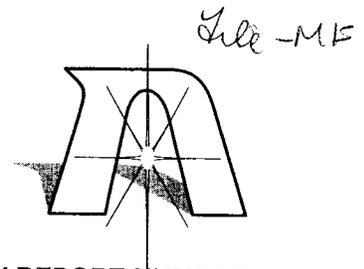


# Northview Pacific Laboratories, Inc.

551 Linus Pauling Drive ✧ Hercules, CA 94547 ✧ 510/964-9000 ✧ Fax 510/964-0551



**SPONSOR** 4151-0

Karen Norkaitis  
W.F. Young, Inc.  
302 Benton Drive  
E. Longmeadow MA 01028

**NV REPORT NUMBER**

X2H377G

**MRP NUMBER**

R2H013G

**REPORT DATE**

October 16, 2002

## TEST ARTICLE

Experimental Patch Lot 23-1  
Sample Code: Lot 23-1  
Lot Number: 23-1

## TEST PERFORMED

Dermal Sensitization - Buehler Method

## STUDY DIRECTOR

Blanca Ramirez, Laboratory Technician  
*In Vivo Services*

## PERFORMING LABORATORY

Northview Pacific Laboratories, Inc.  
551 Linus Pauling Drive  
Hercules, CA 94547

*A Northview Biosciences Company*



*Atlantic, Midwest, and Pacific*

F.D.A. REGISTRATION No. 29-14117  
Reports are submitted to clients on a confidential basis. No reference to the work, the results, or to Northview Pacific Laboratories in any form of advertising, news release, or other public announcement may be made without our written authorization. Test results are applicable only to the samples being tested within the limits of the testing procedures identified and are not necessarily indicative of the characteristics of any other samples from the same or other lots. Northview Pacific Laboratories, Inc. shall not be liable under any circumstances for any amount in excess of the cost of the test performed.



Printed on  
recycled paper

**TABLE OF CONTENTS**

Study Dates ..... 3

Purchase Order Number ..... 3

Test Article Identification ..... 3

Control Article Identification..... 3

Protocol..... 3

Deviations from Protocol..... 3

Data Disposition ..... 3

Summary of Results..... 3

Dermal Sensitization - Buehler Method..... 4

Materials and Methods..... 5

    Test System ..... 5

    Justification for Test System ..... 5

    Table 1: Study Design ..... 5

    Table 2: Study Schedule..... 6

    Sample Preparation and Dosing Procedure ..... 6

    Table 3: Induction and Challenge Phase Dosing Scheme ..... 7

    Observation and Scoring ..... 7

    Table 4: Scoring Key..... 8

    Interpretation and Analysis..... 8

Results and Discussion ..... 8

    Primary Irritancy Screen..... 8

    Induction Phase Scores..... 8

    Challenge Phase Scores..... 8

    Table 5: Induction Phase and Primary Challenge Scores ..... 9

    Table 5: Induction Phase and Primary Challenge Scores (continued)..... 10

    Incidence Score and Severity Index ..... 10

    Table 6: Incidence Scores..... 10

    Table 7: Severity Index ..... 10

    Clinical Observation and Body Weights ..... 11

    Table 8: Animal Weights..... 12

    Table 8: Animal Weights (continued) ..... 13

Conclusion ..... 13

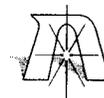
References..... 13

Quality Assurance Unit GLP Inspection and Audit Summary..... 14

Final Report Audit ..... 14

Northview Pacific Laboratories Staff Participating in this Study ..... 15

Statement of Compliance..... 15



**STUDY DATES**

Study Authorization: Signed Protocol  
Date Sample Received: August 28, 2002  
Study Initiation Date: August 30, 2002  
Date On Test: September 10, 2002  
Date Off Test: October 10, 2002  
Report Date: October 16, 2002

**PURCHASE ORDER NUMBER**

L-764

**TEST ARTICLE IDENTIFICATION**

Name: Experimental Patch Lot 23-1  
Physical Description: patch  
Total Quantity Received for Testing: 4 pouches of 6 patches each  
Total Quantity Used for This Study: 17 patches  
Sample Code: Lot 23-1  
Lot Number: 23-1  
Storage Condition: Room Temperature

**CONTROL ARTICLE IDENTIFICATION**

Name: 1-Chloro-2, 4-Dinitrobenzene  
Physical Description: yellow crystalline flakes  
Quantity/Container: 5 g/amber glass jar  
Expiration Date: 1/06  
Lot Number: 118H1358  
Storage Conditions: Room Temperature

**PROTOCOL**

This test was conducted according to Protocol Number R2H013G, which incorporates by reference Northview Standard Operating Procedure 16G-12 and is on file at Northview Pacific Laboratories, Inc. There were no amendments to the protocol.

