FINAL REPORT

Contents:	Text, Tables, and Appendices A - D
Study Title:	Skin Sensitization Study of [ ] in Albino Guinea Pigs (Modified Buehler Method)
Study Number:	WIL-187159
Study Director:	Teresa D. Morris, BS
Data Requirements:	OECD Guidelines Section 406
Study Initiation Date:	24 June 2013
Study Completion Date:	13 January 2014
Performing Laboratory:	WIL Research 1407 George Road Ashland, OH 44805-8946
Sponsor Number:	[ ]
Sponsor:	[ ]

COMPLIANCE STATEMENT

This study, designated WIL-187159, was conducted in compliance with the OECD Principles of GLP [C(97) 186/Final], 26 November 1997; WIL Research's SOPs; and the protocol as approved by the Sponsor, with the following exception. Analytical confirmation of the concentration, homogeneity, and stability of the dosing mixtures was not performed. A Certificate of Analysis was provided by the Sponsor (presented in Appendix B); the characterization analyses were performed in compliance with UK GLP standards (Schedule 1, Good Laboratory Practice Regulations 1999 (SI 1999/3106 as amended by SI 2004/0994)). These regulations are in accordance with GLP standards published as OECD Principles of GLP (revised 1997, ENV/MC/CHEM(98)17).

Teresa D. Morris

Teresa D. Morris, BS
Assistant Director, General Toxicology
Study Director

13 Jan 2014
Date

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1. SUMMARY

1.1. OBJECTIVE

The objective of this study was to determine the ability of the test substance to induce delayed contact hypersensitivity when applied in close contact to the skin of Hartley [Crl:HA] albino guinea pigs (Buehler, 1965).

1.2. TEST GUIDELINES

The protocol was designed to be in general compliance with the OECD Guidelines for Testing of Chemicals, Section 406 (1992).

1.3. STUDY DESIGN

The sensitization potential of [ ] was evaluated in this modified Buehler method dermal sensitization study.

A Test Group of 10 male and 10 female Hartley albino guinea pigs was dosed topically with the test substance 1 time per week for 3 weeks for a total of 3 induction exposures. The duration of each exposure was approximately 6 hours. Approximately two weeks after the last induction exposure, the animals were challenge-dosed for detection of sensitization by topical application of the test substance to a previously unexposed area of skin. The test substance, [ ] was administered at a concentration of 100% (Induction) and at a concentration of 75% (Challenge) prepared w/v in mineral oil.

A Naive Control-I Group of 5 male and 5 female guinea pigs was dosed with the test substance at the challenge phase in the same manner as the Test Group and served as an irritation control.

A Positive Control Group of 5 male and 5 female guinea pigs was included to demonstrate the reliability of the experimental design. The Positive Control Group was induced and challenged on a similar regimen as the Test Group. The positive control material, α-hexylcinnamaldehyde (HCA), was administered at a concentration of 100%

(Induction) and at concentrations of 10% and 20% (Challenge) prepared w/v in 70% acetone/30% PEG 400.

Reactions to challenge dosing were evaluated at approximately 24 and 48 hours after completion of exposure. Body weights and clinical observations were recorded on the day prior to randomization (study day -1) or study day 0 and prior to study termination (study day 30 or 34).

Prior to conducting the induction and challenge phases of the study, two sets of 4 male and 4 female guinea pigs were dosed to determine the appropriate concentrations of test substance to be used for dosing.

1.4. RESULTS

There were no deaths, test substance-related clinical findings, or remarkable body weight changes during the study period. The Incidence Index for the Test Group was 0% (0/20) following challenge dosing.

1.5. CONCLUSIONS

Under the conditions of this study, [ ] was a nonsensitizer in albino guinea pigs.

2. INTRODUCTION

2.1. GENERAL STUDY INFORMATION

This study presents the data from "Skin Sensitization Study of [ ] in Albino Guinea Pigs (Modified Buehler Method)". Due to software spacing constraints, the study title is presented as "Skin Sensitization Study of [ ] in Guinea Pigs Buehler Method" on report tables. The study protocol and deviation from the protocol are presented in Appendix A.

A list of abbreviations potentially used in this report is presented in Section 11. (Abbreviations).

For the data collection process, each phase of the study was separated into what were termed WIL Research computer protocols. The computer protocol reference numbers and types of data collected were identified as follows:

Computer Protocol(s)	Type of Data Collected
WIL-187159	Main study data

2.2. KEY STUDY DATES

Date(s)	Event(s)
24 June 2013	Study initiation date (date protocol signed by Study Director)
25 June 2013	Experimental starting date (Primary Irritation Phase I - animals shaved)
26 June 2013	Experimental start date (Primary Irritation Phase I dosing)
21 August 2013	Experimental termination (completion) date (scheduled euthanasia)

2.3. WIL RESEARCH KEY STUDY PERSONNEL

Bryan P. Fennell, BS	Group Manager, Formulations Laboratory
Heather L. Johnson, BS, RQAP-GLP	Assistant Director, Quality and Regulatory Compliance
Sally A. Keets, AS	Senior Operations Manager, Central Scheduling
Erica L. Lashley, BS, LAT	Senior Animal Operations Manager
Gwendalyn M. Maginnis, DVM	Attending Veterinarian
Theresa M. Rafeld, CPhT	Night Manager, Nonclinical Operations
Robert A. Wally, BS	Operations Manager, Reporting & Technical Support Services

3. EXPERIMENTAL PROCEDURES - MATERIALS AND METHODS

3.1. TEST SUBSTANCE AND POSITIVE CONTROL MATERIAL IDENTIFICATION

The test substance, [ ] was received from the Sponsor on 17 June 2013, as follows:

Identification	Physical Description
[ ] Lot no. TS12003 Exp. date: 01 March 2014 [WIL ID no. 130175]	Light brown viscous liquid

Documentation regarding the purity and stability of the test substance is on file with the Sponsor and WIL Research. A Certificate of Analysis for the test substance was provided by the Sponsor and is presented in Appendix B. The purity of the test substance was 100%; the test substance formulations were not corrected for purity. The test substance was stored at room temperature. A reserve sample of the test substance was collected and stored in the WIL Research Archives.

Mineral oil (lot no. 2AF0729, exp. date 03 August 2013, and lot no. 2BC0539, exp. date 28 January 2015, received from Spectrum Quality Products Inc., New Brunswick, NJ) was used to prepare the test substance formulation for the Primary Irritation and Challenge Phases.

3.2. POSITIVE CONTROL IDENTIFICATION

The positive control material was α-hexylcinnamaldehyde (HCA) (lot no. MKBJ8846V, exp. date: 20 November 2013, received from Sigma Aldrich, Milwaukee, WI).

Acetone (lot no. 2BA0738 , exp. date 18 January 2014, received from Spectrum Quality Products Inc., New Brunswick, NJ) and PEG 400 (lot no. 2BA0610, exp. date 21 October 2014, received from Spectrum Quality Products Inc., New Brunswick, NJ) was used to prepare the HCA formulation for the Challenge Phase.

3.3. PREPARATION

3.3.1. PRIMARY IRRITATION PHASES

The test substance, [ ] was prepared for dosing the Primary Irritation Phases as weight-to-volume (w/v) mixtures in mineral oil at concentrations of 1, 2.5, 5, 10, 25, 50, 75, and 100%. The appropriate amount of test substance for each concentration was weighed and vehicle was added in sufficient quantity to obtain the desired concentrations. Sufficient undiluted test substance was also dispensed for dosing.

3.3.2. INDUCTION PHASE

A sufficient amount of undiluted test substance, [ ] and undiluted positive control material, α-hexylcinnamaldehyde (HCA), were dispensed for induction dosing.

3.3.3. CHALLENGE PHASE

The test substance, [ ] was prepared for dosing the Challenge Phase as a weight-to-volume (w/v) mixture in mineral oil at a concentration of 75%. The appropriate amount of test substance was weighed out and sufficient vehicle was added to obtain the desired concentration.

The positive control material, HCA, was prepared for challenge dosing as 10% and 20% w/v solutions in 70% acetone/30% PEG 400. The appropriate amount of HCA with was weighed for each concentration and sufficient vehicle was added to obtain the desired concentration.

3.4. TEST SYSTEM, ANIMAL RECEIPT, AND ACCLIMATION

Hartley [Crl:HA] albino guinea pigs were used as the test system on this study. The animal model, the Hartley albino guinea pig, is generally recognized as appropriate for skin sensitization studies. The animals were approximately 5 to 9 weeks old at the initiation of dose administration.

The albino guinea pigs utilized for this study were received in good health from Charles River Laboratories, Inc., Saint Constant, Canada, on 18 June 2013. The guinea pigs were

inspected by a qualified technician upon receipt, weighed and uniquely identified by a cage card displaying the animal number. The guinea pigs were acclimated to laboratory conditions for a minimum of 5 days. During the acclimation period, the guinea pigs were observed twice daily for mortality and moribundity.

3.5. METHOD OF TEST SUBSTANCE ADMINISTRATION

The study consisted of 3 phases (Primary Irritation, Induction and Challenge Phases) and 3 treatment groups (Test and Naive Control-I and Positive Control Groups) as illustrated by the following tables. The Test Group consisted of 20 animals that were treated topically in the Induction and Challenge Phases with the test substance. The Naive Control-I Group consisted of 10 animals that remained untreated during the Induction Phase. These animals were treated topically in the Challenge Phase with the test substance. The Positive Group consisted of 10 animals that were treated topically in the Induction and Challenge Phases with the positive control material. An additional group of 10 animals was available for use as a second Naive Control-II Group if rechallenge dosing was required. No rechallenge was conducted; therefore, data from these animals are not included in this report.

The fur of the guinea pigs was removed from the test area using an electric clipper on the day prior to each scheduled dosing.

Primary Irritation Phase I and II				
Group	Number of Animals		Exposure Concentration	Vehicle
	Male	Female		
1	4	4	100, 75, 50, 25, 10, 5, 2.5, and 1% Test Substance	Mineral Oil

Induction and Challenge Phases						
	Number of Animals		Induction		Challenge	
Group	Male	Female	Exp. Conc.	Vehicle	Exp. Conc.	Vehicle
Test	10	10	100% Test Substance	None	75% Test Substance	Mineral Oil
Naive Control-I	5	5	Untreated	Untreated	75% Test Substance	Mineral Oil
Positive Control	5	5	100% HCA	None	10% and 20% HCA	70%/30% acetone/ PEG 400

Exp. Conc. = Exposure Concentration
HCA = α-hexylcinnamaldehyde

Primary Irritation Phase I consisted of single applications of multiple concentrations of the test substance to determine an irritation threshold.

The prepared concentrations of test substance were administered at 0.3 mL/site. Doses were applied under 25-mm Hill Top Chambers®, occluded with plastic wrap and overwrapped with nonirritating elastic tape. There were 4 sites per guinea pig and 4 sites per concentration. The period of exposure was approximately 6 hours, after which the bandages were removed and the sites wiped with disposable paper towels moistened with mineral oil and/or tepid tap water. On the following day, at least 3 hours prior the 24-hour scoring, a commercial depilatory was applied to the area of each animal. After approximately 5-8 minutes, the depilatory was removed using a stream of warm running water, and the animals were gently dried using disposable towels and returned to their cages.

Due to the degree of irritation (erythema) noted during scoring of Primary Irritation Phase I and the potential reaction with the depilatory, the irritation screen was repeated. Primary Irritation Phase II was conducted in the same manner as Primary Irritation Phase, with the exception that the fur of the guinea pigs was removed from the test area using an electric clipper prior to the 24-hour scoring. The results of Primary Irritation Phase II were used to select the test substance concentrations for the Induction and Challenge

Phases. The highest, slightly irritating concentration of the test substance was selected as the dose level for induction and an essentially nonirritating concentration of the test substance was chosen as the dose level for the Challenge Phase.

No depilatory was used for any other phase of this study.

3.5.1. INDUCTION PHASE

The Induction Phase consisted of multiple applications of test substance to stimulate the immune system.

