A 03

MR 7037

TSCA HEALTH & SAFETY STUDY COVER SHEET

TSCA CBI STATUS:

☐ CHECK IF THIS PAGE CONTAINS CONFIDENTIAL BUSINESS INFORMATION (CBI)

Clearly mark the confidential information with bracketing and check the box in the appropriate section (☐ Contains CBI).
Submit a sanitized cover sheet with CBI deleted. Mark the sanitized copy, "Public Display Copy" in the heading.

1.0 SUBMISSION TYPE ☐ Contains CBI

☒ 8(d) ☐ 8(e) ~~XX-FYI~~ ☐ 4 ☐ OTHER: Specify FYI-0698-1332

X - Intial Submission ☐ Follow-up Submission ☐ Final Report Submission

Previous EPA Submission Number or Title if update or follow-up: Docket Number, if any: #

☐ continuation sheet attached

2.1 SUMMARY/ABSTRACT ATTACHED (may be required for 8(e): optional for §4, 8(d) & FYI)	2.2 SUBMITTER TRACKING NUMBER OR INTERNAL ID	2.3 FOR EPA USE ONLY
☐ YES X NO	Cert# P 917006739 98-12-3	98 JUN 12 AM 11:33

3.0 CHEMICAL/TEST SUBSTANCE IDENTITY ☐ Contains CBI

Reported Chemical Name (specify nomenclature if other than CAS name):

CAS#: IPDI Homopolymer

Purity 3.7% %

Contains No CBI

☐ Single Ingredient

☐ Commerical/Tech Grade

X -Mixture Trade Name: Isophorone diisocyanate

Other chemical(s) present in tested mixture	CAS Number	NAME	% WEIGHT
	4098-71-9	Desmodur I (monomer)	

☐ continuation sheet attached

4.0 REPORT/STUDY TITLE ☐ Contains CBI

Dermal Sensitization Study with NB# 654991 Lot 655000E in Guinea Pigs, Study Number 97-324-ON

☐

5.1 STUDY/TSCATS INDEXING TERMS

[CHECK ONE]

HEALTH EFFECTS (HE): X ENVIRONMENTAL EFFECTS (EE): ENVIRONMENTAL FATE (EF):

5.2 STUDY/TSCATS INDEXING TERMS (see instructions for 4 digit codes)

STUDY TYPE: Other: SUBJECT ORGANISM (HE, EE only): Other: ROUTE OF EXPOSURE (HE only): Other: VEHICLE OF EXPRE (HE) Other:

6.0 REPORT/STUDY INFORMATION ☐ Contains CBI - Study is GLP

Laboratory Bayer Toxicology, Stilwell, KS Report/Study Date 5/18/98

Source of Data/Study Sponsor (if different than submitter) Bayer Number of pages : 28

☐ continuation sheet attached

7.0 SUBMITTER INFORMATION ☐ Contains CBI

Submitter: Francis J. Rattay Title: Manager, Reg. Affairs Phone: (412) 777-7471

Company Name: Bayer Corporation Company Address: 100 Bayer Road

Pittsburgh, PA 15205-9741 Submitter Address (if different):

Technical Contact: Francis J. Rattay Phone: (412)777-7471

☐ continuation sheet attached

8.0 ADDITIONAL/OPTIONAL STUDY COMMENTS ☐ Contains CBI

This is an FYI only – sunset date passed.

☐ continuation sheet attached

Submitter Signature: F J Rattay Date: 6/8/98

Page 1 of 1

cc: C. Keffer, H. E. Myer

FYI-98-001332

84980000034

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Study Title

Dermal Sensitization Study with
NB #654991 Lot 655000 B in Guinea Pigs

Data Requirement

40 CFR Part 158
US-EPA-FIFRA, Section 158.340, Guideline 81-6

Authors

L. P. Sheets and V. Avila

Study Completion Date

May 13, 1998

Test Facility

Bayer Corporation
Agriculture Division
Toxicology
17745 South Metcalf
Stilwell, KS 66085-9104

Study Number

97-324-ON

A [illegible]

[illegible] Corporation
97 [illegible] CK

S[illegible] [illegible]NT OF [illegible]TA [illegible]

[illegible] is claim[illegible] [illegible] confidential [illegible] the [illegible] its falling wit[illegible] the scope of [illegible] (0(d)([illegible] (A), [illegible]), or (c).

Company: [illegible]ayer Corporation

Company Agent: J. H. Thyssen Date: 5-1[illegible]-98

Vice President, Toxicology

[signature]
Signature

Bayer Corporation
97-324-ON

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

This study was conducted in compliance with the FIFRA Good Laboratory Practice Standards of 40 CFR Part 160, TSCA Good Laboratory Practice Standards of 40 CFR Part 792, the OECD Principles of Good Laboratory Practice, GD(92)32, (Paris, 1992), and Japanese Ministry of Agriculture, Forestry, and Fisheries (JMAFF), 59 NohSan No. 3850, Good Laboratory Practice Standards for Toxicological Studies on Agricultural Chemicals (August 10, 1984).