**DEVIATIONS FROM PROTOCOL**

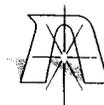
There were no deviations.

**DATA DISPOSITION**

Raw data and the final report from this study are archived at Northview Pacific Laboratories, Inc., 551 Linus Pauling Drive, Hercules, CA 94547, under Northview Report Number X2H377G. The test article(s) will be disposed of by Northview Pacific Laboratories, Inc., unless the client requests that the test article(s) be returned. It is the responsibility of the client to maintain test articles in accordance with GLP regulations.

**SUMMARY OF RESULTS**

The results of this test indicate that the test article does not have the potential to be a contact sensitizer in Hartley albino guinea pigs.



## DERMAL SENSITIZATION - BUEHLER METHOD

This test is designed to determine the potential of a test article to elicit skin sensitization or delayed contact hypersensitivity in guinea pigs using a modified Buehler patch procedure.

In this test, the test article and control solutions were applied directly onto intact sites on the animals' sides. The results were compared to positive control data derived from a historical study, X2B079G performed in February and March 2002. The positive control, dinitrochlorobenzene (DNCB) was prepared as a solution of 9.5% aqueous ethanol.

In the induction phase, the test article and the positive control solution were applied in six-hour exposures given on Days 0, 7, and 14. Twenty-four hours after each dose, the sites were scored for erythema and edema.

Fourteen days after the last induction exposure, the animals received a challenge exposure. In the challenge phase, the test article and positive control solutions were administered to skin sites that were not used during the induction phase. As in the induction phase, the duration of exposure was six hours. The dosing sites were scored for erythema and edema 24 and 48 hours after the dose application.

An incidence score and a severity index score were calculated, based on the erythema and edema scores, to assess the test article's potential to elicit a skin sensitization response in guinea pigs.

After the challenge dosing, none of the test group or naïve control group animals exhibited significant reactions (scores of 1 or greater). The results of this test indicate that the test article does not have a potential to be a contact sensitizer in Hartley albino guinea pigs.

Under the same conditions, all of the positive control animals exhibited response scores of 2 or 3. None of the positive naïve controls exhibited scores greater than 0.5. These results indicate that a positive response can be elicited to a known sensitizer.



## MATERIALS AND METHODS

### Test System

Species	Guinea Pig
Strain	Hartley, Albino
Source	Elm Hill Breeding Labs, Chelmsford, MA TKW Farms, Novato, CA
Number Used	42
Sex	Male and female
Age	Six weeks or older
Initial Weight	300 to 500 grams
Housing	In suspended cages; 18 – 26°C, 50 ± 20% relative humidity
Feed and Water	Laboratory Guinea Pig Diet (Teklad) and water supplied in bottles <i>ad libitum</i>
Photoperiod	Diurnal (12 hours on – 12 hours off)
Quarantine Period	Seven days
Identification	Ear tag

### Justification for Test System

Guinea pigs are the preferred species for skin sensitization tests. They are required for the Buehler Patch Test (Buehler, 1965), the ISO and EPA FIFRA guidelines. The test requires an intact immune system. It is not possible to recreate the conditions of the test in an *in vitro* system.