The test substance (100% [ ] or positive control material (100% HCA) was administered at 0.3 mL/site. Doses were applied under 25-mm Hill Top Chambers®, occluded with plastic wrap and overwrapped with nonirritating elastic tape. Induction doses were applied to the same site on the anterior left flank of all Test and Positive Control Group animals. Test and Positive Control group animals received 3 induction doses spaced 1 week apart over a period of 3 weeks. All induction exposures were approximately 6 hours, after which the bandages were removed and the sites wiped with disposable paper towels moistened with mineral oil and/or tepid tap water. Naive Control-I Group animals remained untreated during the Induction Phase.

3.5.2. CHALLENGE PHASE

The Challenge Phase consisted of a single application of the maximal essentially nonirritating concentration of test substance to determine if delayed contact hypersensitivity had occurred.

Two weeks after the final induction dose, a 75% concentration of the test substance, [ ] in mineral oil was administered to previously unexposed sites on the posterior left flank of the Test and Naive Control-I Group animals at 0.3 mL/site. For the Positive Control group, 10% and 20% concentrations of HCA in 70% acetone/30% PEG 400 were administered to previously unexposed sites on the posterior right and left flanks, respectively, at 0.3 mL/site. Doses were applied under 25-mm Hill Top Chambers® that

were occluded with plastic wrap and overwrapped with nonirritating elastic tape. All challenge exposures were approximately 6 hours, after which the bandages were removed and the sites wiped with disposable paper towels moistened mineral oil and/or tepid tap water.

3.6. ROUTE AND RATIONALE OF TEST SUBSTANCE ADMINISTRATION

The selected route of administration for this study was topical application. This study was intended to provide information on the health hazards likely to arise from exposure to the test substance by the dermal route. The modified Buehler method is an accepted procedure for evaluating the potential of test substances to induce immunologically mediated dermal sensitization. The experimental design used the procedures and standards required by the current federal and international regulations.

3.7. ANIMAL HOUSING

Upon arrival, all animals were housed in individual suspended wire-mesh cages. The animals were maintained by the animal husbandry staff of WIL Research in accordance with SOPs. The animal facilities at WIL Research are accredited by AAALAC International.

3.8. DIET, DRINKING WATER, AND MAINTENANCE

The basal diet used in this study, PMI Nutrition International, LLC, Certified Guinea Pig LabDiet® 5026, is a certified feed with appropriate analyses performed by the manufacturer and provided to WIL Research. Municipal water supplying the facility was analyzed for contaminants according to WIL Research SOPs. The results of the diet and water analyses are maintained at WIL Research. No contaminants were present in animal feed or water at concentrations sufficient to interfere with the objectives of this study. The basal diet and municipal water, delivered by an automatic watering system, were provided *ad libitum* throughout the acclimation period and during the study.

3.9. ENVIRONMENTAL CONDITIONS

All animals were housed throughout the acclimation period and during the study in an environmentally controlled room. The room temperature and humidity controls were set to maintain environmental conditions of 66 ± 5ºF (19 ± 3ºC) and 50 ± 20%, respectively. Room temperature and relative humidity data were monitored continuously and were scheduled for automatic collection on an hourly basis. These data are summarized in Appendix C. Actual mean daily temperature ranged from 65.8ºF to 69.9ºF (18.8ºC to 21.1ºC) and mean daily relative humidity ranged from 47.0% to 68.4% during the study. Fluorescent lighting provided illumination for a 12-hour light (0600 hours to 1800 hours)/12-hour dark photoperiod. Lighting conditions were recorded every 15 minutes. The 12-hour light/12-hour dark photoperiod was interrupted if necessary to allow for the performance of protocol-specified activities. Air handling units were set to provide a minimum of 10 fresh air changes per hour.

3.10. ASSIGNMENT OF ANIMALS TO TREATMENT GROUPS

Animals used on the Primary Irritation Phases were arbitrarily selected from available stock. Animals used on the main study were selected from available stock and assigned to groups by stratified randomization through use of WTDMS™. The selected animals were young adult; body weight values ranged from 433 g to 542 g for males and from 337 g to 487 g for females at randomization excluding Primary Irritation Phase animals.

4. PARAMETERS EVALUATED

4.1. MORTALITY

The guinea pigs were observed twice daily, once in the morning and once in the afternoon, for mortality and moribundity during all phases of the study.

4.2. CLINICAL OBSERVATIONS

Detailed physical examinations were performed on all animals prior to initiation of dosing and at study termination (study day 2 , 30, or 34).

4.3. BODY WEIGHTS

Body weights were obtained and recorded on study day -1 (the day prior to selection/randomization) or 0 and near study termination (study day 2, 30, or 34).

4.4. DERMAL OBSERVATIONS

All application sites were examined and graded in accordance with the score scale presented below at approximately 24 and 48 hours after completion of exposure

Score Scale

0	No reaction
±	Very slight, dispersed erythema
1	Discrete (slight confluent) or moderate patchy erythema
2	Moderate and confluent erythema
3	Severe erythema and/or moderate to severe edema

Prior to evaluation of the Primary Irritation II and Challenge Phase skin reactions, the hair was clipped from the backs of the guinea pigs with an electric shaver.

The sensitization potential of the test substance was based on the dermal responses observed on the test and naive control animals at challenge. Generally, dermal scores of ≥1 in the test animals with scores of 0 to ± noted in the controls were considered indicative of sensitization. A dermal score of 1 in both the test and naive control animals was generally considered equivocal unless a higher dermal response (≥ Grade 2) was noted in the test animals. Group mean dermal scores were calculated for challenge. A

response of at least 15% in a nonadjuvant test would be expected for a mild to moderate sensitizer.

The concentration used at challenge for the Positive Control Group was a known non-irritating concentration; therefore, grades of 1 or greater in the Positive Control Group indicated sensitization responses.

4.4.1. INCIDENCE INDEX

The Incidence Index is the percentage of animals in the Test Group showing post-challenge sensitization responses at either 24 or 48 hours after completion of exposure. Reactions in the Test Group are considered positive when they are more intense than the responses to the test substance in the Naive Control Groups.

4.4.2. SEVERITY INDEX

The Severity Index is the sum of the post-challenge test grades divided by the total number of animals tested (rounded to the nearest tenth). This index was calculated for each group separately at 24 and 48 hours. Grades of ± were equal to 0.5 for calculation purposes.

4.5. NECROPSY

Following study termination, the guinea pigs were euthanized by carbon dioxide inhalation and discarded without examination.

4.6. DATA ACQUISITION AND REPORTING

Program/System	Description
Archive Management System (AMS)	In-house developed application for storage, maintenance, and retrieval of information for archived materials (*e.g.*, lab books, study data, wet tissues, slides, *etc.*).
Formulations Dose Dispensing Management System (FDDMS)	In-house developed system used to assign unique barcodes to formulation containers and individual containers used for dispensing dosing formulations.

Program/System	Description
InSight® Publisher	Electronic publishing system (output is Adobe Acrobat, PDF).
Master Schedule	Maintains the master schedule for the company.
Metasys DDC Electronic Environmental Control System	Controls and monitors animal room environmental conditions.
Microsoft® Office 2007	Used in conjunction with the publishing software to generate study reports.
Provantis Dispense™	Comprehensive system (Instem LSS Limited) to manage test materials, including receipt, formulation instructions, and accountability.
WIL Formulations Dispense System (WFDS)	In-house developed system for use in conjunction with Provantis Dispense™ to ensure proper storage and use of formulations.
WIL Metasys	In-house developed system used to record and report animal room environmental conditions.
WIL Toxicology Data Management System™ (WTDMS™)	In-house developed system used for collection and reporting of in-life and *postmortem* data.

Note: Version numbers of WTDMS™ programs used for the study are presented on the report data tables (reporting programs); version numbers and release dates are otherwise maintained in the study records and/or facility records.

5. RESULTS AND DISCUSSION

5.1. MORTALITY

Data: Table 1, Table 5, Appendix D

There were no deaths during the study.

5.2. CLINICAL OBSERVATIONS

Data: Table 1, Table 5, Appendix D

There were no test substance-related clinical findings observed during the study.

During Primary Irritation Phase I, male no. 22783 had a reddened right ear on study days 0 (prior to dosing) and 2.

In the main study, one Positive Control Group male (no. 22836) had dried brown material on the hindlimb(s) on study day 30. One Test Group male (no. 22804) had dried yellow material on the anogenital area and one Test Group female (no. 22871) had reddened ears on study day 34.

There were no other clinical observations.

5.3. BODY WEIGHTS

Data: Table 2, Table 6

There were no remarkable body weight changes noted during the study.

5.4. DERMAL OBSERVATIONS AND SEVERITY INDICES

Data: Table 3, Table 7, Table 8, Table 9

Challenge Dosing

Dermal Scores

Group	Material	24-hour 0	±	1	2	3	48-hour 0	±	1	2	3	Severity Index 24-hour	48-hour	Incidence Index
Test	[75%]	14	5	1	0	0	17	3	0	0	0	0.2	0.1	0%
Naive Control-I	[75%]	4	4	2	0	0	6	3	1	0	0	0.4	0.3	NA
Positive Control	10% HCA	3	3	4	0	0	5	5	0	0	0	0.6	0.3	40%
Positive Control	20% HCA	1	2	4	3	0	3	3	4	0	0	1.1	0.6	70%

NA = Not Applicable

6. CONCLUSIONS

Under the conditions of this study, [ ] was a nonsensitizer in albino guinea pigs.

7. REPORT REVIEW AND APPROVAL

Report Authored and Approved By:

Teresa D. Morris, BS
Assistant Director, General Toxicology
Study Director

13 Jan 2014
Date

Report Prepared By:

Charlene A. Weygandt, BS
Lead Analyst and Scientific Advisor,
Reporting & Technical Support Services

13 Jan 2014
Date

Reviewed By:

Jonathan M. Hurley, BS
Staff Toxicologist &
Head of Acute Toxicology

13 Jan 2014
Date

8. QUALITY ASSURANCE STATEMENT

Date(s) of Inspection(s)	Phase Inspected	Date(s) Findings Reported to Study Director and Management
24-Jun-2013	Protocol	24-Jun-2013
26-Jun-2013	Test Substance Administration	26-Jun-2013
10-Jul-2013	Protocol Amendment 1	10-Jul-2013
15-Aug-2013	Dermal Observations	15-Aug-2013
06-Sep-2013	Study Records (Rx-1)	06-Sep-2013
06-Sep-2013, 09-Sep-2013	Study Records (I-1)	09-Sep-2013
26-Sep-2013, 27-Sep-2013	Draft Report	27-Sep-2013
09-Jan-2014	Final Report	09-Jan-2014

This study was inspected in accordance with the United States OECD Principles of GLP [C(97) 186/Final], WIL Research's SOPs, and the Sponsor's protocol, with the following exception. The data located in Appendix B (Certificate of Analysis) were the responsibility of the Sponsor. Yearly internal facility inspection are conducted by the WIL Research Quality Assurance Department. A status report is submitted to management monthly.

This report accurately reflects the data generated during the study. The methods and procedures used in the study were those specified in the protocol, its amendments, and WIL Research's SOPs.

Dustin Risner, BA
Quality Assurance Representative

13 Jan 2014
Date

9. DATA RETENTION

The Sponsor has title to all documentation records, raw data, specimens, or other work product generated during the performance of the study. All remaining work product generated by WIL Research, including raw paper data and specimens, are retained in the WIL Research Archives as specified in the study protocol.

A reserve sample of the test substance, pertinent electronic storage media, and the original final report are retained in the WIL Research Archives in compliance with regulatory requirements.

10. REFERENCES

Buehler, E.V. Delayed contact hypersensitivity in the guinea pig. *Archives of Dermatology* **1965,** *91*, 171-7.