SUBMITTER

BAYER CORPORATION

J.H. Thyssen: _____
Vice President, Toxicology

Date: 5-18-98

SPONSOR

AGRICULTURE DIVISION

J.H. Thyssen: _____
Vice President, Toxicology

Date: 5-18-98

STUDY DIRECTOR

L.P. Sheets: _____

Date: 5/18/98

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Bayer Corporation
97-324-ON

SPONSOR

Bayer Corporation
Industrial Chemicals Division
100 Bayer Road
Pittsburgh, Pennsylvania 15205-9741

TEST FACILITY

Bayer Corporation
Agriculture Division
Toxicology
17745 South Metcalf
Stilwell, Kansas 66085-9104

DATES

Experimental Start Date:	November 18, 1997
Experimental Termination Date:	December 19, 1997

PERSONNEL AND RESPONSIBILITIES

Toxicology and Sponsor Representative:	J. H. Thyssen	Jurgen H. Thyssen
Toxicology Laboratory:	G. K. Sangha	for G.K.S. Jurgen H. Thyssen
Study Director:	L. P. Sheets	Larry P. Sheets
Study Conduct:	V. Avila	Vincent
Pathology Services:	B. P. Stuart	B. P. Stuart by S.G. Lake
Animal Care:	R. E. Mueller	Robert E. Mueller
Quality Assurance:	D. M. Wallace	Debra M. Wallace

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Bayer Corporation
97-324-ON

QUALITY ASSURANCE STATEMENT

Audit reports have been submitted to the Study Director and Laboratory Management documenting the status of compliance with applicable departmental standard operating procedures, the study protocol, and Good Laboratory Practice regulations.

The quality assurance unit monitored at least one phase of this dermal sensitization study, selected phases of the range finder study, and at least annually, all phases of the dermal senstization study type including the functions of all support areas for this study type. The following are the audit dates, phases inspected, auditors, and report dates of Quality Assurance inspections of this study and, if applicable, of this study type as well as relevant support areas:

AUDITS		STUDY	REPORT TO
Date	Phases	Auditor	DIRECTOR/ MANAGEMENT
	Phase of Study for 97-324-ON		
11/14/97	Active Ingredient Date Check	L.A. Berry	11/14/97
12/05/97	Scoring for Irritation	L.A. Berry	12/09/97
12/19/97	Body Weights	L.A. Berry	12/19/97
02/05/98	Final Report Review	L.A. Berry	02/05/98
	Phase of Study for 97-924-MX		
07/29/97	Unwrapping and Dose Removal	L.A. Berry	07/29/97
	Phase of Study Type		
02/10/97	Animal Examination and Release for Study Use	L.A. Berry	02/10/97
02/10/97	Shaving and Test Animal Inventory	L.A. Berry	02/12/97
03/12/97	Depilation and Rack Change	L.A. Berry	03/12/97
03/25/97	Technician Training Records Review	L.A. Berry	03/25/97
07/08/97	Unwrapping and Dose Removal	L.A. Berry	07/10/97
09/18/97	Dose Preparation and Test Material Inventory	L.A. Berry	09/18/97
09/22/97	Euthanasia	L.A. Berry	09/22/97
10/07/97	Dosing and Wrapping	L.A. Berry	10/07/97

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Bayer Corporation
97-324-ON

Date	AUDITS Phases	Auditor	REPORT TO STUDY DIRECTOR/ MANAGEMENT
	Animal Care Support Functions		
01/27-28/97	Guinea Pig - Animal Receipt, Shipment Exam, AM/PM Obs, Feeding, Cage Identification, Animal Inventory, Randomization, Quarantine	C.A. Cox	01/28/97
01/28/97	Guinea Pig Animal Indentification (Ear-tagging)	C.A. Cox	01/30/97
01/28/97	Guinea Pig Rack/Cage Prep	C.A. Cox	01/28/97
01/29/97	Guinea Pig Rack/Bedding Change and Special Procedures	C.A. Cox	01/30/97
01/29/97	Guinea Pig Room Disinfection & Special Procedures for Rooms Containing Guinea Pigs	C.A. Cox	01/30/97
02/03/97	Cleaning of Guinea Pig Racks and Cages	C.A. Cox	02/03/97
02/07/97	Guinea Pig Room Filter Check/Change	C.A. Cox	02/08/97
02/19/97	Cleaning of Guinea Pig Feeding Bowls	C.A. Cox	02/20/97
05/19/97	Animal Care Training Records	C.A. Yount	05/19/97
07/23/97	Data review of Vermin Control	C.A. Yount	07/23/97
07/23/97	Data Review of Water Analysis	C.A. Yount	07/23/97
08/05/97	Edstrom Water Filter Changes	C.A. Yount	08/07/97
12/09/97	Equipment Maintenance: Cage Wash	C. A. Yount	12/09/97

In compliance with the Good Laboratory Practice regulations, this final report for study numbers 97-324-ON and 97-924-MX have been reviewed by the Quality Assurance Unit. The results presented in this report accurately describe the methods and standard operating procedures and reflect the raw data collected during the conduct of the studies.

