-hour exposure sites. Due to the severity of the dermal reactions at the 4-hour exposure sites, the animals were immediately sacrificed for humane reasons.

SIGNATURE

Steven M. Glaza
Study Director
Covance Laboratories Inc.

08 DEC 2000
Date

REFERENCES

1. *Code of Federal Regulations*, 49 CFR 173.136 (1997).

2. United States Environmental Protection Agency; Prevention, Pesticides and Toxic Substances; "OPPTS 870.2500 Acute Dermal Irritation"; *Health Effects Test Guidelines* (August 1998).

3. "Acute Dermal Irritation/Corrosion," *Organisation for Economic Co-operation and Development Guidelines for Testing of Chemicals*, Section 4, Health Effects, Number 404, Paris Cedex (July 17, 1992).

4. Draize, J. H., "Primary Irritation of the Skin," In: *Appraisal of the Safety of Chemicals in Foods, Drugs, and Cosmetics - Dermal Toxicity*, Association of Food and Drug Officials of the U.S., pp. 46-47 (1959).

Primary Dermal Irritation Scoring Scale
(Draize Technique)

(1) Erythema and Eschar Formation

No erythema	0
Very slight erythema (barely perceptible)	1
Well-defined erythema	2
Moderate to severe erythema	3
Severe erythema (beet redness) to slight eschar formation (injuries in depth)	4
Highest possible erythema score	4

(2) Edema Formation

No edema	0
Very slight edema (barely perceptible)	1
Slight edema (edges are well defined by definite raising)	2
Moderate edema (raised approximately 1 mm)	3
Severe edema (raised approximately 1 mm and extending beyond area of exposure)	4
Highest possible edema score	4

Corrosion will be considered to have resulted if the substance in contact with the rabbit skin has caused destruction of irreversible alteration of the tissue. Tissue destruction is considered to have occurred if, at any of the readings, there is ulceration or necrosis. Tissue destruction does not include merely sloughing of the epidermis, or erythema, edema, or fissuring.

C 01

Final Report

Dermal Sensitization Study of CGI 1397 in Guinea Pigs - Maximization Test

SANITIZED
COPY

PREPARED FOR:
Ciba Specialty Chemicals Corporation

COVANCE STUDY NUMBER:
01107721

ISSUE DATE:
20 March 2001

Covance Laboratories Inc.
Box 7545
Madison, WI 53707-7545
Deliveries: 3301 Kinsman Blvd., Madison, WI 53704
Tel: 608/241-4471 Fax: 608/241-7227

COVANCE
THE DEVELOPMENT SERVICES COMPANY

Sponsor:

Ciba Specialty Chemicals Corporation
Tarrytown, New York

FINAL REPORT

Study Title:

Dermal Sensitization Study of CGI 1397
in Guinea Pigs – Maximization Test
(EPA/EC Guidelines)

Author:

Steven M. Glaza

Study Completion Date:

20 March 2001

Testing Facility:

Covance Laboratories Inc.
3301 Kinsman Boulevard
Madison, Wisconsin 53704-2595

Laboratory Project Identification:

Covance 01107721

C 03

COMPLIANCE STATEMENT

Dermal Sensitization Study of CGI 1397
in Guinea Pigs - Maximization Test
(EPA/EC Guidelines)

This study was conducted in accordance with the following Good Laboratory Practice Standards with the exception that analysis of the test and control material mixtures for concentration, homogeneity/solubility, and stability was not conducted and that specific purity and stability data for the test material were not provided to Covance Laboratories Inc. at the time the study was conducted:

United States Environmental Protection Agency TSCA:
Good Laboratory Practice Standards, 40 CFR 792

Organisation for Economic Co-operation and Development Principles of Good Laboratory Practice, ENV/MC/CHEM(98)17

Steven M. Glaza
Study Director
Covance Laboratories Inc.

20 MAR 2001
Date

Richard Bolcomb
Sponsor's Representative

24 March 2001
Date

QUALITY ASSURANCE STATEMENT

This report has been reviewed by the Quality Assurance Unit of Covance Laboratories Inc., in accordance with the Environmental Protection Agency (EPA) Good Laboratory Practice Standards, 40 CFR 792 and the Organisation for Economic Cooperation and Development (OECD) Principles of Good Laboratory Practice, ENV/MC/CHEM(98)17. The following inspections were conducted and findings reported to the study director and study director management.