11. ABBREVIATIONS

The following abbreviations may apply to this report:

µ	-	micro
AAALAC	-	Association for Assessment and Accreditation of Laboratory Animal Care
BMDS	-	Bio Medic Data Systems
cm	-	centimeter
dB	-	decibels
dL	-	deciliter
g	-	gram
GLP	-	Good Laboratory Practices
hr	-	hour(s)
kg	-	kilogram
L	-	liter
M	-	molar
mg	-	milligram
mL	-	milliliter
mm	-	millimeter
ms	-	milliseconds
mM	-	millimolar
NA	-	not applicable
OECD	-	Organisation for Economic Cooperation and Development
ppm	-	parts per million
SOP	-	standard operating procedure
WTDMS™	-	WIL Toxicology Data Management System

TABLES 1 - 9

TABLE 1 (PRIMARY IRRITATION PHASES)
SUMMARY OF CLINICAL FINDINGS: TOTAL OCCURRENCE/NO. OF ANIMALS

Primary Irritation Phase I - Males

Table Range	Day 0	Day 2
Normal		
- No Significant Clinical Observations	3/3	3/3
Eyes/Ears/Nose		
- Reddened right ear	1/1	1/1

Primary Irritation Phase I- Females

Table Range	Day 0	Day 2
Normal		
- No Significant Clinical Observations	4/4	4/4

Primary Irritation Phase II - Males

Table Range	Day 0	Day 2
Normal		
- No Significant Clinical Observations	4/4	4/4

Primary Irritation Phase II- Females

Table Range	Day 0	Day 2
Normal		
- No Significant Clinical Observations	4/4	4/4

TABLE 2 (PRIMARY IRRITATION PHASES)
INDIVIDUAL BODY WEIGHTS (G)

Primary Irritation Phase I			
Animal	Sex	Day 0	Day 2
22781	M	385.7	383.2
22782	M	353.8	358.9
22783	M	357.6	364.8
22784	M	342.5	347.8
22851	F	343.3	344.5
22852	F	336.3	333.5
22853	F	374.9	372.3
22854	F	333.0	314.7
Primary Irritation Phase II			
Animal	Sex	Day 0	Day 2
22785	M	424.5	440.5
22786	M	491.2	494.5
22788	M	449.7	452.3
22791	M	433.2	435.1
22857	F	438.6	451.7
22858	F	420.0	428.1
22860	F	428.2	437.7
22863	F	443.4	450.3

M = Male
F = Female

TABLE 3 (PRIMARY IRRITATION PHASE I)
INDIVIDUAL DERMAL REACTIONS

Primary Irritation Phase

Males				
	Material: [ ]			
Animal	Sex	Site/Conc. (%)	24-Hour Observation [a]	48-Hour Observation
22781	M	A/100	±	±
		B/75	±	±
		C/50	±	±
		D/25	±	±
22782	M	A/25	1	±
		B/100	1	±
		C/75	1	±
		D/50	±	±
22783	M	A/10	1	±
		B/5	1	±
		C/2.5	1	±
		D/1	±	±
22784	M	A/1	1	±
		B/10	1	0
		C/5	1	±
		D/2.5	1	0

M = Male

[a] = All animals were scored at 24 hours after administration; the backs of the animals were reddened, and thus the sites were re-evaluated approximately 1 hour later. If the score changed, the latter score appears on this table.

Head
A C
B D
Tail

TABLE 3 (PRIMARY IRRITATION PHASE I)
INDIVIDUAL DERMAL REACTIONS

Primary Irritation Phase

Females				
	Material: [ ]			
Animal	Sex	Site/Conc. (%)	24-Hour Observation [a]	48-Hour Observation
22851	F	A/50	1	±
		B/25	1	±
		C/100	1s	±s
		D/75	1	±
22852	F	A/75	±	±
		B/50	±	±
		C/25	±	0
		D/100	0	0
22853	F	A/2.5	±	±
		B/1	±	0
		C/10	±	0
		D/5	±	0
22854	F	A/5	±	0
		B/2.5	0	0
		C/1	±	0
		D/10	±	0

F = Female; s= scabbing noted at perimeter of dose site

[a] = All animals were scored at 24 hours after administration; the backs of the animals were reddened, and thus the sites were re-evaluated approximately 1 hour later. If the score changed, the latter score appears on this table.

Head
A C
B D
Tail

TABLE 4 (PRIMARY IRRITATION PHASE II)
INDIVIDUAL DERMAL REACTIONS

Primary Irritation Phase

Males				
	Material: [ ]			
Animal	Sex	Site/Conc. (%)	24-Hour Observation	48-Hour Observation
22785	M	A/100	±	0
		B/75	±	±
		C/50	±	0
		D/25	±	0
22786	M	A/25	±	±
		B/100	±	±
		C/75	±	±
		D/50	±	±
22788	M	A/10	±	±
		B/5	0	0
		C/2.5	±	±
		D/1	±	±
22791	M	A/1	±	±
		B/10	±	±
		C/5	±	±
		D/2.5	±	0

M = Male

Head
A C
B D
Tail

TABLE 4 (PRIMARY IRRITATION PHASE II)
INDIVIDUAL DERMAL REACTIONS

Primary Irritation Phase

Females				
	Material: [ ]			
Animal	Sex	Site/Conc. (%)	24-Hour Observation	48-Hour Observation
22857	F	A/50	±	±
		B/25	±	±
		C/100	±	±
		D/75	±	±
22858	F	A/75	±	±
		B/50	±	±
		C/25	±	±
		D/100	±	±
22860	F	A/2.5	0	0
		B/1	±	0
		C/10	±	±
		D/5	0	0
22863	F	A/5	±	0
		B/2.5	±	0
		C/1	0	0
		D/10	0	0

F = Female

Head
A C
B D
Tail

TABLE 5 (MAIN STUDY)

PROJECT NO.:WIL-187159 SKIN SENSITIZATION STUDY OF [ ] IN GUINEA PIGS BUEHLER METHOD PAGE 1

SPONSOR:[ ]

SUMMARY OF CLINICAL FINDINGS: TOTAL OCCURRENCE/NO. OF ANIMALS

SPONSOR NO.:[ ]

----- M A L E -----

TABLE RANGE:	07-16-13 TO 08-21-13		
GROUP:	1	2	4
NORMAL			
-NO SIGNIFICANT CLINICAL OBSERVATIONS	19/10	10/ 5	9/ 5
DISPOSITION			
-EUTHANIZED BY CO2 AND DISCARDED	10/10	5/ 5	5/ 5
BODY/INTEGUMENT			
-DRIED BROWN MATERIAL HINDLIMB(S)	0/ 0	0/ 0	1/ 1
-DRIED YELLOW MATERIAL ANOGENITAL AREA	1/ 1	0/ 0	0/ 0

1- TEST GROUP 2-NAIVE CONTROL-I 3-POSITIVE CONTROL

----- F E M A L E -----

TABLE RANGE:	07-16-13 TO 08-21-13		
GROUP:	1	2	4
NORMAL			
-NO SIGNIFICANT CLINICAL OBSERVATIONS	19/10	10/ 5	9/ 5
DISPOSITION			
-EUTHANIZED BY CO2 AND DISCARDED	10/10	5/ 5	5/ 5
BODY/INTEGUMENT			
-DRIED YELLOW MATERIAL ANOGENITAL AREA	0/ 0	0/ 0	1/ 1
EYES/EARS/NOSE			
-REDDENED LEFT EAR	1/ 1	0/ 0	0/ 0
-REDDENED RIGHT EAR	1/ 1	0/ 0	0/ 0

1- TEST GROUP 2-NAIVE CONTROL-I 3-POSITIVE CONTROL

TABLE 6 (MAIN STUDY)

PROJECT NO.:WIL-187159 SKIN SENSITIZATION STUDY OF [ ] IN GUINEA PIGS BUEHLER METHOD PAGE 1

SPONSOR:[ ]

SPONSOR NO.:[ ]

INDIVIDUAL BODY WEIGHTS [G]

MALE GROUP: TEST GROUP

DAY	-1	34
ANIMAL		
22790	447.	666.
22796	468.	668.
22802	472.	624.
22804	454.	573.
22809	462.	566.
22812	479.	647.
22827	498.	690.
22829	507.	778.
22841	542.	770.
22843	537.	760.
MEAN	487.	674.
S.D.	33.3	76.7
N	10	10

TABLE 6 (MAIN STUDY)

PROJECT NO.:WIL-187159 SKIN SENSITIZATION STUDY OF [ ] IN GUINEA PIGS BUEHLER METHOD

SPONSOR:[ ]

SPONSOR NO.:[ ]

INDIVIDUAL BODY WEIGHTS [G]

MALE GROUP:NAIVE CONTROL-I

DAY	-1	30
ANIMAL		
22819	498.	677.
22831	439.	592.
22832	530.	745.
22839	455.	638.
22846	474.	669.
MEAN	479.	664.
S.D.	35.9	56.2
N	5	5

TABLE 6 (MAIN STUDY)

PROJECT NO.:WIL-187159 SKIN SENSITIZATION STUDY OF [ ] IN GUINEA PIGS BUEHLER METHOD

SPONSOR:[ ]

SPONSOR NO.:[ ]

INDIVIDUAL BODY WEIGHTS [G]

MALE GROUP:POSITIVE CONTROL

DAY	-1	30
ANIMAL		
22789	466.	561.
22795	433.	585.
22821	475.	705.
22836	510.	653.
22837	528.	690.
MEAN	482.	639.
S.D.	37.4	63.5
N	5	5

TABLE 6 (MAIN STUDY)

PROJECT NO.:WIL-187159 SKIN SENSITIZATION STUDY OF [ ] IN GUINEA PIGS BUEHLER METHOD PAGE 4

SPONSOR:[ ]

SPONSOR NO.:[ ]

INDIVIDUAL BODY WEIGHTS [G]

FEMALE GROUP: TEST GROUP

DAY	-1	34
ANIMAL		
22856	448.	610.
22861	337.	386.
22868	418.	499.
22871	454.	620.
22881	423.	530.
22890	487.	620.
22898	465.	618.
22904	397.	481.
22910	426.	541.
22916	427.	562.
MEAN	428.	547.
S.D.	41.2	76.6
N	10	10

TABLE 6 (MAIN STUDY)

PROJECT NO.:WIL-187159 SKIN SENSITIZATION STUDY OF [ ] IN GUINEA PIGS BUEHLER METHOD PAGE 5

SPONSOR:[ ]

SPONSOR NO.:[ ]

INDIVIDUAL BODY WEIGHTS [G]

FEMALE GROUP:NAIVE CONTROL-I

DAY	-1	30
ANIMAL		
22872	389.	478.
22902	437.	525.
22908	460.	611.
22913	414.	496.
22914	436.	578.
MEAN	427.	538.
S.D.	26.8	55.8
N	5	5

TABLE 6 (MAIN STUDY)

PROJECT NO.:WIL-187159 SKIN SENSITIZATION STUDY OF [ ] IN GUINEA PIGS BUEHLER METHOD

SPONSOR:[ ]

SPONSOR NO.:[ ]

INDIVIDUAL BODY WEIGHTS [G]

FEMALE GROUP:POSITIVE CONTROL

DAY	-1	30
ANIMAL		
22866	478.	607.
22883	437.	560.
22887	414.	536.
22893	436.	546.
22903	420.	511.
MEAN	437.	552.
S.D.	25.0	35.6
N	5	5

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TABLE 7 (INDUCTION PHASE)
INDIVIDUAL DERMAL REACTIONS

Induction Phase – Test Group

Material: 100% [ ] Test Site: Anterior Left Flank

Animal	Sex	Induction					
		1		2		3	
	Interval:	24-Hour	48-Hour	24-Hour	48-Hour	24-Hour	48-Hour
22790	M	0	0	0	±	1	±
22796	M	0	0	±	±	±	±
22802	M	0	0	1	±	±	±
22804	M	0	0	±	±	±	0
22809	M	±	0	±	0	1	±
22812	M	0	0	0	0	±	0
22827	M	0	±	±	±	±	±
22829	M	0	±	±	0	±	±
22841	M	±	±	±	0	±	±
22843	M	0	0	1	±	1	±
22856	F	±	0	1	±	±	0
22861	F	±	0	±	0	±	±
22868	F	0	0	0	0	±	±
22871	F	0	0	±	0	±	±
22881	F	0	0	1	0	±	0
22890	F	0	0	±	0	0	0
22898	F	0	±	1	0	±	0
22904	F	0	0	±	0	±	0
22910	F	0	0	1	±	1	±
22916	F	±	0	1	0	±	0