D.M. Wallace for L.A. Berry — 5/15/98

L.A. Berry, Quality Assurance — Date

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Bayer Corporation
97-324-ON

ABSTRACT

The potential for NB #654991 lot 655000 B to induce dermal hypersensitivity and thereby signify a potential for dermal sensitization was evaluated using the Buehler Topical Closed-Patch Technique. A total of 30 adult Hartley albino guinea pigs (15 males and 15 females) were assigned to induced (10/sex) or noninduced (control; 5/sex) groups. The dose was 0.3 g of the undiluted test substance for each induction exposure and 0.1 g of the undiluted test substance for challenge, with an exposure duration of six hours each. Induced animals received a single application per week for three consecutive weeks (on nominal days 0, 7, and 14) and then, after a 14-day "rest" period (day 28), were challenged to assess the development of sensitization. Noninduced controls received only the challenge application on day 28. Animals were evaluated for clinical signs at the dose site and weighed on days 0 and 31.

There was no difference in body weight gain for induced animals relative to noninduced controls.

During the third induction phase, dry scaly skin was evident at the dose site of six induced males and five induced females. There were no compound-related clinical signs (other than erythema) at the dose site for any other animals at any time.

Slight (grade 1) erythema was evident in six males and seven females during the third week of induction. For the challenge exposure, erythema was evident at the dose site of three (of ten; 30%) induced males and females at the first observation. Erythema persisted and increased in incidence in induced males and females through 72 hours following treatment. Erythema was not evident after challenge in any noninduced control and edema was not apparent at the dose site of any animal at any time. Thus, at challenge, induced males and females had cumulative irritation incidences of 40% and 50% and severity indices of 0.37 and 0.43, respectively.

Extent of Responses at Challenge

Induced	Cumulative incidence	Average Severity
Males	40%	0.37
Females	50%	0.43
Noninduced		
Males	0%	0.00
Females	0%	0.00

Incidence: Percent of observations of erythema.
Severity: Average erythema score from all observations (Grades 0 to 3).

Based on (1) the notable response in induced animals, as compared to noninduced controls, and (2) a response in induced animals following the first observation period [2,5], a positive sensitization response to the test substance was evident at challenge in 40% of the induced males and 50% of the induced females and in none of the noninduced controls. These results indicate that NB #654991 lot 655000 B has the potential to induce dermal hypersensitivity in guinea pigs, as assessed by the Buehler Topical Closed-Patch Technique.

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MATERIALS

I. The test substance was supplied by the sponsor with the following information:

Test Substance:

Identification:	NB# 654991
Physical Appearance:	Transparent Pale Yellow Liquid
Lot Number:	655000 B
Active Ingredient:	3.7% Isophorone diisocyanate (January 1997) 3.3% Isophorone diisocyanate (February 1998)
Storage Conditions/ Stability:	Room Temperature Under Inert Gas

Active Ingredient:

Common Name:	Desmodur I (monomer)
Chemical Name:	Isophorone Diisocyanate Based Polyisocyanate
CAS Registry Number:	4098-71-9

II. The test substance was stored under inert gas at room temperature and a sample was archived at room temperature. Doses were prepared for use on the day administered.

PURPOSE

The purpose of this study was to experimentally assess the potential for the test substance to induce dermal sensitization as assessed by the Buehler Topical Closed-Patch Technique.

TEST GUIDELINES

This study was conducted in accordance with:

1) US-EPA-FIFRA, Pesticide Assessment Guidelines, Subdivision F, Hazard Evaluation: Human and Domestic Animals, Guideline 81-6, November 1984.

2) US-EPA-TSCA, Health Effects Testing Guidelines, 40 CFR Section 798.4100.

3) OECD Guidelines for Testing of Chemicals, Section 4, Guideline 406, July, 1992.

4) Japan, Ministry of Agriculture, Forestry and Fisheries, Guidance on Toxicology Study Data for Application of Agricultural Chemical Registration, 59 NohSan No. 4200, January 1985.

In addition, certain changes proposed by the U.S. EPA (OPPTS) in draft (June 1996) Health Effects Test Guidelines (OPPTS; 870.2600) for a dermal sensitization study were incorporated into the study design.

METHODS

Animal Information

Source, Number and Age

Thirty (15 males and 15 females) young-adult Hartley albino guinea pigs (Charles River Laboratories, Inc., Portage, Michigan) were used in this study. These animals were approximately six weeks of age at the initiation of treatment.

Examination and Acclimation

Upon receipt, animals were examined and sacrificed if general appearance and/or behavior were considered abnormal. Those animals considered acceptable were then placed into suspended polycarbonate shoe box cages and acclimated to their ambient laboratory conditions and unlimited supply of food as described below for at least six days prior to placement on the study. For the holding period, animal care personnel observed the animals at least once daily for moribundity and mortality. Upon completion of the acclimation period, a veterinarian reviewed the status of the animals prior to their release for study.

Care and Housing

While on study, guinea pigs were individually housed in suspended polycarbonate shoe box cages with ALPHA-dri bedding material. The room, cages and cage racks were thoroughly cleaned and disinfected before arrival of the animals. The cages and racks were replaced at least two times weekly with clean, disinfected cages and racks. The room was disinfected with a quaternary ammonium compound at least once every two weeks. The ambient laboratory conditions were set (temperature 18-26°C, relative humidity 30-70% 12-hr light/dark cycle) and continuously monitored. Teklad Guinea Pig Diet and tap water were provided for *ad libitum* consumption.

Identification

Each animal was identified by cage card and ear tag.

Randomization

Animals were randomly assigned to cages using a software program [1]. Animals that satisfied study requirements were assigned to the study from consecutively-numbered cages.