Inspection Dates From	Inspection Dates To	Phase	Date Reported to Study Director and Study Director Management
30 Nov 00	30 Nov 00	Protocol Review	01 Dec 00
19 Dec 00	19 Dec 00	Animal Observation	19 Dec 00
02 Mar 01	02 Mar 01	Protocol Amendment Review	02 Mar 01
05 Mar 01	07 Mar 01	Data Review	07 Mar 01
05 Mar 01	07 Mar 01	Report Review	07 Mar 01

Representative, Quality Assurance Unit

20 Mar 2001
Date

STUDY IDENTIFICATION

Dermal Sensitization Study of CGI 1397
in Guinea Pigs - Maximization Test
(EPA/EC Guidelines)

Test Material:	CGI 1397
Sponsor:	Ciba Specialty Chemicals Corporation 540 White Plains Road Tarrytown, New York 10591
Sponsor's Representative:	Richard Balcomb Ciba Specialty Chemicals Corporation Telephone No.: 914.785.4496 Facsimile No.: 914.785.4147
Study Location:	Covance Laboratories Inc. 3301 Kinsman Boulevard Madison, Wisconsin 53704-2595
Study Director:	Steven M. Glaza Covance Laboratories Inc. Telephone No.: 608.241.7292 Facsimile No.: 608.242.7936

Study Timetable

Study Initiation Date:	01 December 2000
Experimental Start Date:	28 November 2000 (selection of animals) 05 December 2000 (initiation of treatment)
Experimental Termination Date:	25 January 2001

KEY PERSONNEL

Study Director, Toxicology:	Steven M. Glaza, BS
Administrative Supervisor, Toxicology:	Rose M. Bridge
In-Life Supervisor, Toxicology Operations:	Jeffrey B. Hicks, LAT
Associate Director, Laboratory Animal Medicine:	Donna J. Clemons, DVM, MS Diplomate, ACLAM

C 07

CONTENTS

COMPLIANCE STATEMENT 2

QUALITY ASSURANCE STATEMENT 3

STUDY IDENTIFICATION 4

KEY PERSONNEL 5

SUMMARY 8

OBJECTIVE 10

TEST AND CONTROL MATERIALS 10
- Test Material 10
- Test Material Purity and Stability 10
- Test Material Storage and Retention 10
- Control Material Identification 11
- Control Material Purity and Stability 11
- Control Material Storage and Retention 11

TEST SYSTEM 11
- Test Animal 11
- Housing 12
- Animal Diet 12
- Group Assignments 12
- Justification for Species Selection 13

PROCEDURES 13
- Irritation Screening Study 13
- Definitive Study 14
- Reason for Route of Administration 16
- Observations 16
- Termination 16
- Statistical Evaluation 16
- Location of Raw Data, Records, and Final Report 17

DISCUSSION 17
- Irritation Screening Study 17
- Definitive Study 17

C 08

CONCLUSION 18

SIGNATURE 19

REFERENCES 19

TABLE
Dermal Reaction Scoring Scale 20
1 Individual Body Weights and Dermal Reactions - Irritation Screening Study 21
2 Individual Body Weights (g) - Definitive Study 22
3 Individual Clinical Signs - Definitive Study 24
4 Summary of Individual Daily Observations of Dermal Reactions at Induction Sites 26
5 Individual Dermal Reactions to Challenge Application - Definitive Study 27

APPENDIX 1 30
Protocol Deviation 30

APPENDIX 2 31
Positive Control Report 32

SUMMARY

In an irritation screening study, a group of four male guinea pigs received 0.1-mL intradermal injections of CGI 1397 prepared at concentrations of 0.1, 0.5, and 1% w/v in mineral oil (three test sites per animal). The test sites were evaluated for dermal reactions at 24 and 48 hours after injection. A second irritation screening group of four male guinea pigs first received duplicate 0.1-mL intradermal injections of a 1:1 dilution of Freund's Complete Adjuvant (FCA) and sterile water on the shaved shoulder area (one injection on each side of the midline). Seven days after administration of the intradermal injections, the animals received a topical application of CGI 1397 at concentrations of 5, 10, 20, and 40% w/w in petrolatum with each animal receiving all four concentrations of the test material (four test sites per animal). The test sites were occluded for 24 hours and dermal irritation was evaluated at 24 and 48 hours after removal of the patches.