M = Male; F = Female

TABLE 7 (INDUCTION PHASE)
INDIVIDUAL DERMAL REACTIONS

Induction Phase – Positive Control Group

Material: 100% HCA Test Site: Anterior Left Flank

Animal	Sex	Induction					
		1		2		3	
	Interval:	24-Hour	48-Hour	24-Hour	48-Hour	24-Hour	48-Hour
22789	M	1	±	2	1	2	2
22795	M	1	±	2	1	3	3d
22821	M	±	±	2	1	3	3
22836	M	±	±	±	0	2	2
22837	M	1	±d	2	1	3	2
22866	F	±	±	2	1	3	3
22883	F	1	1	1	±	3	3
22887	F	1	1	3	2	3e	3e
22893	F	±	±	1	±	2	1d
22903	F	1	1d	2	1	3d	3d

M = Male; F = Female
d = desquamation; e = eschar

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TABLE 8 (CHALLENGE DOSING)
INDIVIDUAL DERMAL REACTIONS

Challenge Dosing – Test Group

Material: 75% [ ]		Test Site: Posterior Left Flank	
		Observation Period	
Animal	Sex	24 Hour	48 Hour
22790	M	±	±
22796	M	±	±
22802	M	0	0
22804	M	0	0
22809	M	1	0
22812	M	0	±
22827	M	0	0
22829	M	±	0
22841	M	0	0
22843	M	±	0
22856	F	0	0
22861	F	±	0
22868	F	0	0
22871	F	0	0
22881	F	0	0
22890	F	0	0
22898	F	0	0
22904	F	0	0
22910	F	0	0
22916	F	0	0

M = Male; F = Female

TABLE 8 (CHALLENGE DOSING)
INDIVIDUAL DERMAL REACTIONS

Challenge Dosing – Naive Control-I

Material: 75% [ ]		Test Site: Posterior Left Flank	
		Observation Period	
Animal	Sex	24 Hour	48 Hour
22819	M	±	0
22831	M	0	0
22832	M	0	0
22839	M	1	1
22846	M	±	±
22872	F	±	±
22902	F	0	0
22908	F	±	0
22913	F	0	0
22914	F	1	±

M = Male; F = Female

TABLE 8 (CHALLENGE DOSING)
INDIVIDUAL DERMAL REACTIONS

Challenge Dosing – Positive Control

Material: HCA					
		Observation Period			
		24 Hour		48 Hour	
Animal	Sex	A	B	A	B
22789	M	1	1	±	0
22795	M	1	2	0	1
22821	M	1	1	±	±
22836	M	0	±	0	0
22837	M	±	2	±	1
22866	F	0	1	0	1
22883	F	1	±	±	±
22887	F	±	2	±	1
22893	F	0	0	0	0
22903	F	±	1	0	±

M = Male; F = Female
A = 10% HCA - Posterior Left Flank; B = 20% HCA - Posterior Right Flank

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TABLE 9 (CHALLENGE DOSING)
INCIDENCE OF DERMAL RESPONSES

Challenge Dosing

Group	Material	Interval	Dermal Scores 0	±	1	2	3	Number of Animals	Incidence Index	Severity Index
Test Group	75% [ ]	24-Hours	14	5	1	0	0	20	0%	0.2
		48-Hours	17	3	0	0	0	20		0.1
Naive Control-I	75% [ ]	24-Hours	4	4	2	0	0	10	NA	0.4
		48-Hours	6	3	1	0	0	10		0.3
Positive Control Group	10% HCA	24-Hours	3	3	4	0	0	10	40%	0.6
		48-Hours	5	5	0	0	0	10		0.3
Positive Control Group	20% HCA	24-Hours	1	2	4	3	0	10	70%	1.1
		48-Hours	3	3	4	0	0	10		0.6

NA = Not applicable

APPENDIX A

Study Protocol and Deviation

DEVIATION FROM THE PROTOCOL

This study was conducted in accordance with the protocol, except for the following.

- **Protocol Section 5.6** states that animals will weigh approximately 300 to 450 g (excluding Primary Irritation Phase animals) at randomization. However, the animals selected for the study weighed 433 g to 542 g for males and 337 to 487 g for females at randomization.

  Impact Statement: Animal body weights were greater than the protocol-specified range due to a delay in the start of the induction exposure. This had no effect on the study since the animals were still young adults.

This deviation did not negatively impact the quality or integrity of the data nor the outcome of the study.

PROTOCOL AMENDMENT 1

Study Number: WIL-187159

Sponsor: [ ]

Sponsor Study Reference Number: [ ]

Title of Study:

Skin Sensitization Study of [ ] in Albino Guinea Pigs (Modified Buehler Method)

Protocol Modifications:

1) **8.1 Primary Irritation Phase:**

   The primary irritation phase will be repeated using the same dose concentrations. Eight (8) additional naïve guinea pigs will be used. All procedures will be the same with the exception that animals will not be depilated the day following dosing.

2) **8.3 Challenge Phase:**

   Remove paragraph 5; animals will not be depilated the day following dosing.

3) **9.2.2 Primary Irritation and Challenge Phase:**

   A commercial depilatory was used as described for the first primary irritation screen. No depilatory will be used for any other phase of this study. For the second irritation screen and the challenge phase, the test sites will be carefully clipped with an electric shaver at least three hours prior to the 24-hour scoring.

Reasons for Protocol Modifications:

1-3) Due to the degree of irritation (erythema) noted during scoring of the primary irritation screen and the potential reaction with the depilatory, the irritation screen will be repeated. The use of a commercial depilatory will be replaced by clipping of the test sites with an electric shaver.

Approval:

Sponsor approval received by the Study Director via Email on 09 July 2013.

WIL Research

Teresa D. Morris, BS
Study Director

10 Jul 2013
Date

Chevron Energy Technology Company

[ ]

Linda Waid, RVT, LAT
Sponsor Representative

1/7/2014
Date

PROTOCOL

Skin Sensitization Study of [ ] in Albino Guinea Pigs (Modified Buehler Method)

[OECD Guideline 406]

WIL Study Number: WIL-187159

Sponsor Study Reference Number: [ ]

Submitted To:

[ ]

WIL Research
1407 George Road
Ashland, OH 44805-8946

1 OBJECTIVE:

To determine the ability of the test substance to induce delayed contact hypersensitivity when applied in close contact to the skin of albino guinea pigs (Buehler, 1965).

This protocol has been designed and the study will be conducted in general compliance with the following guideline:

Organisation for Economic Cooperation and Development (OECD) Guidelines for Testing of Chemicals, Section 406 (1992).

The study will be conducted in compliance with the OECD [C(97) 186/Final] Good Laboratory Practice Regulations; with the exception that analytical confirmation of the concentration, homogeneity and stability of the dosing mixture (if prepared) will not be performed.

2 PERSONNEL INVOLVED IN THE STUDY:

2.1 Sponsor Representative:

[ ]

2.2 WIL Study Director:

Teresa D. Morris, BS
Assistant Director, General Toxicology
Tel: (419) 289-8700
Fax: (419) 289-3650
E-mail: teresa.morris@wilresearch.com

2.3 WIL Departmental Responsibilities:

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Bennett J. Varsho, MPH, DABT
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Sally A. Keets, AS
Senior Operations Manager, Central Scheduling

Erica L. Lashley, BS, LAT
Senior Animal Operations Manager

Bryan P. Fennel, BS
Group Manager, Formulations Laboratory

Patrick A. Swyers, BS
Manager, Gross Pathology Toxicology Laboratory

Gwendalyn M. Maginnis, DVM
Attending Veterinarian

Robert A. Wally, BS
Operations Manager, Reporting
& Technical Support Services

Heather L. Johnson, BS, RQAP-GLP
Assistant Director of Quality and Regulatory Compliance

3 STUDY SCHEDULE:

Proposed Experimental Starting (Transfer from Stock) Date:	25 June 2013
Proposed Experimental Start (First Day of Dosing) Date:	26 June 2013
Proposed Experimental Completion/Termination Date:	02 August 2013
Proposed Audited Draft Report Date:	13 September 2013

4 TEST SUBSTANCE:

Unless otherwise noted, the identity, strength, purity, composition, stability and method of syntheses (fabrication and/or derivation) of the test substance will be documented by the Sponsor. A Certificate of Analysis for the test substance will be provided to WIL Research for inclusion in the final report.

4.1 Identification:

[ ]

4.2 Lot Number:

To be provided by the Sponsor

4.3 Purity:

Responsibility of the Sponsor

4.4 Stability:

Responsibility of the Sponsor

4.5 Physical Description:

To be documented by WIL Research

4.6 Storage Conditions:

To be provided by the Sponsor

4.7 Personnel Safety:

At minimum, appropriate gloves, eye protection and long sleeves (lab coat) are to be worn during dose administration. Refer to Material Safety Data Sheet for complete available information.

4.8 Retention Samples:

A retention sample of the test substance (as received) will be collected in accordance with WIL Research SOP No. T2-001. Dosing preparation samples will not be collected.

4.9 Unused Test substance:

The unused portion of the test substance will be returned following the issuance of the final study report. Alternatively, the test substance may be retained for subsequent studies.

5 TEST SYSTEM:

5.1 Species:

Albino guinea pig

5.2 Strain:

Hartley (Crl:HA)

5.3 Source:

Charles River Laboratories
(Documentation of the specific breeding facility will be maintained in the study records and included in the final report.)

5.4 Number on Study:

A total of 58 animals will be obtained from the acute stock colony. Eight (8) animals will be assigned to the Primary Irritation Group, twenty (20) animals to the Test Group, ten (10) animals to the Naïve Control-I Group and ten (10) animals to the Positive Control Group. Ten (10) animals will be maintained for use, in the Naïve Control-II Group, should rechallenge dosing be conducted.

5.5 Sex:

Equal number of males and females in each group. Females will be nulliparous and nonpregnant.

5.6 Body Weight Range:

Approximately 300 to 450 grams (excluding Primary Irritation Phase animals) at randomization.

5.7 Approximate Age:

Young adult

5.8 Identification System:

Individual cage cards will be affixed to each cage and will display the animal number, sex, group and study number.

5.9 Justification for Selection:

This species and strain is generally recognized as appropriate for skin sensitization studies. The number of animals selected is the number required to satisfy regulatory guidelines. The experimental design uses the procedures and standards required by the current federal and international regulations.

6 SPECIFIC MAINTENANCE SCHEDULE:

6.1 Animal Housing:

The animals will be housed individually in suspended wire-mesh cages in an environmentally controlled room. Animals will be housed in clean cages elevated above ground corncob bedding or other suitable material that will be changed at least three times each week. Animals will be changed out into clean cages approximately every two weeks. The facilities at WIL Research are fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC International).

6.2 Environmental Conditions:

Controls will be set to maintain the temperature at 66 ± 5°F (19 ± 3°C) and the relative humidity at 50 ± 20%. Temperature and relative humidity will be monitored continuously. Data for these two parameters will be scheduled for automatic collection on an hourly basis. Fluorescent lighting will provide illumination for a 12-hour light/dark photoperiod. Temporary adjustments to the light/dark cycles may be made to accommodate protocol-specified activities. The ventilation rate will be set at a minimum of 10 room air changes per hour, 100% fresh air.

6.3 Drinking Water:

Municipal water will be available *ad libitum*. Filters servicing the automatic watering system will be changed regularly according to standard operating procedures (SOPs). Municipal water supplying the laboratory is analyzed for contaminants according to SOPs to ascertain that none are present at concentrations that would be expected to affect the outcome of the study and the results are maintained on file.

6.4 Basal Diet:

PMI Nutrition International, LLC Certified Guinea Pig LabDiet® 5026 will be offered *ad libitum* during the study. Standard Operating Procedures provide specifications for acceptable levels of heavy metals and pesticides that are reasonably expected to be present in the diet without interfering with the purpose or conduct of the study. Analyses are performed and provided by the manufacturer and the results are maintained on file.

7 EXPERIMENTAL DESIGN:

7.1 Animal Receipt and Acclimation:

Each animal will be inspected by a qualified technician upon receipt. Guinea pigs judged to be in good health and suitable as test animals will be acclimated to laboratory conditions for a minimum of five days. All animals will be weighed initially and identified with a cage card displaying the animal number and sex. During the acclimation period, each guinea pig will be observed twice daily for changes in general appearance and behavior.