Dose Selection

An irritation test was performed under range-finder study number 97-924-MX, to establish doses for the present sensitization study. This test study was performed to select (1) the lowest irritating dose and (2) the highest non-irritating dose for use as the induction and challenge applications, respectively. Procedurally, the treatment of animals was identical to that of the study animals during the challenge week. Each animal was observed for evidence of dermal irritation 24 and 48 hours following treatment. The test substance was administered undiluted to ten animals (five/sex) at separate dose sites with dose amounts of 0.1, 0.2, 0.3 and 0.4 g.

Individual doses were measured by weight rather than volume for greater accuracy due to the high viscosity of the test substance. The test substance was not diluted with water because this would cause the test substance to polymerize. The undiluted test substance was observed to remain primarily on the surface of the patch following application; thus ensuring good contact with the surface of the dose site.

In this test, the 0.3 g of the test substance elicited slight (grade 1) irritation in one male and one female, the 0.2 g dose produced slight (grade 1) irritation in one male and the 0.1 g dose did not produce erythema at the dose site for any animal. Based on these results, the 0.3 g of the test substance was selected as a minimally-irritating concentration for use as each induction application and the 0.1 g of the test substance was selected as the highest non-irritating concentration for use as the challenge application.

Test Substance Analysis

The concentration of active ingredient in the test substance was measured within eleven months of initial administration and again after the study.

Analysis of Test Substance in the Vehicle

The test substance was administered neat (undiluted). Therefore, analysis of the dose was not necessary.

Experimental Design

Route, Dose, and Number of Animals

The test substance was evaluated using the Buehler Topical Closed-Patch technique [2-5]. Hair from the appropriate area of each animal was removed on the day prior to each treatment using electric clippers equipped with a number 40 blade. On the day of treatment, 0.3 g of the undiluted test substance (induction) or 0.1 g of the undiluted test substance (challenge) was applied to an occlusive Webril patch (a 2 cm x 2 cm gauze pad backed with hypoallergenic tape). After [illegible]ition of the test substance to the patch, the patch was placed onto the appropriate shorn [illegible] area (see below). The animal was then wrapped with an elastic bandage that was secured with adhesive tape.

A total of 40 adult Hartley albino guinea pigs (20 males and 20 females) were assigned to induced or noninduced (control) groups. The induced and the noninduced groups each consisted of 10 males and 10 females. The noninduced groups included challenge and rechallenge control groups that consisted of 5 males and 5 females each. Since a rechallenge was not necessary, only 30 animals are reported (20 induced and 10 noninduced control).

Animals in the induction group received three induction applications (6-hour duration each) on study days 0, 7, and 14, followed by a challenge application (6-hour duration) on day 28. Animals in the noninduced group received only the one 6-hour application at challenge on day 28. The dose site for all three induction applications was adjacent to the left shoulder and the challenge dose site was adjacent to the left hip. These sites did not overlap. Following exposure, the bandages and patches were removed and the dose site was wiped using paper towels dampened with tap water to remove as much test substance residue as feasible without damaging the skin.

Erythema Assessment, Clinical Signs and Body Weights

Approximately 24, 48, and 72 hours after unwrapping for each induction and challenge treatment, erythema was graded according to the following criteria:

0 = No erythema.
1 = Slight, barely perceptible erythema.
2 = Moderate, clearly perceptible erythema.
3 = Severe, marked erythema which extends beyond the dose site.

Adjacent untreated skin was used for reference. These criteria are consistent with the reference test method [2].

Challenge: On the morning following unwrapping of the challenge treatment, the dose site and surrounding naive area were depilated with a commercial depilatory to facilitate grading of erythema.

If present, lesions or other signs of exposure at the dose site were recorded for each animal at each inspection for erythema.

All animals were weighed on days 0 and 31.

Data Reporting and Evaluation

Responses following each treatment are expressed as incidence and severity. Incidence is defined as the percent of animals in the group showing a response grade of one or greater divided by the number of animals per group. Incidence is tabulated for each day of observation at challenge as well as presented as a summary value (cumulative incidence) that represents the portion of animals per group that displayed a response grade of one or greater at any observation point during challenge. Severity is defined as the sum of all grades divided by the number of observations.

A substance is classified as a potential contact sensitizer if it produces consistent [illegible] in the induced group in excess of those observed from the [illegible] criteria for identification of positive responses at challenge include: 1) an [illegible] of a grade [illegible] response or higher in more than 15% of the induced animals, and 2) persistence of at least one of the responses for more than one day [2,5].

Method Validation

To support the validity and sensitivity of the methods and techniques used for this assessment, an ancillary study was conducted using α-1-hexylcinnamaldehyde (HCA) as a positive control test substance (See Appendix I)[7]. This study was conducted within a six-month time frame relative to the conduct of the current study with the NB #654991 lot 655000 B. The techniques and methods of study conduct and response assessment were similar or identical to those described herein and the same personnel were involved in performing both studies. With HCA, slight (grade 1) erythema was evident during the first week of induction in one male and female, and the incidence of erythema increased thereafter during subsequent weeks of induction. A moderate incidence (40-

50%) and severity (grades 1-2) of erythema were observed for induced males and females at challenge while noninduced controls had no response. These results demonstrate a sensitization response that compares well with those of others who used these test procedures to test HCA for sensitization [8,9]. Therefore, the results from this study serve to support the validity and sensitivity of test procedures and training of personnel involved with the present dermal sensitization study using the Buehler Topical Closed-Patch Technique.