Based on the results of the irritation screening studies, the test material was administered as a 1% w/v mixture in mineral oil and in 1:1 FCA in sterile water for the intradermal injection phase. The test material was administered as a 40% w/w mixture in petrolatum for the topical induction and challenge applications in the definitive study. Twenty animals were assigned to the test group and 10 animals were assigned to the irritation control group. On Day 1, the animals in the test and irritation control groups received the following duplicate 0.1-mL intradermal injections on the shoulder area:

	Description of Injections	
	Test Group	Irritation Control Group
Anterior Sites (A and B)	1:1 dilution of FCA in sterile water	
Medial Sites (C and D)	1% w/v suspension of test material in mineral oil	Vehicle (mineral oil)
Posterior Sites (E and F)	1% w/v suspension of test material in mineral oil and 1:1 FCA/sterile water	1:1 dilution of mineral oil in 1:1 FCA/sterile water

Six days later, the animals in the test and irritation control groups were pretreated with 10% w/w sodium lauryl sulfate (SLS) in petrolatum applied topically at the injection sites. On Day 8, a 40% w/w mixture of the test material in petrolatum was applied topically over the injection sites of the animals in the test group and petrolatum was applied over the injection sites of the animals in the irritation control group. All induction sites were then occluded for 48 hours.

Two weeks after the topical induction application, all animals received a challenge dose. A 40% w/w mixture of the test material in petrolatum was applied to the right side and the control material (petrolatum) was applied to the left side of each animal in the test and initial irritation control groups. All test and control sites were occluded for 24 hours. Any residual material was then removed using water, liquid Ivory® soap, and disposable paper towels. The challenge sites were examined for dermal reactions at 24 and 48 hours after patch removal.

To further examine the sensitizing potential of this test material, a second challenge dose was conducted 19 days after the initial challenge application. A new naive site was selected on the test animals and on 5 new irritation control animals. Each animal in the test and additional irritation control groups received an application of a 25% w/w mixture of the test material in petrolatum in the same manner as the initial challenge application. The initial irritation control animals were not treated during this phase of the study.

Five of the 20 animals (25%) in the test group exhibited intense dermal reactions and 15 of 20 test animals exhibited mild to moderate dermal reactions to the initial challenge application of the test material. Subcutaneous hemorrhaging, desquamation, and/or fissuring were also observed within the challenge sites of six animals in the test group. Four of the 10 animals in the initial irritation control group exhibited mild to moderate dermal reactions to the test material at challenge.

All animals (100%) in the test group exhibited mild to intense dermal reactions to the second challenge application of the test material. Subcutaneous hemorrhaging, blanching, and/or fissuring were also observed within the test sites of 12 animals in the test group. None of the 5 animals in the second irritation control group exhibited a dermal reaction to the second challenge application of the test material.

Five of the 20 test animals were considered to have had sensitization responses to the initial challenge application of the test material. During the second challenge phase, all 20 animals in the test group were considered to have had sensitization responses to the second challenge application of the test material.

The EC guideline requires a response of at least 30% (i.e., six of 20 guinea pigs) to classify a compound as a skin sensitizer by an adjuvant-type test method (Magnusson and Kligman maximization assay[1]). Based on the number of animals in the test group that are

considered to have exhibited sensitization reactions during the second challenge phase (20 of 20 animals), CGI 1397 is considered to be a skin sensitizer and the risk phrase R43 "may cause sensitization by skin contact" is appropriate under terms of the General Classification and Labeling Requirements for Dangerous Substances and Preparations, as stated in Annex IV to Commission Directive 93/21/EEC.[2]

OBJECTIVE

The objective of this study was to assess the contact sensitization potential of a test material when administered by intradermal injection and topical application in guinea pigs.[3,4]

All procedures used in this study were in compliance with the Animal Welfare Act, the Guide for the Care and Use of Laboratory Animals, and the Office of Laboratory Animal Welfare. In the opinion of the sponsor and study director, the study did not unnecessarily duplicate any previous work. All procedural times presented in this report fall within the acceptable ranges as specified in Covance standard operating procedure (SOP).

TEST AND CONTROL MATERIALS

Test Material

The test material was identified as CGI 1397, Lot No. ADJ042-036, and described as a yellow powder.