All relevant records and data collected during the acclimation period for animals used on this study will be maintained on file.

7.2 Veterinary Care:

Animals will be monitored by the technical staff for any condition requiring possible veterinary care. If any such condition is identified, a staff veterinarian will be notified for an examination and evaluation. Animals will be treated as outlined in the Animal Welfare Act Compliance section of the protocol.

7.3 Route and Rationale of Test substance Administration:

The route of administration will be topical application. This study is intended to provide information on the health hazards likely to arise from exposure to the test substance by the dermal route. The modified Buehler method is an accepted procedure for evaluating the potential of test substances to induce immunologically mediated dermal sensitization.

7.4 Study Phases:

The study will consist of three phases:

1. Primary Irritation Phase – Range-finding experimentation to determine the appropriate level of test substance to be used in the Induction and Challenge Phases.

2. Induction Phase – Multiple topical applications of the test substance to attempt to stimulate the immune system.

3. Challenge Phase – Topical application of the test substance to investigate the elicitation of a sensitization response. If necessary to clarify the results of the challenge, a rechallenge may be performed.

7.5 Treatment Groups:

Eight animals will be selected arbitrarily from available stock for the Primary Irritation Phase. Additional Primary Irritation Phase experimentation may be conducted if acceptable levels for induction or challenge dosing are not obtained. This will be done upon sponsor authorization.

Following the acclimation period, animals for the main study (Induction and Challenge Phases) will be selected from available stock based upon health and body weight. All animals will be naïve. Animals will be randomly assigned to the following treatment groups using the WIL Toxicology Data Management System (WTDMS™).

7.5.1 Test Group (10 animals/sex):

Animals in the Test Group will be treated topically in the Induction and Challenge Phases with the test substance.

7.5.2 Naïve Control-I Group (5 animals/sex):

Animals in the Naïve Control-I Group will remain untreated during the Induction Phase. These animals will be treated topically in the Challenge Phase with the test substance.

7.5.3 Naïve Control-II Group (5 animals/sex):

Animals in the Naïve Control-II Group will remain untreated during the Induction and Challenge Phases. Should a rechallenge be necessary, animals will be treated topically with the test substance.

7.5.4 Positive Control Group (5 animals/sex)

Animals in the Positive Control Group will be treated topically in the Induction and Challenge Phases with the positive control material. In addition to reporting Positive Control Group data with this study, data collected from these animals will be included in WIL Research historical control data.

7.6 Treatment Levels:

Induction and challenge dose levels for the Test and Naïve Control Groups will be determined from the results of the Primary Irritation Phase. Generally, the dose level selected for the Induction Phase will be the highest concentration, which produced mild to moderate irritation. The dose level for the Challenge Phase will be the highest essentially nonirritating concentration, which produced no reaction to slight, patchy erythema. All selected dose concentrations will be documented in the study records.

Positive control animals will be induced with 100% and challenged with 10 and 20% concentrations of the positive control article hexylcinnamaldehyde (HCA).

7.7 Test Substance Preparation:

The methods used for preparation of all test and control material dosing mixtures will be documented in the study records and described in the final report.

The test and control substances will be dosed undiluted as received or diluted in an appropriate vehicle. The methods used for preparation of all test and control material dosing mixtures will be documented in the study records and described in the final report. The test substance will be diluted in a vehicle of mineral oil. A vehicle of 70/30 (v/v) acetone/PEG 400 will be utilized for the preparation of the positive control article mixtures. All dilutions will be prepared on a w/v basis.

The methods that will be used are expected to result in the intended test substance concentration in the dosing mixtures. Dosing mixtures will be mixed during preparation and throughout dose administration to ensure homogeneity. Any visible evidence of instability (e.g., evolution of heat, formation of gas, color change, etc.) will be noted in the study records. The dosing mixtures will be prepared on the day of dosing. Actual preparation procedures will be documented in the study records and presented in the final report. Analysis of dosing preparations will not be performed.

8 METHOD OF ADMINISTRATION:

8.1 Primary Irritation Phase:

Animals used for the primary irritation phase will be observed for mortality/moribundity twice daily (morning and afternoon). Moribund animals will be removed from study and euthanized by carbon dioxide inhalation. A detailed physical examination and body weight will be collected prior to dosing and at termination. Upon termination, these animals will be euthanized by carbon dioxide inhalation and discarded.

Eight guinea pigs will be utilized to evaluate the irritation potential of the test substance at levels of 100%, 75%, 50%, 25%, 10%, 5%, 2.5% and 1% when prepared in a vehicle of mineral oil. Each guinea pig will receive different concentrations of test substance at four sites; anterior right flank, posterior right flank, anterior left flank and posterior left flank. The concentrations will be rotated among the various test sites to minimize possible site-to-site variation in responsiveness.

If all levels tested for use during any study phase prove to be unacceptable, additional levels may need to be tested. The irritation evaluations may be conducted before or during the study phases, as appropriate.

The day prior to test substance exposure, the hair will be removed from each of the animal's backs and flanks using a small animal clipper. A 0.3 mL quantity of each test preparation will be applied onto the cotton pad of a 25-mm Hill Top Chamber®. The chambers will then be placed on the appropriate test site, occluded with plastic wrap (or other suitable occlusive dressing) and securely held in place with sufficient wrappings of a nonirritating elastic tape.

After approximately six hours, the chambers, plastic wrap and tape will be removed from the animals. Any chambers that may not have been totally occluded will be noted. Residual test substance will be cleansed from the application sites using clean, disposable paper towels moistened with mineral oil. An alternate solvent (such as the vehicle) may be used to remove residual test substance upon approval by the Study Director.

On the following day, the animals will be depilated as described in protocol section 9.2. The sites will be evaluated for skin reactions at 24 and 48 hours (±2 hours) after completion of exposure as described in protocol section 9.2.

8.2 Induction Phase:

The test substance will be applied to the same site on the anterior left flank, one time per week for three consecutive weeks (total of three exposures) for each

animal in the Test Group. Positive Control Group animals will be treated concurrently in an identical manner with the positive control article. At the discretion of the Study Director, the test sites may be moved slightly to avoid irritated or damaged areas following the first or second induction dosing. Animals in the Naïve Control Groups will remain untreated during this phase.

The test substance will be administered to the Test Group at the level chosen during the primary irritation phase. The undiluted positive control article, hexylcinnamaldehyde (HCA), will be administered to the Positive Control Group.

The day prior to each exposure, the hair will be removed from the test site, anterior left flank, using a small animal clipper. The test substance or positive control article will be administered by applying a 0.3 mL quantity of the appropriate material onto the cotton pad of a 25-mm Hill Top Chamber®. The chambers will then be placed on the appropriate test site, occluded with plastic wrap (or other suitable occlusive dressing) and securely held in place with sufficient wrappings of a nonirritating elastic tape.

After approximately six hours, the chambers, plastic wrap and tape will be removed from the animals. Any chambers that may not have been totally occluded will be noted. Residual test substance will be cleansed from the application sites using clean, disposable paper towels moistened with mineral oil. An alternate solvent (such as the vehicle) may be used to remove residual test substance upon approval by the Study Director. The positive control article will be removed using disposable towels moistened with tepid tap water.

Irritation will be scored 24 and 48 hours (±2 hours) after bandage removal as described in protocol section 9.2. A depilatory will not be used prior to these observations. The sole purpose of these scores is to document irritation produced during the induction phase.

8.3 Challenge Phase:

Approximately two weeks after the last induction exposure, the previously exposed animals in the Test Group and untreated animals in the Naïve Control-I Group will be dosed with test substance. The test substance will be applied to a previously unexposed site on the posterior left flank of each animal in the Test and Naïve Control-I Groups. Positive Control Group animals will be treated concurrently in a similar manner with the positive control article at individual sites on the posterior left flank and posterior right flank. Animals in the Naïve Control-II Group will remain untreated.

The day prior to exposure, the hair will be removed from the test site, posterior left flank, using a small animal clipper. The test substance or positive control article will be administered by applying a 0.3 mL quantity of the appropriate material

onto the cotton pad of a 25-mm Hill Top Chamber®. The chambers will then be placed on the appropriate test site, occluded with plastic wrap (or other suitable occlusive dressing) and securely held in place with sufficient wrappings of a nonirritating elastic tape.

The test substance will be administered to the Test Group and Naïve Control–I Group at the level chosen during the primary irritation phase. The positive control article (HCA) will be administered to the Positive Control Group as 10% and 20% w/v formulation in 70/30 (v/v) acetone/PEG 400.

After approximately six hours, the chambers, plastic wrap and tape will be removed from the animals. Any chambers that may not have been totally occluded will be noted. Residual test substance will be cleansed from the application sites using clean, disposable paper towels moistened with mineral oil. An alternate solvent (such as the vehicle) may be used to remove residual test substance upon approval by the Study Director. The positive control article will be removed using disposable towels moistened with tepid tap water.

The following day the animals will be depilated as described in section 9.2. The sites will be evaluated for skin reactions at 24 and 48 hours (±2 hours) after completion of exposure as described in protocol section 9.2.

If requested by the Sponsor, animals in the Test Group may be rechallenged at previously unexposed sites on the right flank to clarify initial challenge results. Previously untreated Naïve Control-II Group animals will be treated in an identical manner. Rechallenge will be performed approximately one to two weeks after the challenge exposure.

9 OBSERVATIONS:

9.1 Viability:

All animals will be observed for mortality/moribundity twice daily (morning and afternoon) for the duration of study. Moribund animals will be removed from study and euthanized by carbon dioxide inhalation.

9.2 Dermal Observations:

9.2.1 Induction Phase:

Reactions will be scored at 24 and 48 hours (±2 hours) after bandage removal using the scoring scale presented below. The sole purpose of these observations is to document irritation produced during the Induction Phase.

9.2.2 Primary Irritation and Challenge Phases:

Sites will be evaluated for skin reactions at 24 and 48 hours (±2 hours) after patch removal using the scoring scale presented below. At least three hours prior to the 24-hour scoring, a commercial depilatory will be applied to the clipped area of each animal. After approximately 5-8 minutes, the depilatory will be removed using a stream of warm running water. The animals will then be gently dried using disposable towels and returned to their cages.

9.2.3 Grading System for Skin Reactions:

The test sites will be evaluated for skin reactions during all phases of the study in accordance with the following grading system:

0 No reaction
± Very slight dispersed erythema
1 Discrete (slight confluent) or moderate patchy erythema
2 Moderate and confluent erythema
3 Severe erythema and/or moderate to severe edema

9.3 Body Weights and Detailed Physical Examinations:

Body weights will be recorded and a detailed physical examination will be performed at randomization (typically no more than two days prior to initiation of dosing) and again at termination. Animals that die on study or are euthanized *in extremis* will be weighed as soon as the animal is found in that condition.

9.4 Gross Pathology:

Animals will be euthanized by carbon dioxide inhalation. A gross necropsy examination on major organ systems of the thoracic and visceral cavities will be conducted on all animals found dead or euthanized *in extremis.* Animals euthanized following study termination will be discarded without further examination. Naïve Control–II Group animals will be returned to the acute stock colony if no rechallenge is performed.

10 EVALUATION OF RESULTS:

The sensitization potential of the test substance will be based on the dermal responses observed on the test and naïve control animals at challenge and rechallenge (if conducted). Generally, dermal scores of ≥1 in the test animals with scores of 0 to ± noted in the controls are considered indicative of sensitization. A dermal score of 1 in both the test and naïve control animals is generally considered equivocal unless a higher dermal response (≥ Grade 2) is noted in the test animals. Group mean dermal scores will be calculated for challenge and rechallenge (if conducted). A response of at least 15% in a nonadjuvant test should be expected for a mild to moderate sensitizer.

The sensitization potential of the test article will be based on the dermal responses observed on the test and naïve control animals at challenge and rechallenge (if conducted).