Statistical Analysis

Group mean body weight changes were evaluated by Student's t-statistic to test for a significant difference between the induced and relevant control groups. Lotus 1-2-3 software [6] was used to compute descriptive statistics and comparative statistics (t-tests) as needed.

In all cases, a p-value less than or equal to 0.05 was considered to be statistically significant.

Archival Procedures

The protocol, raw data, a sample of the test substance, and the final report are archived at locations specified by Bayer Corporation, Agriculture Division, Toxicology, 17745 South Metcalf, Stilwell, Kansas 66085-9104.

RESULTS AND DISCUSSION

Animal Care: Housing and Contaminants

The Animal Care Report is provided in Appendix V. Continuous monitoring of the animal quarters used during this study showed no deviations from the [illegible] ranges for temperature (18 - 26 °C) and humidity (30 - 70%). Levels of possible contaminants found in commercially-available food and municipal water are not expected to affect the outcome of this study.

Clinical Signs and Body Weights

Results of clinical signs and body weight measurements are summarized in Table 1. Individual body weight data are listed in Appendix II.

During the third induction phase, dry scaly skin was evident at the dose site of six induced males and five induced females. There were no compound-related clinical signs (other than erythema) at the dose site for any other animals at any time. Body weight gain for induced males and females was comparable to that of noninduced animals.

Induction and Challenge Response

Summaries of erythema scores for each group are presented in Table 2. Individual erythema grades for induced and noninduced (control) males and females are shown in Appendices III and IV, respectively.

Slight (grade 1) erythema was evident in six males and seven females during the third week of induction. For the challenge exposure, erythema was evident at the dose site of three (of ten; 30%) induced males and females at the first observation. Erythema persisted and increased in incidence in induced males and females through 72 hours following treatment. Thus, at challenge, induced males and females had irritation incidences of 40% and 50% and severity indices of 0.37 and 0.43, respectively. Erythema was not evident after challenge in any noninduced control and edema was not apparent at the dose site of any animal at any time.

Based on (1) the notable response in induced animals, as compared to noninduced controls, and (2) a response that persisted in induced animals following the first observation period [2,5], a positive sensitization response to the test substance was evident at challenge in 40% of the induced males and 50% of the induced females and in none of the noninduced controls. These results support the conclusion that, NB #654991 lot 655000 B does have the potential to induce dermal hypersensitivity in guinea pigs, as assessed by the Buehler Topical Closed-Patch Technique.

SUMMARY AND CONCLUSIONS

The Buehler Topical Closed-Patch Technique was used to examine the potential for the development of dermal sensitization in response to NB #654991 lot 655000 B in guinea pigs. A total of 30 adult Hartley albino guinea pigs (15 males and 15 females) were assigned to induced (10/sex) or noninduced (control; 5/sex) groups. The dose was 0.3 g of the undiluted test substance for each induction exposure and 0.1 g of the undiluted test substance for challenge, with an exposure duration of six hours each. These doses were selected based on the results of primary dermal irritation tests in guinea pigs to select (1) the lowest irritating dose and (2) the highest non-irritating dose for use as the induction and challenge applications, respectively.

No decrement in body weight gain was evident for induced males or females following treatment, relative to noninduced controls. Dry scaly skin at the dose site in six induced males and five induced females was the only evidence of a compound-related clinical sign (other than erythema) at any time during the study. During the induction phase, the undiluted NB #654991 lot 655000 B provoked slight (grade 1) erythema in six males and seven females during the third week. For the challenge exposure, erythema was evident at the dose site in three (of ten; 30%) induced males and females at the first observation. Erythema persisted and increased in incidence in induced males and females through 72 hours following treatment. Thus, at challenge, induced males and females had irritation incidence indices of 40% and 50% and severity indices of 0.37 and 0.43, respectively. Erythema was not evident after challenge in any noninduced control and edema was not observed in any animal at any time.

Based on the response in induced males and females, NB #654991 lot 655000 B has the potential to induce dermal sensitization in guinea pigs, as assessed by the Buehler Topical Closed-Patch Technique.

REFERENCES

1. SAS Institute Inc., Cary, North Carolina.

2. Buehler, E.V., "Delayed Contact Hypersensitivity in the Guinea Pig," *Arch. Dermatol., 91,* 1965, pp. 171-177.

3. Ritz, H.L. and E.V. Buehler, "Planning, Conduct and Interpretation of Guinea Pig Sensitization Patch Tests," *Current Concepts in Cutaneous Toxicity,* V.A. Drill and V. Lazar (eds.), Academic Press, NY, 1980, pp. 25-40.

4. Klecak, G. (1991). Identification of Contact Allergens: Predictive Tests in Animals. In F.N. Marzulli and H.I. Maibach (Eds.), *Dermatotoxicology,* 4th ed. (pp 363-413). New York, Hemisphere Publishing Corporation.

5. Robinson, M.K., Nusair, T.L., Fletcher, E.. .. and Ritz, H.L. A Review of The Buehler Guinea Pig Skin Sensitization Test And Its Use In a Risk Assessment Process For Human Skin Sensitization. *Toxicology, 61,* 1990, pp 91-107.