Test Material Purity and Stability

The sponsor is responsible for the purity and stability of the test material (including under test conditions). Specific purity and stability information was not provided to Covance at the time of this study conduct.

Test Material Storage and Retention

The test material was stored in a refrigerator set to maintain a temperature of 2 to 8°C. A reserve sample of the test material was taken and maintained in a freezer set to maintain a

temperature of -10 to -30°C in accordance with Covance SOP. Any unused test material will be discarded after issuance of the final report according to Covance SOP.

Control Material Identification

The control materials used in this study were mineral oil (Mallinckrodt, Lot No. 6357 KXPM, expiration date 15 August 2001); and petrolatum (Fisher, Lot. No. 980347, expiration date 19 March 2001).

Control Material Purity and Stability

The purity and stability of the mineral oil and petrolatum are the responsibility of Covance and were deemed adequate for the conduct of this study.

Control Material Storage and Retention

The mineral oil and petrolatum were stored at room temperature. A reserve sample of the control materials was taken and will be retained in a freezer set to maintain a temperature of -10 to -30°C in accordance with Covance SOP. Any unused control material will be retained by Covance for future tests until the indicated expiration date is reached.

TEST SYSTEM

Test Animal

Young adult albino guinea pigs of the Crl:(HA)BR strain were procured from Charles River Laboratories, as follows:

Identification	Date Received	Supplier Location
Irritation screens	14 November 2000	Raleigh, North Carolina
Test, initial irritation control	04 December 2000	Portage, Michigan
Second irritation control	09 January 2001	Kingston, New York

Housing

After receipt, the animals were acclimated for a period of at least 5 days. During acclimation and throughout the study, the animals were individually housed in suspended, stainless-steel cages. Environmental controls for the animal room were set to maintain a temperature of 18° to 26°C, a relative humidity of 30 to 70%, and a 12-hour light/12-hour dark 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, Purina Mills, Inc.) and water. The diet is routinely analyzed by the manufacturer for nutritional components and environmental contaminants. Samples of the water are periodically analyzed. There were no known contaminants in the diet or water at levels that would be expected to interfere with or affect the results of the study. Vegetables were provided as environmental enrichment, but did not require analysis.

Group Assignments

Thirty-eight healthy, acclimated male albino guinea pigs, weighing from 354 to 499 g and approximately 5 to 6 weeks of age, were identified by animal number and corresponding ear tag and divided into the following treatment groups:

Identification	Number of Animals
Irritation screening study	
Intradermal injection group	4
Topical application group	4
Definitive study	
Test group	20
Irritation control group	10

In addition, a new irritation control group of 5 healthy, acclimated male albino guinea pigs was added to the study on Day 41 for the second challenge phase. These animals

were identified by animal number and corresponding ear tag, weighed from 485 to 583 g, and were approximately 7 weeks of age on Day 41.

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 eight animals was conducted to determine the irritation threshold of the test material. The purpose of the irritation screening study was to find the highest test material concentration that does not produce excessive irritation to use for intradermal injections, a moderately irritating concentration to use for the topical induction application, and the highest nonirritating topical concentration to be used for the challenge application.

One irritation screening subgroup of four animals was treated intradermally with the test material at concentrations of 0.1, 0.5, and 1% w/v in mineral oil. Concentrations greater than 1% could not be administered by injection. Each animal received all three concentrations of the test material. Each test material concentration was administered as a 0.1-mL intradermal injection into a single shaved site on each animal. The injection sites were evaluated for erythema and edema reactions at 24 and 48 hours after injection.

A second irritation screening subgroup of four animals first received duplicate 0.1 mL intradermal injections of a 1:1 dilution of FCA in sterile water on the shaved shoulder area (one injection on each side of the midline). Seven days after administration of the intradermal injections, the animals received a topical application of CGI 1397 at concentrations of 5, 10, 20, and 40% w/w in petrolatum. The 40% w/w mixture was deemed to be the highest concentration that could be uniformly prepared and administered. Four application sites were selected on the shaved back of each animal (two on the right side and two on the left side). Each animal received all four concentrations of the test material. Each test material concentration was applied in a thick even layer to 2-cm x 2-cm Whatman No. 3 filter papers, placed over the respective

application sites, covered with Blenderm™ tape, and wrapped with Elastoplast® tape. The patches remained in place for 24 hours, after which they were removed. Any residual test material was then removed from the application sites using water, Ivory® soap, and disposable paper towels. The application sites were observed for dermal reactions at 24 and 48 hours after patch removal. The sites were shaved before the 24-hour dermal observation.

