TEST FACILITY

NAMSA
6750 Wales Road
Northwood, OH 43619
419.666.9455

SPONSOR

Jim Baker
Ensinger Industries Inc.
365 Meadowlands Blvd.
Washington, PA 15301

STUDY TITLE

ISO Maximization Sensitization Study - Extract

TEST ARTICLE NAME

Black Tecanyl MT XRO

TEST ARTICLE IDENTIFICATION

Lot: 17940

TABLE OF CONTENTS Page

Summary

A guinea pig maximization test of Black Tecanyl MT XRO, Lot: 17940, was conducted to evaluate the potential for delayed dermal contact sensitization. This study was conducted based on the requirements of the International Organization for Standardization 10993: Biological Evaluation of Medical Devices, Part 10: Tests for Irritation and Delayed-Type Hypersensitivity.

The test article was extracted in 0.9% sodium chloride USP (SC) and sesame oil, NF (SO). Each extract was intradermally injected and occlusively patched to ten test guinea pigs (per extract) in an attempt to induce sensitization. The vehicle was similarly injected and occlusively patched to five control guinea pigs (per vehicle). Following a recovery period, the test and control animals received a challenge patch of the appropriate test article extract and the reagent control. All sites were scored at 24 and 48 hours after patch removal.

Under the conditions of this study, the SC and SO test article extracts showed no evidence of causing delayed dermal contact sensitization in the guinea pig.

Study and Supervisory Personnel:

Lindsey E. Roberts, B.S.
Shelli L. Petterle, A.A., B.A.
Carrie A. Fetter
Nicole L. Pence, A.A.
Jennifer M. Henry, B.S.
Laura A. Breitigan, B.S.
Tiffany M. Kitchen, RVT, A.A.S.
Christine M. Szych, RVT, A.A.S., LAT
Mark J. Menzel, RVT, A.A.S.
Jessica L. Peters, A.A.
Michael D. Hendershot
Molly F. Corvo, B.S.
Nicole L. Pence, A.A.
Natasha N. Norris, B.S.
Diane L. Miller
Colleen M. Stevenson, A.A.
Lisa A. Severhof, B.A.
Don R. Pohl, B.S.
Courtney M. Hood, B.S.
Jane A. Kervin, B.S., M.B.A.

Approved by:

Christina L. Ovall, B.A.
Technical Writer

11-27-07
Date Completed

1. Introduction

Purpose
A guinea pig maximization test of the material identified below was conducted to evaluate the potential to cause delayed dermal contact sensitization. This study was conducted based on the requirements of the International Organization for Standardization 10993: Biological Evaluation of Medical Devices, Part 10: Tests for Irritation and Delayed-Type Hypersensitivity.

Dates
The test article was received on October 11, 2007. Treatment began on October 30, 2007, and the observations were concluded November 25, 2007.

2. Materials

The test article provided by the sponsor was identified and handled as follows:

Test Article Name: Black Tecanyl MT XRO

Test Article Identification: Lot: 17940

Storage Conditions: Room Temperature

Vehicles: 0.9% sodium chloride USP solution (SC)
Sesame oil, NF (SO)

Preparation: Based on a ratio of 4 g:20 ml, a 2.0 g portion of the test article was covered with 10 ml of the vehicle at each preparation interval. The test article was extracted in SC and SO at 121°C for 1 hour. The vehicles (without test article) were similarly prepared to serve as the reagent control.

Condition of Extracts:

	SC Test	SC Control
Induction I:	clear with black particles	clear
Induction II:	clear with black particles	clear
Challenge:	clear with black particles	clear

	SO Test	SO Control
Induction I:	clear with black particles	clear
Induction II:	clear with black particles	clear
Challenge:	clear	clear

Additional Materials: Freund's Complete Adjuvant (FCA) was mixed 50:50 (v/v) with the chosen vehicle and used at induction I. A 10% (w/w) sodium lauryl sulfate (SLS) suspension in petrolatum was used for induction II. These materials were provided by the test facility.