6. Lotus Development Corp., Cambridge, Massachusetts.

7. Sheets, L.P. and Avila, V., Dermal Sensitization Study with α-1-hexylcinnamaldehyde: Verification of Test Methods for Buehler Topical Closed-Patch Technique, Bayer Corp., Agriculture Division Report Number 107781, U.S.EPA MRID Number 44409907, 1997.

8. Basketter, D.A., and G.F. Gerberick, "An interlaboratory evaluation of the Buehler test for the identification and classification of skin sensitizers," *Contact Dermatitis, 35,* 1996, pp. 146-151.

9. Basketter, D.A., E. Selbie, E.W. Scholes, D. Lees, I. Kimber, and P.A. Botham, "Results with OECD recommended positive control sensitizers in the Maximization, Buehler, and local lymph node assays," *Fd. Chem. Toxic., 31,* 1993, pp. 63-67.

Table 1

Summary of Clinical Signs and Body Weights for a Dermal Sensitization Study with NB #654991 Lot 655000 B in Guinea Pigs

	Clinical Signs			Body weights[2]
	Incidence[1]	Sign	First Observation	Average Gain (g) from Day 0 to 31
Males				
Induced	6/10	Dry scaly skin at dose site.	Week 3	235 ± 30
Noninduced (controls)	0/5	---	---	233 ± 57
Females				
Induced	5/10	Dry scaly skin at dose site.	Week 3	162 ± 37
Noninduced (controls)	0/5	---	---	160 ± 42

* *Significantly different from control ($p \leq 0.05$).*
[1] *The number of animals displaying the sign / the total number of animals.*
[2] *The mean ± 1 standard deviation.*

Table 2

Occurrence of Erythema During a Dermal Sensitization Study with NB #654991 Lot 655000 B in Guinea Pigs

Group	Number of Animals	Average Dermal Grades[a]						Cumulative Incidence and Average Severity at Challenge
		Induction Week			Challenge Day			
		1st	2nd	3rd	1st	2nd	3rd	
Males								
Induced	10	0%/0.0	0%/0.0	60%/0.53	30%/0.30	40%/0.40	40%/0.40	40%/0.37
Noninduced (controls)	5	NA	NA	NA	0%/0.0	0%/0.0	0%/0.0	0%/0.0
Females								
Induced	10	0%/0.0	0%/0.0	70%/0.63	30%/0.30	50%/0.50	50%/0.50	50%/0.43
Noninduced (controls)	5	NA	NA	NA	0%/0.0	0%/0.0	0%/0.0	0%/0.0

[a] *Presented as incidence(%)/severity*
Incidence: Percent of positive observations of erythema.
Severity: Average erythema scores from all observations.
NA Not Applicable

Appendix I

Positive Control Validation Study with α-1-Hexylcinnamaldehyde (HCA)

Performance of the testing facility (Bayer Corporation, Agriculture Division, Toxicology see above for address and affiliation) to conduct dermal sensitization studies by the Buehler Topical Closed-Patch Technique was assessed using HCA as a positive control (reference) substance. Standardized techniques and procedures for study conduct were followed and these methods represent those used during study conduct with experimental test substances. This positive control study was conducted from June 10 - July 11, 1997, using the laboratory study number 97-924-LX (Bayer Agriculture Division Report Number 107781) [7].

For this study, 15 male and 15 female adult Hartley albino guinea pigs were assigned to induced (10/sex) or noninduced (controls; 5/sex) groups. A volume of 0.4 ml of 50% and 5% (v/v) solutions of HCA in 70/30 (v/v) acetone/PEG 400 was used for induction and challenge applications, respectively. Techniques, as well as the regimen for solution application and response assessment, were as described earlier in this report.

Individual animal scores following each application of HCA are included (Appendices IA and IB). Slight (grade 1) erythema was evident during the first week of induction in one male and one female, and the incidence of erythema increased thereafter during subsequent weeks of induction. A moderate incidence of slight (grade 1) erythema was evident for induced males and females at challenge while noninduced controls had no positive response. No animal had evidence of edema at any time. Incidence and severity indices for erythema from each group are as follows (calculations as described previously):

Extent of Responses at Challenge: HCA Positive Control Study

Induced	Cumulative Incidence	Average Severity
Males	40%	0.27
Females	50%	0.47
Controls		
Males	0%	0.00
Females	0%	0.00

Incidence: Percent of observations of erythema.
Severity: Average erythema score from all observations.

Thus, approximately half of the induced males and females had erythema at challenge, whereas no responses were noted for any control. As HCA is known to provoke dermal sensitization in guinea pigs, these findings verify the sensitivity and reliability of laboratory techniques and the training of personnel applied to investigate the development of dermal sensitization in response to xenobiotics. This positive-control (reference) experiment demonstrates and documents the capability of this test facility to conduct dermal sensitization experiments in guinea pigs using the Buehler Topical Closed-Patch Technique.