Criteria for determining if a skin reaction in a guinea pig is indicative of skin sensitization under conditions where there is no confounding irritation in the concurrent irritation control animals are as follows:

- The skin reaction score is greater than a ± in the induced animals.
- The skin reaction scores in the concurrent irritation control animals are not greater than a ± (if skin reaction scores in the concurrent irritation control animals are greater than a ±, then a rechallenge at a lower concentration is required and different criteria may apply).
- Special consideration will be given to the skin reaction score for individual animals that persist at both 24-hour and 48-hour observations in the induced animals.
- Skin reactions indicative of skin sensitization in individual animals are present at both challenge and rechallenge.

The concentration used at challenge for the Positive Control Group is a known non-irritating concentration; therefore, grades of 1 or greater in the Positive Control Group indicate sensitization responses.

Criteria for determining if a test material is classifiable as skin sensitizer:

- 15% of the induced animals have skin reactions indicative of skin sensitization at both challenge and rechallenge, and there is no confounding irritation present in the concurrent irritation control animals

10.1 Incidence Index:

The percentage of animals in the Test or Positive Control Group showing sensitization responses at either 24 or 48 hours. (By definition and design, Naïve Control animals cannot be sensitized; therefore Incidence Indices are not calculated for these groups.) The higher the Incidence Index, the stronger the sensitizing potential of the test substance.

10.2 Severity Index:

The sum of the reaction grades divided by the total number of animals tested in a given group determined separately for both 24 and 48 hours (grades of ± are equal to 0.5 for calculation of severity indices).

11 REPORT:

The final report will include, but will not necessarily be limited to, the following: compliance statement, summary, objective, test substance identification and receipt information, methods, clinical observations, mortality, body weights, gross necropsy findings, individual and summary irritation scores, indices, results and discussion, key personnel, a signed QA statement and protocol deviations, if any.

The report will include historical positive control data conducted within six months of this study by WIL Research.

WIL Research will submit one electronic copy (PDF with an MS Word copy of the report text for editing and comments) of an audited draft report in a timely manner upon completion of data collection prior to issuance of the final report. It is expected that the Sponsor will review the draft report and provide comments to WIL Research within a two-month time frame following submission. Within one month following receipt of the Sponsor's comments, WIL Research shall provide a revised draft report that incorporates the Sponsor's reasonable revisions and suggestions. One revision will be permitted as part of the cost of the study; additional changes or revisions may be made, at extra cost. WIL Research will submit the final report within two weeks of receiving authorization from the Sponsor. If the Sponsor's comments and/or authorization to finalize the report have not been received at WIL Research within one year following submission of the draft report, WIL Research may elect to finalize the report following appropriate written notification to the Sponsor. An electronic copy (hyperlinked and bookmarked PDF) of the final report will be provided. Requests for paper copies of the final report may result in additional charges.

12 RECORDS TO BE MAINTAINED:

All original raw data records (as defined by the applicable GLPs and WIL SOPs) generated by WIL Research will be collected and maintained in the WIL Research Archives as described in the following section.

13 WORK PRODUCT:

Sponsor will have title to all documentation records, raw data, slides, specimens, or other work product generated during the performance of the study. All work product including raw paper data, pertinent electronic storage media and specimens will be retained at no charge for a period of six months following issuance of the final report in the WIL Research Archives. Thereafter, WIL Research will charge a monthly archiving fee for retention of all work product. All work product will be stored in compliance with regulatory requirements.

Any work product, including documents, specimens, and samples, that are required by this protocol, its amendments, or other written instructions of the Sponsor, to be shipped by WIL Research to another location will be appropriately packaged and labeled as defined by WIL's SOPs and delivered to a common carrier for shipment. WIL Research will not be responsible for shipment following delivery to the common carrier.

14 QUALITY ASSURANCE:

The study will be audited by the WIL Research Quality Assurance Department while in progress to assure compliance with applicable Good Laboratory Practices and adherence to the protocol and to WIL Research SOPs. The raw data and draft report will be audited by the WIL Research Quality Assurance Department to assure that the final report accurately describes the conduct and the findings of the study.

15 PROTOCOL MODIFICATION:

Modification of the protocol may be accomplished during the course of this investigation. However, no changes will be made in the study design without the verbal or written permission of the Sponsor. In the event that the Sponsor verbally requests or approves changes in the protocol, such changes will be made by appropriate documentation in the form of protocol amendments. All alterations of the protocol and reasons for the modification(s) will be signed by the Study Director and the Sponsor Representative.

16 ANIMAL WELFARE ACT COMPLIANCE:

This study will comply with all applicable sections of the Final Rules of the Animal Welfare Act regulations (9 CFR). The Sponsor should make particular note of the following:

- The Sponsor signature on this protocol documents for the Study Director the Sponsor's assurance that, for the study described in this protocol, there are no acceptable non-animal alternatives and the study does not unnecessarily duplicate previous experiments.

- Whenever possible, procedures used in this study have been designed to avoid or minimize discomfort, distress or pain to animals. All methods are described in this study protocol or in written laboratory SOPs.

- Animals that experience severe or chronic pain or distress that cannot be relieved will be painlessly euthanized as deemed appropriate by the veterinary staff and Study Director. The Sponsor will be advised by the Study Director of all circumstances which could lead to this action in as timely a manner as possible.

- Methods of euthanasia used during this study are in conformance with the above-referenced regulation.

- The Sponsor/Study Director has considered alternatives to procedures that may cause more than momentary or slight pain or distress to the animals and has provided a written narrative description (AWA covered species) of the methods and sources used to determine that alternatives are not available.

17 REFERENCES:

Buehler, E.V. Delayed Contact Hypersensitivity In The Guinea Pig. *Archives of Dermatology* **1965,** *91*, 171-7.

18 PROTOCOL APPROVAL:

Sponsor approval received by the Study Director via Email on 24 June 2013.

[ ]

for L.W. Bernadette D. Munoz
Linda Waid, RVT, LAT
Sponsor Representative

25 JUNE 2013
Date

WIL Research

Teresa D. Morris
Teresa D. Morris, BS
Study Director

24 June 2013
Date

APPENDIX B

Certificate of Analysis (Sponsor-Provided Data)

DETERMINATION OF INFRARED SPECTRUM AND DENSITY

CERTIFICATE OF ANALYSIS

Test Item	:	[ ]
Harlan Study Number	:	41203840
Study Director	:	B J O'Connor
Number of Pages	:	5

Sponsor:

[ ]

Test Facility:
Harlan Laboratories Ltd.
Shardlow Business Park
Shardlow
Derbyshire
DE72 2GD
UK
Telephone: +44 (0) 1332 792896
Facsimile: +44 (0) 1332 799018

Introduction and Summary

The infrared spectrum and density of the test item has been determined.

Experimental testing was conducted between 13 July 2012 and 23 July 2012.

The infrared spectrum of the test item was consistent with the proposed chemical composition.

The density of the test item was determined to be 942 kg/m^3 at 20.0 ± 0.5 °C.

Test Item Details

Name	[ ]
Description	
Lot Number	
Purity	
Date Received	
Storage Conditions	
Expiry Date	

Compositional information was supplied by the Sponsor for interpretation of the infrared spectrum. The integrity of supplied data relating to the identity, purity and stability of the test item is the responsibility of the Sponsor.

Testing Details

Infrared Spectrum

The infrared spectrum was acquired using a Perkin-Elmer Spectrum One Fourier-transform infrared spectrophotometer. The test item was scanned directly on the diamond/zinc selenide crystal over the wavenumber range 4000 to 400 cm^{-1}.

[ ]

Conclusion

It was not possible to positively confirm the test item composition from the infrared spectrum alone. However, no evidence was found in the infrared spectrum that contradicted the proposed chemical composition of the test item. The infrared spectrum of the test item was consistent with the proposed chemical composition.

Density

The determination was carried out using a procedure based on the pycnometer method, Method A3 Relative Density of Commission Regulation (EC) No 440/2008 of 30 May 2008 and Method 830.7300 of the OPPTS Guidelines. A glass pycnometer was weighed to a constant mass. Distilled water and test item were equilibrated at 20.0 ± 0.5°C. A calibration was carried out by determining the mass of distilled water required to fill the pycnometer. The pycnometer was then emptied and dried to constant mass. The mass of test item required to fill the pycnometer was then determined.

Results of duplicate determinations of density are shown in the following table:

Measurement	Determination	
	1	2
Mass of pycnometer (g)	17.0620	18.2211
Mass of pycnometer and distilled water at 20.0 °C (g)	27.2313	28.1330
Mass of pycnometer and test item at 20.0 °C (g)	26.6579	27.5694
Density of water at 20.0 °C (kg/m^3)	998.20	998.20
Density (kg/m^3)	941.92	941.45
Relative density	0.942	0.941

Temperature: 20.0 ± 0.5 °C
Mean density: 942 kg/m^3

Conclusion

The density of the test item was determined to be 942 kg/m^3 at 20.0 ± 0.5 °C.

Archive Statement

Unless instructed otherwise by the Sponsor, all original data and the Certificate of Analysis will be retained in the Harlan Laboratories Ltd, Shardlow, UK archives for five years after which instructions will be sought as to further retention or disposal. Further retention or return of the data will be chargeable to the Sponsor.

GLP Compliance Statement

Harlan Laboratories Ltd., Shardlow Business Park, Shardlow, Derbyshire, DE72 2GD, UK

Harlan Study Number:	41203840
Study Title:	[ ]
	Determination of Infrared Spectrum and Density

The study described was performed in compliance with UK GLP standards (Schedule 1, Good Laboratory Practice Regulations 1999 (SI 1999/3106 as amended by SI 2004/0994)). These Regulations are in accordance with GLP standards published as OECD Principles on Good Laboratory Practice (revised 1997, ENV/MC/CHEM(98)17).

This Certificate of Analysis fully and accurately reflects the procedures used and data generated. There were no circumstances considered to have affected the integrity of the study or the validity of the data.

Study Director: B J O'Connor

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Date: 11 OCT 2012

Quality Assurance Report

Harlan Laboratories Ltd., Shardlow Business Park, Shardlow, Derbyshire, DE72 2GD, UK

Harlan Study Number: 41203840
Study Title: [ ]
Determination of Infrared Spectrum and Density

The general facilities are inspected at least once a year and the results are reported to management.

Study-related procedures were audited and inspected. The details of these audits and inspections are given below.

Dates and Types of QA Inspections			**Reported to the Study Director and Test Facility Management**
Date of Inspection	Type of Inspection	Phase Inspected	Report Date
11 July 2012	Verification	Study Plan	11 July 2012
13 July 2012	Process – based	Infrared Spectrum	13 July 2012
05 July 2012	Process – based	Density	05 July 2012
01 October 2012	Confirmation	Draft Report	01 October 2012

This statement confirms that this final Certificate of Analysis reflects the raw data and the procedures followed.