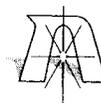
**Table 1: Study Design**

Group	Number of Animals	Induction Phase (Days 0, 7 and 14)			Number of Exposures	Challenge Phase (Day 28)		
		Concentration (% wt/vol)	Duration (hrs)	Dosing Site		Concentration (% wt/vol)	Duration (hrs)	Dosing Site
Test Article	20	1 inch <sup>2</sup>	6	R	3	1 inch <sup>2</sup>	6	L
Test Article Naïve Control	10	NA	NA	NA	NA	1 inch <sup>2</sup>	6	L
Positive Control	6	0.075%	6	R	3	0.050%	6	L
Positive Naïve Control	6	NA	NA	NA	NA	0.050%	6	L

R = right flank

L = left flank

NA = not applicable



**Table 2: Study Schedule**

Time	Procedure
Induction Phase	
Day -1	Test and positive control groups clipped
Day 0	Test and positive control groups shaved and dosed
Day 1	24 hour post-induction scoring
Day 6	Test and positive control groups clipped
Day 7	Test and positive control groups shaved and dosed
Day 8	24 hour post-induction scoring
Day 13	Test and positive control groups clipped
Day 14	Test and positive control groups shaved and dosed
Day 15	24 hour post-induction scoring
Challenge Phase	
Day 27	All groups clipped
Day 28	All groups shaved and dosed
Day 29	24 hour post-challenge scoring
Day 30	48 hour post-challenge scoring

### Sample Preparation and Dosing Procedure

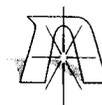
*Study Design* – The animal assignments, route of dose administration, and dosing schedule are summarized in Table 1. The animals were divided into 4 groups; twenty test animals, ten naïve control animals, six positive control animals and six naïve positive control animals. The positive controls were tested as part of a historical positive control study (NV Study No. X2B079G) conducted February and March 2002.

*Primary Irritancy Screen* – In the case of certain pharmaceuticals (or any test article with potential to cause extensive destruction of the skin), preliminary testing is required to determine the concentration of the test article to be used in the main test. If the irritant properties of the test article are known this preliminary testing is not necessary.

The test article was provided by the sponsor as an intact patch, for which the dosing concentration could not be altered. Therefore no primary irritancy screen was required.

In historical control study, X2B079G a primary irritancy screen was performed on the positive control material to find suitable concentrations to be used for the induction and challenge phases. For the induction phase, the concentration used was one, which produced moderate irritation but induced a response no greater than a grade of 2. For the challenge exposure, the concentration selected was the highest non-irritating concentration that produced a response of no more than 2 grades of 0.5 out of four animals.

The dosing concentrations, determined from that screen were: 0.075% weight/volume for the induction phase and 0.050% weight/volume for the challenge.



*Sample Preparation* – The test article was cut into 1-inch square pieces.

The positive control solution was weighed and dissolved in 95% ethanol. The ethanol/DNCB solution was then diluted with deionized water to achieve a 9.5% aqueous ethanol solution. A fresh solution of DNCB was prepared for each application. For the induction phase, the final concentration used was 0.075% (weight/volume). For the challenge phase, the final concentration used was 0.050% (weight/volume).

*Animal Preparation* – Hair at the dosing site on the flank of each guinea pig was clipped the day before the dosing. On the morning of the test, the dosing sites were shaved.

*Dosing Procedure* – The procedure for the induction and challenge phase dosing is illustrated in Table 3. The test article patches and Hill Top Chambers™, containing 0.3 mL volumes of the control solution were applied to the shaved sites on the animals. Fresh preparations of the solutions were used for each exposure.

The trunks of the animals were wrapped with gauze, which was held in place with tape. Six hours after dosing, the animals were unwrapped and marked with a felt pen in order to locate the sites for scoring.

For the induction phase, the animals were dosed on the right side as described above on Days 0, 7, and 14. The animals were reclipped and reshaved prior to each dosing. The doses for the second (Day 7) and third (Day 14) induction exposures were applied to the same sites as for the first exposure. In cases where scores of 3 were seen, the dose was administered to a new site.

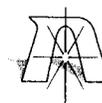
For the challenge phase, on Day 28 of the study the animals were dosed at a previously unexposed site on the left side in the same manner as described above. In addition to the test and positive control groups, groups of previously unexposed animals were dosed with the test article and positive control solution to serve as naïve controls.