Appendix IA

Individual Erythema Grades from Males Treated with α-1-HEXYLCINNAMALDEHYDE: All Observations

Group	Nominal Animal Number	Week of Induction: First 24-Hr	First 48-Hr	First 72-Hr	Second 24-Hr	Second 48-Hr	Second 72-Hr	Third 24-Hr	Third 48-Hr	Third 72-Hr	Challenge 24-Hr	Challenge 48-Hr	Challenge 72-Hr
Induced	1	0	0	0	1	1	1	1	0	0	0	0	0
	2	0	0	0	0	0	0	0	0	0	0	1	0
	3	0	0	0	0	0	0	1	1	0	0	0	0
	4	0	0	0	1	1	0	1	1	1	0	0	0
	5	0	0	0	1	1	1	1	1	1	0	0	0
	6	0	0	0	0	0	0	1	1	0	0	0	0
	7	0	1	1	1	1	1	1	1	1	1	1	1
	8	0	0	0	1	1	1	1	1	1	0	0	0
	9	0	0	0	1	1	1	1	1	1	1	1	1
	10	0	0	0	1	1	1	2	1	1	0	1	0
Noninduced (Controls)	11	NA	NA	NA	NA	NA	NA	NA	NA	NA	0	0	0
	12	NA	NA	NA	NA	NA	NA	NA	NA	NA	0	0	0
	13	NA	NA	NA	NA	NA	NA	NA	NA	NA	0	0	0
	14	NA	NA	NA	NA	NA	NA	NA	NA	NA	0	0	0
	15	NA	NA	NA	NA	NA	NA	NA	NA	NA	0	0	0

NA: Not Appropriate, no observation taken.

Key

0 = No erythema.

1 = Slight, barely perceptible erythema.

2 = Moderate, clearly perceptible erythema.

3 = Severe, marked erythema which extends beyond the dose site.

Appendix IB

Individual Erythema Grades from Females Treated with α-1-HEXYLCINNAMALDEHYDE: All Observations

Group	Nominal Animal Number	Week of Induction: First 24-Hr	First 48-Hr	First 72-Hr	Second 24-Hr	Second 48-Hr	Second 72-Hr	Third 24-Hr	Third 48-Hr	Third 72-Hr	Challenge 24-Hr	Challenge 48-Hr	Challenge 72-Hr
Induced	1	0	1	0	1	1	1	1	1	1	0	0	0
	2	0	0	0	1	1	1	1	1	1	0	1	1
	3	0	0	0	1	1	1	1	1	1	1	1	1
	4	0	0	0	0	0	0	1	0	0	1	1	1
	5	0	0	0	1	1	0	2	2	2	1	1	1
	6	0	0	0	1	1	1	1	0	0	0	0	0
	7	0	0	0	1	1	1	1	1	0	0	0	0
	8	0	0	0	1	1	1	2	2	1	1	1	1
	9	0	0	0	0	1	0	1	1	0	0	0	0
	10	0	0	0	0	0	0	1	1	1	0	0	0
Noninduced (Controls)	11	NA	NA	NA	NA	NA	NA	NA	NA	NA	0	0	0
	12	NA	NA	NA	NA	NA	NA	NA	NA	NA	0	0	0
	13	NA	NA	NA	NA	NA	NA	NA	NA	NA	0	0	0
	14	NA	NA	NA	NA	NA	NA	NA	NA	NA	0	0	0
	15	NA	NA	NA	NA	NA	NA	NA	NA	NA	0	0	0

NA: Not Appropriate, no observation taken.

Key

0 = No erythema.

1 = Slight, barely perceptible erythema.

2 = Moderate, clearly perceptible erythema.

3 = Severe, marked erythema which extends beyond the dose site.

Appendix II

Individual Body Weights (g) in a Dermal Sensitization Study with NB #654991 Lot 655000 B in Guinea Pigs

Treatment Group	Nominal Animal Number	Males Reference Day 0	Males Reference Day 31	Females Reference Day 0	Females Reference Day 31
Induced	1	372	555	369	584
	2	419	680	342	509
	3	415	641	375	528
	4	395	647	364	457
	5	382	625	277	437
	6	372	570	387	536
	7	408	634	370	555
	8	399	625	339	505
	9	450	700	387	594
	10	419	706	348	470
Noninduced (Controls)	11	394	606	364	518
	12	414	673	380	593
	13	409	665	374	517
	14	406	699	392	577
	15	392	537	337	441

Weight in grams.
Initial treatment is referenced as day 0.

Appendix III

Individual Erythema Grades from Males Treated with NB #654991 Lot 655000 B: All Observations

Group	Nominal Animal Number	Week of Induction: First 24-Hr	First 48-Hr	First 72-Hr	Second 24-Hr	Second 48-Hr	Second 72-Hr	Third 24-Hr	Third 48-Hr	Third 72-Hr	Challenge 24-Hr	Challenge 48-Hr	Challenge 72-Hr
Induced	1	0	0	0	0	0	0	1	1	0	1	1	1
	2	0	0	0	0	0	0	0	0	0	1	1	1
	3	0	0	0	0	0	0	0	0	0	0	1	1
	4	0	0	0	0	0	0	1	1	1	0	0	0
	5	0	0	0	0	0	0	0	0	0	0	0	0
	6	0	0	0	0	0	0	1	1	0	1	1	1
	7	0	0	0	0	0	0	0	0	0	0	0	0
	8	0	0	0	0	0	0	1	1	1	0	0	0
	9	0	0	0	0	0	0	1	1	1	0	0	0
	10	0	0	0	0	0	0	1	1	1	0	0	0
Noninduced (Controls)	11	NA	NA	NA	NA	NA	NA	NA	NA	NA	0	0	0
	12	NA	NA	NA	NA	NA	NA	NA	NA	NA	0	0	0
	13	NA	NA	NA	NA	NA	NA	NA	NA	NA	0	0	0
	14	NA	NA	NA	NA	NA	NA	NA	NA	NA	0	0	0
	15	NA	NA	NA	NA	NA	NA	NA	NA	NA	0	0	0

NA: Not Appropriate, no observation taken.