3. Test System

Test System

Species:	Guinea pig (*Cavia porcellus*)
Strain:	Hla®:(HA)CVF®
Source:	Hilltop Lab Animals, Inc.
Sex:	Female (nulliparous)
Body Weight Range:	301 grams to 400 grams at study initiation
Age:	Young adult
Acclimation Period:	Minimum 5 days
Number of Animals:	Thirty
Identification Method:	Ear punch

Justification of Test System
The Hartley albino guinea pig has been used historically for sensitization studies (Magnusson and Kligman, 1970). The guinea pig is believed to be the most sensitive animal model for this type of study. The susceptibility of the Hartley guinea pig strain to a known sensitizing agent, 1-chloro-2,4-dinitrobenzene (DNCB), has been substantiated at NAMSA with this method under lab number 07T_34120_02 completed on July 27, 2007.

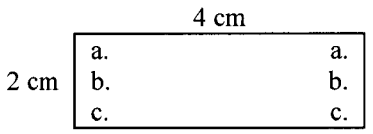
4. Animal Management

Husbandry:	Conditions conformed to Standard Operating Procedures that are based on the "Guide for the Care and Use of Laboratory Animals."
Food:	A commercially available guinea pig feed was provided daily.
Water:	Potable water was provided *ad libitum* through species appropriate water containers or delivered through an automatic watering system.
Contaminants:	Reasonably expected contaminants in feed or water supplies did not have the potential to influence the outcome of this test.
Housing:	Animals were housed in groups in stainless steel suspended cages identified by a card indicating the lab number, animal numbers, test code, sex, animal code and first treatment date.
Environment:	The room temperature was monitored daily. The temperature range for the room was within a range of 64-79°F. The room humidity was monitored daily. The humidity range for the room was 30-70%. The light cycle was controlled using an automatic timer (12 hours light, 12 hours dark).
Accreditation:	NAMSA is an AAALAC International accredited facility and is registered with the United States Department of Agriculture. Additionally, NAMSA maintains an approved Animal Welfare Assurance on file with the National Institutes of Health, Office for Laboratory Animal Welfare.
Personnel:	Associates involved were appropriately qualified and trained.
Selection:	Only healthy, previously unused animals were selected.
Sedation, Analgesia or Anesthesia:	Sedation, analgesia or anesthesia was not necessary during the routine course of this procedure.
Veterinary Care:	In the unlikely event that an animal became injured, ill, or moribund, care was conducted in accordance with current veterinary medical practice. If warranted for humane reasons, euthanasia was conducted in accordance with the current report of the American Veterinary Medical Association's Panel on Euthanasia. The objective of the study will be given due consideration in any decision and the study sponsor will be advised.
IACUC:	This procedure has been approved by NAMSA Institutional Animal Care and Use Committees (IACUC), and is reviewed at least annually by the same committees. Any significant changes to this procedure were approved by the IACUC prior to conduct.

5. Method

On the first day of treatment, fifteen guinea pigs per extract (ten test, five control) were weighed and identified. The fur over the dorsoscapular region was removed with an electric clipper.

Induction I
The test animals were injected with the test article extract and the control animals were injected with the reagent control. Three rows of intradermal injections (two per row) were given to each animal within an approximate 2 cm x 4 cm boundary of the fur clipped area as illustrated below:



Control Animals:
a. 0.1 ml of 50:50 (v/v) mixture of FCA and the chosen vehicle
b. 0.1 ml of vehicle
c. 0.1 ml of a 1:1 mixture of the 50:50 (v/v) vehicle/FCA mixture and the vehicle

Test Animals:
a. 0.1 ml of 50:50 (v/v) mixture of FCA and the chosen vehicle
b. 0.1 ml of test extract
c. 0.1 ml of a 1:1 mixture of the 50:50 (v/v) vehicle/FCA mixture and the test extract

To minimize tissue sloughing the "a" and "c" injections were slightly deeper than "b". Site "c" was injected slightly more caudal than site "b".