**Table 3: Induction and Challenge Phase Dosing Scheme**

Group	Number of Sites	Volume/Site	Induction Phase	Challenge
			Article	Article
Test	1	1 in <sup>2</sup>	Test Article	Test Article
Naive Control	1	1 in <sup>2</sup>	NA	Test Article
Positive Control	1	0.3 mL	0.075% DNCB	0.050% DNCB
Naive Positive Control	1	0.3 mL	NA	0.050% DNCB

**Observation and Scoring**

*Scoring Procedure* – Twenty-four hours after dose application, the sites were scored according to the criteria in Table 4. The scoring was repeated 48 hours after application for the challenge phase.



**Table 4: Scoring Key**

Description	Score
No Reaction	0
Slight patchy erythema	0.5
Slight confluent or moderate patchy erythema	1
Moderate erythema	2
Erythema, edema, or cracking of the skin	3

*Weights* – All of the animals were weighed at the beginning and at the end of the study.

*Clinical Observations* – All of the animals were observed for toxic signs immediately after dosing and daily until the end of the study.

### Interpretation and Analysis

Two different scores were calculated to analyze test results. These were determined for both the 24 and 48-hour readings.

The Incidence Score represents the number of animals in each group showing responses of 1 or greater, at either 24 or 48 hours, expressed as a fraction of the total number of animals tested in the group. The highest possible value for the incidence score is 1.0.

The Severity Index is the sum of the test grades for animals in a group, at either 24 or 48 hours, divided by the total number of animals in that group. The highest possible value for the Severity Index is 3.0.

## RESULTS AND DISCUSSION

### Primary Irritancy Screen

The test article was provided in a form in which the concentration could not be altered. Therefore, a primary irritancy screen could not be performed.

### Induction Phase Scores

The results of the scoring for the induction phase are shown in Table 5.

*Test Group* - The strongest reaction exhibited was slight patchy erythema (scores of 0.5). Most of the animals had no reaction.

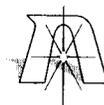
*Positive Control Group* – Scores after the first induction exposure were 0.5. The intensity of the reaction increased with each exposure so that all six test group animals presented with scores of 2 after the third exposure.

### Challenge Phase Scores

The results of the 24 and 48-hour observations are shown in Table 5.

*Test and Test Naïve Control Groups* – The strongest reaction exhibited was slight patchy erythema (scores of 0.5). Most of the animals had no reaction.

*Positive and Naïve Positive Control Groups* – After both the 24 and 48 hour observations, all six positive control animals exhibited scores of 2. None of the positive naïve control animals exhibited scores greater than 0.5.



**Table 5: Induction Phase and Primary Challenge Scores**

Animal Number	INDUCTION PHASE			PRIMARY CHALLENGE	
	Exposures			Observations	
	First	Second	Third	24 Hours	48 Hours
Test Group					
78960	0	0.5	0.5	0	0
78962	0	0	0	0.5	0
78965	0	0.5	0.5	0	0.5
78968	0	0.5	0.5	0	0
78969	0	0	0	0	0
78970	0.5	0.5	0	0.5	0
78979	0	0.5	0	0.5	0.5
78985	0	0	0	0.5	0.5
78989	0.5	0	0	0	0
78990	0	0.5	0	0	0.5
79159	0.5	0	0	0.5	0
79161	0	0	0	0	0
79163	0	0	0	0	0.5
79164	0	0	0	0	0
79165	0	0	0	0	0
79167	0	0	0	0	0.5
79168	0	0	0	0	0
79169	0	0	0	0	0
79170	0	0	0	0	0
79171	0	0	0	0	0
Naïve Control Group					
78958	NA	NA	NA	0.5	0
78980	NA	NA	NA	0.5	0.5
78981	NA	NA	NA	0.5	0.5
78984	NA	NA	NA	0.5	0.5
78991	NA	NA	NA	0	0
79157	NA	NA	NA	0.5	0
79166	NA	NA	NA	0	0
79172	NA	NA	NA	0	0
79173	NA	NA	NA	0.5	0.5
79174	NA	NA	NA	0	0



**Table 5: Induction Phase and Primary Challenge Scores (continued)**

Animal Number	INDUCTION PHASE			PRIMARY CHALLENGE	
	Exposures			Observations	
	First	Second	Third	24 Hours	48 Hours
Positive Control Group					
75764	0.5	2	2	2	2
75768	0.5	2	2	2	2
75769	0.5	2	2	2	2
75773	0.5	2	2	2	2
75774	0.5	2	2	2	2
75778	0.5	2	2	2	2
Positive Naïve Control Group					
75664	NA	NA	NA	0.5	0
75762	NA	NA	NA	0.5	0
75763	NA	NA	NA	0	0
75766	NA	NA	NA	0.5	0.5
75767	NA	NA	NA	0	0
75775	NA	NA	NA	0.5	0

**Incidence Score and Severity Index**

*Incidence Score* – The incidence scores are shown in Table 6.