Definitive Study

Based on the results obtained from the irritation screening study, the test material was administered as a 1% w/v mixture in mineral oil and as a 1% w/v mixture in mineral oil and 1:1 FCA/sterile water for the intradermal injections and as a 40% w/w mixture in petrolatum for the topical induction and challenge applications.

Intradermal Injections. On Day 1, an approximate 4-cm x 6-cm area was clipped along the midline over the shoulder region of each animal in the test and irritation control groups. Six intradermal injections were made within the boundaries of a 2-cm x 4-cm area, one row of three injections on each side of the midline. The animals in the test group received the following injections:

- Anterior Sites - 0.1 mL of a 1:1 dilution of FCA in sterile water
- Medial Sites - 0.1 mL of a 1% w/v suspension of test material in mineral oil
- Posterior Sites - 0.1 mL of a 1% w/v suspension of the test material in mineral oil and 1:1 FCA/sterile water

The animals in the irritation control group received the following injections:

- Anterior Sites - 0.1 mL of a 1:1 dilution of FCA in sterile water
- Medial Sites - 0.1 mL of mineral oil
- Posterior Sites - 0.1 mL of a 1:1 dilution of mineral oil in the prepared 1:1 sterile water/FCA emulsion

Topical Application. Based on the results of the irritation screening study, the areas of the intradermal injections in the test and irritation control animals were closely shaved on Day 7 and were then pretreated with a 10% w/w sodium lauryl sulfate (SLS) suspension in petrolatum before the topical induction application. The SLS was massaged into the skin with a glass rod; no bandage was applied. On Day 8, any remaining SLS was wiped away using disposable paper towels moistened with water and the animals in the test and irritation control groups received a topical induction application. A 40% w/w mixture of the test material in petrolatum or control material (petrolatum) was applied in a thick, even layer to 2-cm x 4-cm Whatman No. 3 filter papers and placed over the injection sites of the animals in the respective test or irritation control groups. The patches were covered with Blenderm™ tape and secured by Elastoplast® tape wrapped around the trunk. Forty-eight hours after application, the dressings were removed. Any residual material was then removed from the application sites using water, liquid Ivory® soap, and disposable paper towels.

Challenge Phase. On Day 22, 2 weeks after the topical induction dose, the animals in the test and irritation control groups received a challenge dose. The hair was removed from an approximate 5-cm x 5-cm area on the right and left sides by shaving as before. A 40% w/w mixture of the test material in petrolatum or control material (petrolatum) was applied in a thick, even layer to 2-cm x 2-cm Whatman No. 3 filter papers. The filter paper containing the test material was placed on the right side and the filter paper containing petrolatum alone was placed on the left side of each animal in the test and irritation control groups. Each patch was covered with Blenderm™ tape and wrapped with Elastoplast® tape wound around the trunk to provide an occlusive dressing. Twenty-four hours after application, the dressings were removed. Any residual material was then removed from the application sites using water, liquid Ivory® soap, and disposable paper towels. The sites were shaved before the 24-hour challenge observation.

All test and control material mixtures used in the irritation screening and definitive phases of the study were thoroughly mixed before application. All prepared mixtures were stored at room temperature until administered.

Second Challenge Phase. To further examine the sensitizing potential of this test material, a second challenge dose was conducted 19 days after the initial challenge application. A new naive site was selected on the test animals and on 5 new irritation control animals. Each animal in the test and additional irritation control groups received

an application of a 25% w/w mixture of the test material in petrolatum in the same manner as the initial challenge application. The initial irritation control animals were not treated during this phase of the study.

Reason for Route of Administration

Historically, and according to the referenced method (Magnusson and Kligman), the intradermal and dermal routes have been the routes of choice for determining contact hypersensitization.

Observations

The challenge sites were examined for dermal reactions at 24 hours following the challenge application patch removal. The sites were examined again at 48 hours after patch removal to detect any weak, slowly developing reactions.

The areas of the intradermal injections and topical induction applications for each animal in the definitive study were evaluated for general irritation from Day 2 through to termination with the exception of Day 9 when the animals were wrapped for the 48-hour topical induction period.