Induction II
The day prior to conducting the Induction II patch, the fur over the dorsoscapular region (same area as used during induction I) was removed with an electric clipper and the area was treated with 0.5 to 1 gram of a 10% sodium lauryl sulfate (SLS) suspension in petrolatum. The SLS suspension, applied to provoke a mild acute inflammation, was massaged into the skin over the injection site. The area was left uncovered.

At 7 days (±1 day) after completion of the Induction I injection, any remaining SLS residue was gently removed with a gauze pad. A 2 cm x 4 cm section of filter paper, saturated with approximately 0.3 ml of freshly prepared test article extract, was then topically applied to the previously injected sites of the test animals. The control animals were similarly patched with the appropriate reagent control. Each patch was secured with a nonreactive tape and the trunk of each animal was wrapped with an elastic bandage. At 48 hours, the binders and patches were removed. Animal number 12 and number 15 (SC, Control) were found unwrapped at time of patch removal. These animals were not rewrapped and duration of exposure was deemed sufficient.

Challenge
At 14 days (±1 day) after unwrapping the Induction II wraps, the fur was removed from the sides and flank areas with an electric clipper. The nonwoven cotton disk contained in a Hill Top Chamber® was saturated with approximately 0.3 ml of the test article extract or reagent control. The test extract was applied to the right flank of each animal and the control vehicle was applied to the left flank of each animal. Each patch was secured to the skin with semiocclusive hypoallergenic adhesive tape. The trunk of each animal was wrapped with an elastic bandage to maintain well-occluded sites. At 24 hours, the wraps and patches were removed and any residue remaining at the sites was removed.

Laboratory Observations
1. Animals were observed daily for general health.
2. Body weights were recorded at pretreatment.
3. Observations for dermal reactions were conducted at 24 and 48 hours after challenge patch removal. Prior to each scoring interval, the sites were wiped with 35% isopropyl alcohol. If necessary, the fur was clipped from each site to facilitate scoring. Scores were recorded in accordance with the criteria shown below:

Patch test reaction	Grading scale
No visible change	0
Discrete or patchy erythema	1
Moderate and confluent erythema	2
Intense erythema and swelling	3

6. Evaluation and Statistical Analysis

The responses from the challenge phase were compared within the test animal group and between test and control conditions. Control conditions were (1) the vehicle control solution on the test animals and (2) the test extract, control solution and biomaterial (if applied) on the control animals.

In the final analysis of data, consideration was given to the overall pattern, intensity, duration and character of reactions of the test as compared to the control conditions. Statistical manipulation of data was not applicable to this study. Grades of 1 or greater in the test group generally indicated sensitization, provided that grades of less than 1 were observed on the control animals. If grades of 1 or greater were noted on control animals, then the reactions of test animals that exceeded the most severe control reaction were considered to be due to sensitization.

7. Results

Body Weights and Clinical Observations
Individual body weights are presented in Appendix 1. All animals appeared clinically normal throughout the study.

Dermal Observations
Individual results of dermal scoring for the challenge phase appear in Appendix 2. No evidence of sensitization was observed.

8. Conclusion

Under the conditions of this study, the SC and SO test article extracts showed no evidence of causing delayed dermal contact sensitization in the guinea pig.

Results and conclusions apply only to the test article tested. Any extrapolation of these data to other samples is the sponsor's responsibility. All procedures were conducted in conformance with good manufacturing practices, certified to ISO 13485:2003 and accredited to ISO 17025:2005.

9. Records

All raw data pertaining to this study and a copy of the final report are retained in designated NAMSA archive files.

10. References

Guide for the Care and Use of Laboratory Animals, Institute for Laboratory Animal Research, National Academy of Sciences (Washington: National Academy Press, 1996).