Quality Assurance:

J. M. CROWTHER

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Date: 11 OCT 2012

APPENDIX C

Animal Room Environmental Conditions

SKIN SENSITIZATION STUDY OF [ ] IN GUINEA PIGS BUEHLER METHOD

PROJECT NO.:WIL- 187159 TEMPERATURE/HUMIDITY - STUDY SUMMARY REPORT

SPONSOR: 187 - [ ]

STUDY SPECIFICATIONS: 187159 DATE IN 06/26/13 TIME IN 08:00

DATE OUT 08/21/13 TIME OUT 16:00

ROOM SPECIFICATIONS: B ROOM 41 LOW TEMPERATURE °F: 61.0 HIGH TEMPERATURE °F: 71.0 LOW HUMIDITY %RH: 30.0

TEST SYSTEM: Guinea Pig LOW TEMPERATURE °C: 16.1 HIGH TEMPERATURE °C: 21.7 HIGH HUMIDITY %RH: 70.0

	PRIMARY TEMP		SECONDARY TEMP		PRIMARY HUM	SECONDARY HUM
DATE	MEAN (°F)	MEAN (°C)	MEAN (°F)	MEAN (°C)	MEAN (%RH)	MEAN (%RH)
06/26/13	68.0	20.0	66.4	19.1	52.6	56.7
06/27/13	67.6	19.8	66.3	19.1	52.6	56.5
06/28/13	67.1	19.5	66.1	18.9	53.7	57.2
06/29/13	65.8	18.8	64.8	18.2	47.0	49.4
06/30/13	65.8	18.8	64.7	18.2	47.8	50.3
07/01/13	65.9	18.8	64.8	18.2	49.9	52.9
07/02/13	66.2	19.0	65.1	18.4	50.7	53.8
07/03/13	66.7	19.3	65.6	18.7	52.9	56.3
07/04/13	66.2	19.0	65.1	18.4	53.7	57.9
07/05/13	66.1	18.9	65.0	18.3	54.3	58.7
07/06/13	66.4	19.1	65.4	18.6	53.6	57.7
07/07/13	66.0	18.9	64.9	18.3	52.4	56.3
07/08/13	66.9	19.4	65.6	18.7	53.1	56.5
07/09/13	67.5	19.7	66.0	18.9	54.0	57.9
07/10/13	67.7	19.8	66.3	19.1	55.9	58.3
07/11/13	66.0	18.9	64.5	18.1	48.2	50.1
07/12/13	67.0	19.4	65.7	18.7	52.2	54.6
07/13/13	68.1	20.1	66.9	19.4	56.5	59.6
07/14/13	68.7	20.4	67.7	19.8	60.0	62.7

	PRIMARY TEMP		SECONDARY TEMP		PRIMARY HUM	SECONDARY HUM
DATE	MEAN (°F)	MEAN (°C)	MEAN (°F)	MEAN (°C)	MEAN (%RH)	MEAN (%RH)
07/15/13	69.7	20.9	68.6	20.3	62.6	65.0
07/16/13	69.4	20.8	68.4	20.2	63.0	65.4
07/17/13	69.8	21.0	68.7	20.4	63.7	65.7
07/18/13	69.9	21.1	68.8	20.4	64.0	66.1
07/19/13	69.7	20.9	68.6	20.3	61.9	64.9
07/20/13	67.5	19.7	66.4	19.1	57.9	61.3
07/21/13	67.0	19.4	65.8	18.8	58.2	61.7
07/22/13	66.4	19.1	65.3	18.5	60.2	63.3
07/23/13	67.7	19.8	66.5	19.2	60.6	63.5
07/24/13	66.1	18.9	64.8	18.2	55.5	58.1
07/25/13	66.1	18.9	64.9	18.3	52.8	54.7
07/26/13	66.7	19.3	65.6	18.7	56.1	58.4
07/27/13	66.7	19.3	65.7	18.7	60.0	62.8
07/28/13	65.8	18.8	64.7	18.2	55.8	58.2
07/29/13	66.2	19.0	65.7	18.7	57.3	58.3
07/30/13	66.9	19.4	67.4	19.7	56.6	55.5
07/31/13	67.6	19.8	68.3	20.2	62.2	60.8
08/01/13	68.1	20.1	68.7	20.4	63.4	61.7
08/02/13	66.7	19.3	67.3	19.6	58.8	58.1
08/03/13	67.9	19.9	68.5	20.3	61.1	60.3
08/04/13	66.5	19.2	66.9	19.4	58.5	57.8
08/05/13	66.7	19.3	67.2	19.6	59.3	57.8
08/06/13	67.6	19.8	68.1	20.1	62.0	60.6

	PRIMARY TEMP		SECONDARY TEMP		PRIMARY HUM	SECONDARY HUM
DATE	MEAN (°F)	MEAN (°C)	MEAN (°F)	MEAN (°C)	MEAN (%RH)	MEAN (%RH)
08/07/13	68.5	20.3	69.1	20.6	68.4	65.7
08/08/13	67.7	19.8	68.2	20.1	62.7	59.8
08/09/13	66.6	19.2	66.8	19.3	56.2	53.9
08/10/13	66.7	19.3	67.0	19.4	59.8	58.3
08/11/13	66.8	19.3	67.1	19.5	60.1	58.5
08/12/13	66.8	19.3	67.1	19.5	60.6	59.0
08/13/13	66.8	19.3	67.1	19.5	59.0	57.3
08/14/13	66.3	19.1	66.7	19.3	58.1	56.3
08/15/13	66.5	19.2	66.9	19.4	55.6	53.6
08/16/13	66.4	19.1	66.8	19.3	58.5	57.2
08/17/13	66.5	19.2	66.8	19.3	58.6	57.3
08/18/13	66.5	19.2	66.9	19.4	59.2	57.8
08/19/13	66.8	19.3	67.0	19.4	60.0	58.3
08/20/13	66.8	19.3	67.1	19.5	59.2	57.5
08/21/13	66.6	19.2	66.9	19.4	60.1	58.4

	PRIMARY TEMP		SECONDARY TEMP		PRIMARY HUM	SECONDARY HUM
DATE	MEAN (°F)	MEAN (°C)	MEAN (°F)	MEAN (°C)	MEAN (%RH)	MEAN (%RH)

SUMMARY OF DAILY MEANS	MEAN	MIN	MAX
PRIMARY TEMP °F:	67.1	65.8	69.9
PRIMARY TEMP °C:	19.5	18.8	21.1
SECONDARY TEMP °F:	66.6	64.5	69.1
SECONDARY TEMP °C:	19.2	18.1	20.6
PRIMARY HUM %RH:	57.3	47.0	68.4
SECONDARY HUM %RH:	58.4	49.4	66.1
N DAYS	57		

B ROOM 41 SUMMARY OF HOURLY VALUES

	PRIMARY TEMP		SECONDARY TEMP		PRIMARY HUM	SECONDARY HUM
MEAN	67.1 °F	19.5 °C	66.6 °F	19.2 °C	57.3 %RH	58.4 %RH
MIN	64.8 °F	18.2 °C	63.6 °F	17.6 °C	44.1 %RH	43.5 %RH
MAX	77.2 °F	25.1 °C	76.5 °F	24.7 °C	92.4 %RH	83.7 %RH
SD	1.51	0.84	1.63	0.91	5.53	5.11
SE	0.04	0.02	0.04	0.02	0.15	0.14
N SAMPLES	1346		1346		1345	1346
FIRST DAY	06/26/13					
LAST DAY	08/21/13					
N DAYS	57					

STUDY 187159 SUMMARY OF HOURLY VALUES

	PRIMARY TEMP		SECONDARY TEMP		PRIMARY HUM	SECONDARY HUM
MEAN	67.1 °F	19.5 °C	66.6 °F	19.2 °C	57.3 %RH	58.4 %RH
MIN	64.8 °F	18.2 °C	63.6 °F	17.6 °C	44.1 %RH	43.5 %RH
MAX	77.2 °F	25.1 °C	76.5 °F	24.7 °C	92.4 %RH	83.7 %RH
SD	1.51	0.84	1.63	0.91	5.53	5.11
SE	0.04	0.02	0.04	0.02	0.15	0.14
N SAMPLES	1346		1346		1345	1346
FIRST DAY	06/26/13					
LAST DAY	08/21/13					
N DAYS	57					

APPENDIX D

Individual Clinical Observations

APPENDIX D (MAIN STUDY)

PROJECT NO.:WIL-187159 SKIN SENSITIZATION STUDY OF [ ] IN GUINEA PIGS BUEHLER METHOD

SPONSOR:[ ]

SPONSOR NO.:[ ]

INDIVIDUAL CLINICAL OBSERVATIONS

TABLE RANGE: 07-16-13 TO 08-21-13

ANIMAL	SEX	GROUP	CATEGORY	DATE	TIME	GRADE	OBSERVATIONS
22790	M	TEST GROUP	NORMAL	07-16-13	9:44	P	NO SIGNIFICANT CLINICAL OBSERVATIONS
				08-20-13	15:26	P	NO SIGNIFICANT CLINICAL OBSERVATIONS
22790	M	TEST GROUP	DISPOSITION	08-21-13	8:01	P	EUTHANIZED BY CO2 AND DISCARDED
22796	M	TEST GROUP	NORMAL	07-16-13	9:45	P	NO SIGNIFICANT CLINICAL OBSERVATIONS
				08-20-13	15:26	P	NO SIGNIFICANT CLINICAL OBSERVATIONS
22796	M	TEST GROUP	DISPOSITION	08-21-13	8:02	P	EUTHANIZED BY CO2 AND DISCARDED
22802	M	TEST GROUP	NORMAL	07-16-13	9:46	P	NO SIGNIFICANT CLINICAL OBSERVATIONS
				08-20-13	15:26	P	NO SIGNIFICANT CLINICAL OBSERVATIONS
22802	M	TEST GROUP	DISPOSITION	08-21-13	8:02	P	EUTHANIZED BY CO2 AND DISCARDED
22804	M	TEST GROUP	NORMAL	07-16-13	9:47	P	NO SIGNIFICANT CLINICAL OBSERVATIONS
22804	M	TEST GROUP	DISPOSITION	08-21-13	8:02	P	EUTHANIZED BY CO2 AND DISCARDED
22804	M	TEST GROUP	BODY/INTEGUMENT	08-20-13	15:27	P	DRIED YELLOW MATERIAL ANOGENITAL AREA
22809	M	TEST GROUP	NORMAL	07-16-13	9:48	P	NO SIGNIFICANT CLINICAL OBSERVATIONS
				08-20-13	15:27	P	NO SIGNIFICANT CLINICAL OBSERVATIONS
22809	M	TEST GROUP	DISPOSITION	08-21-13	8:02	P	EUTHANIZED BY CO2 AND DISCARDED
22812	M	TEST GROUP	NORMAL	07-16-13	9:49	P	NO SIGNIFICANT CLINICAL OBSERVATIONS
				08-20-13	15:28	P	NO SIGNIFICANT CLINICAL OBSERVATIONS
22812	M	TEST GROUP	DISPOSITION	08-21-13	8:02	P	EUTHANIZED BY CO2 AND DISCARDED
22827	M	TEST GROUP	NORMAL	07-16-13	9:51	P	NO SIGNIFICANT CLINICAL OBSERVATIONS
				08-20-13	15:28	P	NO SIGNIFICANT CLINICAL OBSERVATIONS
22827	M	TEST GROUP	DISPOSITION	08-21-13	8:02	P	EUTHANIZED BY CO2 AND DISCARDED
22829	M	TEST GROUP	NORMAL	07-16-13	9:51	P	NO SIGNIFICANT CLINICAL OBSERVATIONS
				08-20-13	15:28	P	NO SIGNIFICANT CLINICAL OBSERVATIONS
22829	M	TEST GROUP	DISPOSITION	08-21-13	8:02	P	EUTHANIZED BY CO2 AND DISCARDED
22841	M	TEST GROUP	NORMAL	07-16-13	9:54	P	NO SIGNIFICANT CLINICAL OBSERVATIONS
				08-20-13	15:29	P	NO SIGNIFICANT CLINICAL OBSERVATIONS
22841	M	TEST GROUP	DISPOSITION	08-21-13	8:02	P	EUTHANIZED BY CO2 AND DISCARDED
22843	M	TEST GROUP	NORMAL	07-16-13	9:54	P	NO SIGNIFICANT CLINICAL OBSERVATIONS
				08-20-13	15:29	P	NO SIGNIFICANT CLINICAL OBSERVATIONS

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INDIVIDUAL CLINICAL OBSERVATIONS