Clinical observations were conducted daily throughout the study. Body weights for the irritation screening animals were determined only on the day of treatment. Body weights on the definitive study animals were determined before the initial test material administration and at termination of the respective inlife phase.

Termination

At termination of the respective inlife phase for each group, all animals were euthanized and discarded.

Statistical Evaluation

No statistical evaluations 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 Covance in accordance with Covance SOP.

DISCUSSION

Irritation Screening Study
Individual body weights and dermal reactions are in Table 1. The irritation screening subgroup of four animals that received CGI 1397 via intradermal injection exhibited mild to moderate dermal reactions to the 0.1, 0.5, and 1% w/v concentrations.

The second irritation screening subgroup of four animals received CGI 1397 at concentrations of 5, 10, 20, and 40% w/w in petrolatum via topical application. No dermal irritation was observed with any of the concentrations applied.

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 in both groups appeared normal throughout the study. Weight losses of 2 to 5 grams were noted for three animals in the additional irritation control group between Day 41 and 44. These weight losses are not considered to be test material related.

Dermal Reactions at Induction Sites. A summary of the irritation observed at the intradermal injection and topical induction application sites for both groups is presented in Table 4. The irritation observed was similar for both groups, consisting of mild to moderate erythema and edema reactions with scab formation at the injection sites.

Dermal Reactions to CGI 1397 at Challenge. Individual dermal reactions to the challenge application are in Table 5. Five of the 20 animals in the test group exhibited intense dermal reactions and 15 of 20 test animals exhibited mild to moderate dermal reactions to the initial challenge application of the test material. Subcutaneous hemorrhaging, desquamation, and/or fissuring were also observed within the test sites of

six animals in the test group. Four of the 10 animals in the irritation control group exhibited mild to moderate dermal reactions to the test material at the initial challenge. None of the animals in the test or initial irritation control groups exhibited a dermal reaction to the control material at the initial challenge. Five of the 20 test animals were considered to have had sensitization responses to the initial challenge application of the test material. These reactions are considered to be sensitization responses due to their increase in severity over that observed in the irritation control group.

During the second challenge phase, all animals in the test group exhibited mild to intense dermal reactions to the second challenge application of the test material. Subcutaneous hemorrhaging, blanching, and/or fissuring were also observed within the challenge sites of 12 animals in the test group. None of the 5 animals in the additional irritation control group exhibited a dermal reaction to the test material at the second challenge. All 20 animals in the test group were thus considered to have had sensitization responses to the second challenge application of the test material.

Positive Control Report. A study report detailing the results of sensitization testing of α-hexylcinnamaldehyde (a known skin sensitizer) using the same maximization method is presented in the appendix of this report. This positive control study was conducted within 6 months of the conduct of this study.

CONCLUSION

The EC guideline requires a response of at least 30% (i.e., six of 20 guinea pigs) to classify a compound as a skin sensitizer by an adjuvant-type test method (Magnusson and Kligman maximization assay). Based on the number of animals in the test group that are considered to have exhibited sensitization reactions during the second challenge phase (20 of 20 animals), CGI 1397 is considered to be a skin sensitizer and the risk phrase R43 "may cause sensitization by skin contact" is appropriate under terms of the General Classification and Labeling Requirements for Dangerous Substances and Preparations, as stated in Annex IV to Commission Directive 93/21/EEC.

SIGNATURE

Steven M. Glaza
Study Director
Covance Laboratories Inc.

20 MAR 2001
Date

REFERENCES

1. Magnusson, B. and Kligman, A., *Allergic Contact Dermatitis in the Guinea Pig*, Charles C. Thomas, pp. 113-117, 120 (1970).

2. "Classification on the Basis of Toxicological Properties," *Official Journal of the European Community*, L 110 A, Volume 36, 4 May 1993.

3. United States Environmental Protection Agency; Prevention, Pesticides and Toxic Substances; "OPPTS 870.2600 Skin Sensitization"; *Health Effects Test Guidelines* (August 1998).

4. "Skin Sensitisation," *Organisation for Economic Cooperation and Development Guidelines for Testing of Chemicals*, Section 4, Health Effects, Number 406, Paris Cedex (July 17, 1992).

Dermal Reaction Scoring Scale

0 = No reaction
1 = Scattered mild redness
2 = Moderate and diffuse redness
3 = Intense redness and swelling