ISO 10993-10 (2002) Biological evaluation of medical devices - Part 10: Tests for irritation and delayed-type hypersensitivity.

Magnusson, B. and A. Kligman, *Allergic Contact Dermatitis in the Guinea Pig* (Springfield: C.H. Thomas, 1970).

OLAW, Public Health Service Policy on Humane Care and Use of Laboratory Animals (NIH Publication)

United States Code of Federal Regulation (CFR) 9: The Animal Welfare Act.

Appendix 1 - Individual Body Weights and Clinical Observations

SC Group

Group	Animal Number	Individual Observation	
		Pretreatment Body Weight (g)	Clinical Observations
Test	1	320	Animal appeared clinically normal throughout the study
	2	306	Animal appeared clinically normal throughout the study
	3	301	Animal appeared clinically normal throughout the study
	4	314	Animal appeared clinically normal throughout the study
	5	354	Animal appeared clinically normal throughout the study
	6	315	Animal appeared clinically normal throughout the study
	7	366	Animal appeared clinically normal throughout the study
	8	341	Animal appeared clinically normal throughout the study
	9	348	Animal appeared clinically normal throughout the study
	10	334	Animal appeared clinically normal throughout the study
Control	11	353	Animal appeared clinically normal throughout the study
	12	370	Animal appeared clinically normal throughout the study
	13	381	Animal appeared clinically normal throughout the study
	14	356	Animal appeared clinically normal throughout the study
	15	359	Animal appeared clinically normal throughout the study

Appendix 1 (continued) - Individual Body Weights and Clinical Observations

SO Group

Group	Animal Number	Individual Observation	
		Pretreatment Body Weight (g)	Clinical Observations
Test	16	372	Animal appeared clinically normal throughout the study
	17	343	Animal appeared clinically normal throughout the study
	18	352	Animal appeared clinically normal throughout the study
	19	347	Animal appeared clinically normal throughout the study
	20	336	Animal appeared clinically normal throughout the study
	21	357	Animal appeared clinically normal throughout the study
	22	400	Animal appeared clinically normal throughout the study
	23	360	Animal appeared clinically normal throughout the study
	24	357	Animal appeared clinically normal throughout the study
	25	351	Animal appeared clinically normal throughout the study
Control	26	343	Animal appeared clinically normal throughout the study
	27	328	Animal appeared clinically normal throughout the study
	28	365	Animal appeared clinically normal throughout the study
	29	407	Animal appeared clinically normal throughout the study
	30	381	Animal appeared clinically normal throughout the study

Appendix 2 - Dermal Reactions – Challenge

SC Group

Group	Animal Number	Hours Following Patch Removal			
		24 Hour Score		48 Hour Score	
		Control	Test Extract	Control	Test Extract
Test	1	0	0	0	0
	2	0	0	0	0
	3	0	0	0	0
	4	0	0	0	0
	5	0	0	0	0
	6	0	0	0	0
	7	0	0	0	0
	8	0	0	0	0
	9	0	0	0	0
	10	0	0	0	0
Control	11	0	0	0	0
	12	0	0	0	0
	13	0	0	0	0
	14	0	0	0	0
	15	0	0	0	0

Appendix 2 (continued) - Dermal Reactions – Challenge

SO Group

Group	Animal Number	Hours Following Patch Removal			
		24 Hour Score		48 Hour Score	
		Control	Test Extract	Control	Test Extract
Test	16	0	0	0	0
	17	0	0	0	0
	18	0	0	0	0
	19	0	0	0	0
	20	0	0	0	0
	21	0	0	0	0
	22	0	0	0	0
	23	0	0	0	0
	24	0	0	0	0
	25	0	0	0	0
Control	26	0	0	0	0
	27	0	0	0	0
	28	0	0	0	0
	29	0	0	0	0
	30	0	0	0	0