TABLE RANGE: 07-16-13 TO 08-21-13

ANIMAL	SEX	GROUP	CATEGORY	DATE	TIME		GRADE OBSERVATIONS
22843	M	TEST GROUP	DISPOSITION	08-21-13	8:02	P	EUTHANIZED BY CO2 AND DISCARDED
22819	M	NAIVE CONTROL-I	NORMAL	07-16-13	9:49	P	NO SIGNIFICANT CLINICAL OBSERVATIONS
				08-16-13	14:31	P	NO SIGNIFICANT CLINICAL OBSERVATIONS
22819	M	NAIVE CONTROL-I	DISPOSITION	08-21-13	8:02	P	EUTHANIZED BY CO2 AND DISCARDED
22831	M	NAIVE CONTROL-I	NORMAL	07-16-13	9:52	P	NO SIGNIFICANT CLINICAL OBSERVATIONS
				08-16-13	14:32	P	NO SIGNIFICANT CLINICAL OBSERVATIONS
22831	M	NAIVE CONTROL-I	DISPOSITION	08-21-13	8:02	P	EUTHANIZED BY CO2 AND DISCARDED
22832	M	NAIVE CONTROL-I	NORMAL	07-16-13	9:52	P	NO SIGNIFICANT CLINICAL OBSERVATIONS
				08-16-13	14:32	P	NO SIGNIFICANT CLINICAL OBSERVATIONS
22832	M	NAIVE CONTROL-I	DISPOSITION	08-21-13	8:02	P	EUTHANIZED BY CO2 AND DISCARDED
22839	M	NAIVE CONTROL-I	NORMAL	07-16-13	9:53	P	NO SIGNIFICANT CLINICAL OBSERVATIONS
				08-16-13	14:32	P	NO SIGNIFICANT CLINICAL OBSERVATIONS
22839	M	NAIVE CONTROL-I	DISPOSITION	08-21-13	8:02	P	EUTHANIZED BY CO2 AND DISCARDED
22846	M	NAIVE CONTROL-I	NORMAL	07-16-13	9:55	P	NO SIGNIFICANT CLINICAL OBSERVATIONS
				08-16-13	14:33	P	NO SIGNIFICANT CLINICAL OBSERVATIONS
22846	M	NAIVE CONTROL-I	DISPOSITION	08-21-13	8:02	P	EUTHANIZED BY CO2 AND DISCARDED
22789	M	POSITIVE CONTROL	NORMAL	07-16-13	9:44	P	NO SIGNIFICANT CLINICAL OBSERVATIONS
				08-16-13	14:37	P	NO SIGNIFICANT CLINICAL OBSERVATIONS
22789	M	POSITIVE CONTROL	DISPOSITION	08-21-13	8:04	P	EUTHANIZED BY CO2 AND DISCARDED
22795	M	POSITIVE CONTROL	NORMAL	07-16-13	9:45	P	NO SIGNIFICANT CLINICAL OBSERVATIONS
				08-16-13	14:37	P	NO SIGNIFICANT CLINICAL OBSERVATIONS
22795	M	POSITIVE CONTROL	DISPOSITION	08-21-13	8:04	P	EUTHANIZED BY CO2 AND DISCARDED
22821	M	POSITIVE CONTROL	NORMAL	07-16-13	9:50	P	NO SIGNIFICANT CLINICAL OBSERVATIONS
				08-16-13	14:37	P	NO SIGNIFICANT CLINICAL OBSERVATIONS
22821	M	POSITIVE CONTROL	DISPOSITION	08-21-13	8:04	P	EUTHANIZED BY CO2 AND DISCARDED
22836	M	POSITIVE CONTROL	NORMAL	07-16-13	9:53	P	NO SIGNIFICANT CLINICAL OBSERVATIONS
22836	M	POSITIVE CONTROL	DISPOSITION	08-21-13	8:04	P	EUTHANIZED BY CO2 AND DISCARDED
22836	M	POSITIVE CONTROL	BODY/INTEGUMENT	08-16-13	14:38	P	DRIED BROWN MATERIAL HINDLIMB(S)
22837	M	POSITIVE CONTROL	NORMAL	07-16-13	9:53	P	NO SIGNIFICANT CLINICAL OBSERVATIONS

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INDIVIDUAL CLINICAL OBSERVATIONS

TABLE RANGE: 07-16-13 TO 08-21-13

ANIMAL	SEX	GROUP	CATEGORY	DATE	TIME	GRADE	OBSERVATIONS
22837	M	POSITIVE CONTROL	NORMAL	08-16-13	14:38	P	NO SIGNIFICANT CLINICAL OBSERVATIONS
22837	M	POSITIVE CONTROL	DISPOSITION	08-21-13	8:04	P	EUTHANIZED BY CO2 AND DISCARDED
22856	F	TEST GROUP	NORMAL	07-16-13	9:56	P	NO SIGNIFICANT CLINICAL OBSERVATIONS
				08-20-13	15:30	P	NO SIGNIFICANT CLINICAL OBSERVATIONS
22856	F	TEST GROUP	DISPOSITION	08-21-13	8:02	P	EUTHANIZED BY CO2 AND DISCARDED
22861	F	TEST GROUP	NORMAL	07-16-13	9:56	P	NO SIGNIFICANT CLINICAL OBSERVATIONS
				08-20-13	15:30	P	NO SIGNIFICANT CLINICAL OBSERVATIONS
22861	F	TEST GROUP	DISPOSITION	08-21-13	8:02	P	EUTHANIZED BY CO2 AND DISCARDED
22868	F	TEST GROUP	NORMAL	07-16-13	9:57	P	NO SIGNIFICANT CLINICAL OBSERVATIONS
				08-20-13	15:30	P	NO SIGNIFICANT CLINICAL OBSERVATIONS
22868	F	TEST GROUP	DISPOSITION	08-21-13	8:02	P	EUTHANIZED BY CO2 AND DISCARDED
22871	F	TEST GROUP	NORMAL	07-16-13	10:03	P	NO SIGNIFICANT CLINICAL OBSERVATIONS
22871	F	TEST GROUP	DISPOSITION	08-21-13	8:02	P	EUTHANIZED BY CO2 AND DISCARDED
22871	F	TEST GROUP	EYES/EARS/NOSE	08-20-13	15:31	P	REDDENED LEFT EAR
				08-20-13	15:31	P	REDDENED RIGHT EAR
22881	F	TEST GROUP	NORMAL	07-16-13	12:29	P	NO SIGNIFICANT CLINICAL OBSERVATIONS
				08-20-13	15:31	P	NO SIGNIFICANT CLINICAL OBSERVATIONS
22881	F	TEST GROUP	DISPOSITION	08-21-13	8:02	P	EUTHANIZED BY CO2 AND DISCARDED
22890	F	TEST GROUP	NORMAL	07-16-13	12:30	P	NO SIGNIFICANT CLINICAL OBSERVATIONS
				08-20-13	15:32	P	NO SIGNIFICANT CLINICAL OBSERVATIONS
22890	F	TEST GROUP	DISPOSITION	08-21-13	8:02	P	EUTHANIZED BY CO2 AND DISCARDED
22898	F	TEST GROUP	NORMAL	07-16-13	12:32	P	NO SIGNIFICANT CLINICAL OBSERVATIONS
				08-20-13	15:32	P	NO SIGNIFICANT CLINICAL OBSERVATIONS
22898	F	TEST GROUP	DISPOSITION	08-21-13	8:02	P	EUTHANIZED BY CO2 AND DISCARDED
22904	F	TEST GROUP	NORMAL	07-16-13	12:34	P	NO SIGNIFICANT CLINICAL OBSERVATIONS
				08-20-13	15:33	P	NO SIGNIFICANT CLINICAL OBSERVATIONS
22904	F	TEST GROUP	DISPOSITION	08-21-13	8:02	P	EUTHANIZED BY CO2 AND DISCARDED
22910	F	TEST GROUP	NORMAL	07-16-13	12:34	P	NO SIGNIFICANT CLINICAL OBSERVATIONS
				08-20-13	15:33	P	NO SIGNIFICANT CLINICAL OBSERVATIONS

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INDIVIDUAL CLINICAL OBSERVATIONS

TABLE RANGE: 07-16-13 TO 08-21-13

ANIMAL	SEX	GROUP	CATEGORY	DATE	TIME		GRADE OBSERVATIONS
22910	F	TEST GROUP	DISPOSITION	08-21-13	8:02	P	EUTHANIZED BY CO2 AND DISCARDED
22916	F	TEST GROUP	NORMAL	07-16-13	12:35	P	NO SIGNIFICANT CLINICAL OBSERVATIONS
				08-20-13	15:33	P	NO SIGNIFICANT CLINICAL OBSERVATIONS
22916	F	TEST GROUP	DISPOSITION	08-21-13	8:02	P	EUTHANIZED BY CO2 AND DISCARDED
22872	F	NAIVE CONTROL-I	NORMAL	07-16-13	12:28	P	NO SIGNIFICANT CLINICAL OBSERVATIONS
				08-16-13	14:33	P	NO SIGNIFICANT CLINICAL OBSERVATIONS
22872	F	NAIVE CONTROL-I	DISPOSITION	08-21-13	8:03	P	EUTHANIZED BY CO2 AND DISCARDED
22902	F	NAIVE CONTROL-I	NORMAL	07-16-13	12:33	P	NO SIGNIFICANT CLINICAL OBSERVATIONS
				08-16-13	14:33	P	NO SIGNIFICANT CLINICAL OBSERVATIONS
22902	F	NAIVE CONTROL-I	DISPOSITION	08-21-13	8:03	P	EUTHANIZED BY CO2 AND DISCARDED
22908	F	NAIVE CONTROL-I	NORMAL	07-16-13	12:34	P	NO SIGNIFICANT CLINICAL OBSERVATIONS
				08-16-13	14:34	P	NO SIGNIFICANT CLINICAL OBSERVATIONS
22908	F	NAIVE CONTROL-I	DISPOSITION	08-21-13	8:03	P	EUTHANIZED BY CO2 AND DISCARDED
22913	F	NAIVE CONTROL-I	NORMAL	07-16-13	12:34	P	NO SIGNIFICANT CLINICAL OBSERVATIONS
				08-16-13	14:34	P	NO SIGNIFICANT CLINICAL OBSERVATIONS
22913	F	NAIVE CONTROL-I	DISPOSITION	08-21-13	8:03	P	EUTHANIZED BY CO2 AND DISCARDED
22914	F	NAIVE CONTROL-I	NORMAL	07-16-13	12:35	P	NO SIGNIFICANT CLINICAL OBSERVATIONS
				08-16-13	14:34	P	NO SIGNIFICANT CLINICAL OBSERVATIONS
22914	F	NAIVE CONTROL-I	DISPOSITION	08-21-13	8:03	P	EUTHANIZED BY CO2 AND DISCARDED
22866	F	POSITIVE CONTROL	NORMAL	08-16-13	14:39	P	NO SIGNIFICANT CLINICAL OBSERVATIONS
22866	F	POSITIVE CONTROL	DISPOSITION	08-21-13	8:04	P	EUTHANIZED BY CO2 AND DISCARDED
22866	F	POSITIVE CONTROL	BODY/INTEGUMENT	07-16-13	9:57	P	DRIED YELLOW MATERIAL ANOGENITAL AREA
22883	F	POSITIVE CONTROL	NORMAL	07-16-13	12:30	P	NO SIGNIFICANT CLINICAL OBSERVATIONS
				08-16-13	14:39	P	NO SIGNIFICANT CLINICAL OBSERVATIONS
22883	F	POSITIVE CONTROL	DISPOSITION	08-21-13	8:04	P	EUTHANIZED BY CO2 AND DISCARDED
22887	F	POSITIVE CONTROL	NORMAL	07-16-13	12:30	P	NO SIGNIFICANT CLINICAL OBSERVATIONS
				08-16-13	14:39	P	NO SIGNIFICANT CLINICAL OBSERVATIONS
22887	F	POSITIVE CONTROL	DISPOSITION	08-21-13	8:04	P	EUTHANIZED BY CO2 AND DISCARDED
22893	F	POSITIVE CONTROL	NORMAL	07-16-13	12:31	P	NO SIGNIFICANT CLINICAL OBSERVATIONS

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INDIVIDUAL CLINICAL OBSERVATIONS

TABLE RANGE: 07-16-13 TO 08-21-13

ANIMAL	SEX	GROUP	CATEGORY	DATE	TIME	GRADE	OBSERVATIONS
22893	F	POSITIVE CONTROL	NORMAL	08-16-13	14:39	P	NO SIGNIFICANT CLINICAL OBSERVATIONS
22893	F	POSITIVE CONTROL	DISPOSITION	08-21-13	8:04	P	EUTHANIZED BY CO2 AND DISCARDED
22903	F	POSITIVE CONTROL	NORMAL	07-16-13	12:33	P	NO SIGNIFICANT CLINICAL OBSERVATIONS
				08-16-13	14:40	P	NO SIGNIFICANT CLINICAL OBSERVATIONS
22903	F	POSITIVE CONTROL	DISPOSITION	08-21-13	8:05	P	EUTHANIZED BY CO2 AND DISCARDED

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