For the test and test naïve control, the scores were 0 for both 24 and 48 hours. For the positive control, the scores were 1.0 at 24 and 48 hours. For the positive naïve control group, the scores were 0.0 for both 24 and 48 hours.

*Severity Index* – The severity indices are shown in Table 7.

For the test group, the severity index scores were 0.1 and 0.2 for 24 and 48 hours, respectively. For the test naïve control group, they were 0.3 and 0.2 for 24 and 48 hours, respectively. For the positive control group, the severity index scores were 2.0 at 24 and 48 hours. For the positive naïve control group, they were 0.3 at 24 hours and 0.1 at 48 hours.

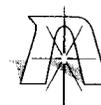
**Table 6: Incidence Scores**

**Table 7: Severity Index**

	Test	Naive	Positive	Positive Naive		Test	Naive	Positive	Positive Naive
24 Hours	0	0	1.0	0.0	24 Hours	0.1	0.3	2.0	0.3
48 Hours	0	0	1.0	0.0	48 Hours	0.2	0.2	2.0	0.1

**Incidence Score:** this is the number of animals in each group showing responses of 1 or greater at 24 or 48 hours, divided by the total number of animals in the group.

**Severity Index:** this is the sum of the test scores divided by the total number of animals treated in a given group.



**Clinical Observation and Body Weights**

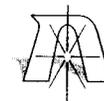
*Clinical Observations* – On Day 7 of the study, animal no. 78968 of the Test Group appeared thin. The animal was weighed, and had lost 46 g since commencement of the study. The animal was treated with 5 mL of Lactated Ringer's Injection subcutaneously daily for six consecutive treatments. By the end of the treatment the animal exhibited normal weight gain. All other test and control animals appeared healthy and no toxic signs were observed in the remaining animals over the duration of the study.

*Body Weight* – Weight measurements are shown in Table 8. All animals showed normal weight gain during the course of the study.



**Table 8: Animal Weights**

Animal Number	Sex	Pre-Test Weight (g)	Post-Test Weight (g)
Test Group			
78960	M	325	575
78962	M	320	546
78965	M	337	606
78968	M	333	472
78969	M	367	651
78970	M	314	501
78979	M	331	546
78985	M	321	583
78989	M	335	525
78990	M	332	548
79159	F	312	455
79161	F	317	504
79163	F	329	530
79164	F	320	455
79165	F	310	430
79167	F	309	468
79168	F	326	503
79169	F	353	550
79170	F	303	434
79171	F	328	535
Naïve Control Group			
78958	M	324	542
78980	M	335	633
78981	M	334	604
78984	M	327	616
78991	M	333	544
79157	F	313	485
79166	F	330	481
79172	F	305	443
79173	F	327	463
79174	F	349	462



**Table 8: Animal Weights (continued)**

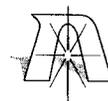
Animal Number	Sex	Pre-Test Weight (g)	Post-Test Weight (g)
Positive Control Group			
75764	F	309	475
75768	F	308	505
75769	F	322	480
75773	F	333	460
75774	F	382	552
75778	F	321	479
Naive Positive Control Group			
75664	F	386	547
75762	F	331	500
75763	F	316	443
75766	F	327	497
75767	F	303	468
75775	F	330	464

## CONCLUSION

The results of this test indicate that the test article does not have the potential to be a contact sensitizer in Hartley albino guinea pigs.