Key

0 = No erythema.

1 = Slight, barely perceptible erythema.

2 = Moderate, clearly perceptible erythema.

3 = Severe, marked erythema which extends beyond the dose site.

Appendix IV

Individual Erythema Grades from Females Treated with NB #654991 Lot 655000 B: All Observations

Group	Nominal Animal Number	Week of Induction: First 24-Hr	First 48-Hr	First 72-Hr	Second 24-Hr	Second 48-Hr	Second 72-Hr	Third 24-Hr	Third 48-Hr	Third 72-Hr	Challenge 24-Hr	Challenge 48-Hr	Challenge 72-Hr
Induced	1	0	0	0	0	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	1	1	1	0	1	1
	3	0	0	0	0	0	0	1	1	1	1	1	1
	4	0	0	0	0	0	0	1	1	0	0	0	0
	5	0	0	0	0	0	0	1	1	1	1	1	1
	6	0	0	0	0	0	0	1	1	1	0	0	0
	7	0	0	0	0	0	0	0	0	0	0	1	1
	8	0	0	0	0	0	0	0	0	0	1	1	1
	9	0	0	0	0	0	0	1	1	0	0	0	0
	10	0	0	0	0	0	0	1	1	1	0	0	0
Noninduced (Controls)	11	NA	NA	NA	NA	NA	NA	NA	NA	NA	0	0	0
	12	NA	NA	NA	NA	NA	NA	NA	NA	NA	0	0	0
	13	NA	NA	NA	NA	NA	NA	NA	NA	NA	0	0	0
	14	NA	NA	NA	NA	NA	NA	NA	NA	NA	0	0	0
	15	NA	NA	NA	NA	NA	NA	NA	NA	NA	0	0	0

NA: Not Appropriate, no observation taken.

Key

0 = No erythema.

1 = Slight, barely perceptible erythema.

2 = Moderate, clearly perceptible erythema.

3 = Severe, marked erythema which extends beyond the dose site.

C 01

Bayer Corporation
97-324-ON

Appendix V

Animal Care Report

Author: R.E. Mueller

C 02

ANIMAL CARE REPORT

Study Number 97-324-ON

Dermal Sensitization Study with NB# 654991 Lot 655000 B in Guinea Pigs

DATES

Animal Arrival Date: November 10, 1997
Shipment Released for Study Date: November 17, 1997
Study Animal Termination Date: December 19, 1997
Room Number: 319 Toxicology Building

ANIMAL INFORMATION

Species/Strain: Cavia porcellus, Hartley Albino

Source: Charles River Laboratories, Portage, Michigan

Number in Shipment: 22 Males, 22 Females received; 22 Males, 22 Females released for study.

Animals Used for this Study: Females: 487-501
Males: 465-479

Method of Shipment: Vendor vehicle

Birth Date: Males and Females: October 3, 1997

Shipment Examination Date: November 10, 1997

Acclimation Period Dates: November 10, to November 17, 1997

Shipment Release Form Signed: November 17, 1997

ANIMAL CARE AND HOUSING

Caging Type: Individually housed in polycarbonate shoe box cage providing 162 square inch area. The required area for guinea pigs weighing more than 350 grams is 101 square inches.

Bedding Type/Changes: ALPHA-dri was used in cages changed at least twice weekly from November 10, 1997, until December 19, 1997.

Room Disinfection: A quaternary ammonium compound was used at least once every two weeks from November 10, 1997, until December 19, 1997.

Rack Changes: Racks were changed at least once each week from November 10, 1997, until December 19, 1997.

Feeding: Animals were fed Teklad Guinea Pig Diet (W) 8602, ad libitum, daily from November 10, 1997, until December 19, 1997.

Lighting: A 12 hour light/dark cycle was maintained from November 10, 1997, until December 19, 1997.

Water: Kansas City water district provides water for our facility. Water is provided ad libitum using an Edstrom automatic watering system.

Environmental Condition:

Temperature: 13 to 26°C
Humidity: 30 to 70% RH
Deviations: None. Edstrom Watchdog report submitted for raw data file

Pest Control Use: None

Identification: Cage cards, ear tags

Randomization: A SAS program was used to generate a random list. Animal Care technicians performed randomization on November 10, 1997.

CONCLUSION:

The animal care procedures and room conditions during this study were in accordance with accepted standards of laboratory animal care in compliance with the "Animal Welfare Act of 1966" (Public Law 89-544) and its amendments, as well as the "Guide for Care and Use of Laboratory Animals" (National Academy Press, 1996), and should not affect the outcome of the study. The facility in which the study was conducted is fully accredited with the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC International) in addition to being a registered research facility with the United States Department of Agriculture, Animal and Plant Health Inspection Service, Animal Care(USDA-APHIS-AC).

Date: January 8, 1998

C 04

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