## REFERENCES

1. NV SOP 16G-12, *Dermal Sensitization – Buehler Method*
2. E.V. Buehler, Delayed Contact Hypersensitivity in the Guinea Pig, *Arch Dermatol*, 91:171, 1995.
3. H.L. Ritz and E.V. Buehler, V.A. Drill and P. Lazar (eds.), Planning, Conduct and Interpretation of Guinea Pig Sensitization Patch Test In *Current Concepts in Cutaneous Toxicity*, Academic Press, New York, 1980, p.25.
4. M.K. Robinson, T.L. Nusair, E.R. Fletcher, and H.L. Ritz, "A Review of the Buehler Guinea Pig Skin Sensitization Test and its Use in a Risk Assessment Process for Human Skin Sensitization", *Toxicology*, 61:91, 1990.



**QUALITY ASSURANCE UNIT GLP INSPECTION AND AUDIT SUMMARY**

This study, X2H377G, was inspected by Quality Assurance at intervals adequate to assure the integrity of the study. The SOP/protocol that the study followed, the phase(s) of the study inspected, and the date(s) of the inspection are provided below.

<u>SOP/Protocol</u>	<u>Phase of Study</u>	<u>Date</u>
16G-12	Induction Exposure #2	9/17/02

QAU inspection findings are routinely reviewed by the management of Northview Pacific Laboratories. Management is notified immediately if there are any deviations which might affect the integrity of the study data.

QAU inspection findings for this study were reported to the Study Director and Management on the following date(s): September 17, 2002

**FINAL REPORT AUDIT**

Quality Assurance has conducted a thorough audit of the test data generated during this study. Northview Report Number X2H377G represents an accurate description of the conduct and final results of the study.

Olympie Heaham  
Quality Assurance Auditor

10/17/02  
Date



### NORTHVIEW PACIFIC LABORATORIES STAFF PARTICIPATING IN THIS STUDY

Some or all of the following staff were involved in the conduct of this study:

1. Gurpreet Ratra, Ph D., Toxicology Manager, *In Vivo* Services
2. Angela Colagross-Schouten, D.V.M., M.P.V.M., Manager, Veterinary Services
3. Cheryl Loughery, Supervisor, *In Vivo* Services
4. Robert Noonan, Ph.D., LATg, Senior Scientist, *In Vivo* Services
5. Roger O'Meara, LATg, Laboratory Technician 3, *In Vivo* Services
6. Fernando D. Salangsang, Laboratory Technician 2, *In Vivo* Services
7. Leah Wilcox, Laboratory Technician 2, *In Vivo* Services
8. Blanca Ramirez, Laboratory Technician 1, *In Vivo* Services
9. Jade David, Laboratory Technician 1, *In Vivo* Services
10. Aria Eshraghi, Laboratory Technician 1, *In Vivo* Services
11. Alicia Chandler, Laboratory Technician 1, *In Vivo* Services
12. Xenia Tan, Laboratory Technician 1, *In Vivo* Services
13. Michelle Pyeatt, Laboratory Technician 1, *In Vivo* Services
14. Zobair Musa, Laboratory Technician 1, *In Vivo* Services
15. Geoffrey J. del Rosario, Laboratory Technician 1, *In Vivo* Services
16. Jessica Houghton, Laboratory Technician 1, *In Vivo* Services
17. Joseph Johnson, Assistant Laboratory Technician 1, *In Vivo* Services
18. Apryl Carlton, Assistant Laboratory Technician 1, *In Vivo* Services
19. Noe Gonzales, Assistant Laboratory Technician 1, *In Vivo* Services
20. Melvin Parker, Assistant Laboratory Technician 1, *In Vivo* Services
21. Darlene Magee, Assistant Laboratory Technician 1, *In Vivo* Services
22. Erin Hung, Laboratory Technician 1, *In Vivo* Services
23. Shannon Murphy, Laboratory Technician 1, *In Vivo* Services
24. Stephanie Lee, Laboratory Technician 1, *In Vivo* Services
25. Arterrias Mason, Laboratory Technician 1, *In Vivo* Services

### STATEMENT OF COMPLIANCE

This study, Northview Report Number X2H377G, has been conducted in accordance with applicable Good Laboratory Practice Regulations.

*Blanca Ramirez*  
 Study Director

10/16/02  
 Study Completion Date

*Olympie Graham*  
 Quality Assurance Unit

10/17/02  
 Date

*G. S. Ratra*  
 Management

10/17/02  
 Date

:cw, 10/16/02 10:46 AM

