TEST FACILITY

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STUDY TITLE

ISO Intracutaneous Study - Extract

TEST ARTICLE NAME

Black Tecanyl MT XRO

TEST ARTICLE IDENTIFICATION

Lot: 17940

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Summary

The test article, Black Tecanyl MT XRO, Lot: 17940, was extracted in 0.9% sodium chloride USP solution and alcohol in saline. These extracts were evaluated for intracutaneous reactivity 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.

A 0.2 ml dose of the appropriate test article extract was injected by the intracutaneous route into five separate sites on the right side of the back of each rabbit. Similarly, the corresponding control was injected on the left side of the back of each rabbit. The injection sites were observed immediately after injection. Observations for erythema and edema were conducted at 24, 48, and 72 hours after injection.

Under the conditions of this study, there was no erythema or edema from the SC extract injected intracutaneously into rabbits. There was very slight erythema and very slight edema from the SO extract injected intracutaneously into rabbits. The test article extracts met the requirements of the test since the difference between the test extracts and corresponding control mean score was 1.0 or less/greater than 1.0.

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1. Introduction

Purpose
The test article identified below was extracted and the extracts were evaluated for biocompatibility 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 purpose of the study was to determine whether leachables extracted from the material would cause local dermal irritant effects following injection into rabbit skin.

Dates
The test article was received on October 11, 2007. The animals were injected on October 17, 2007 and the observations were concluded on October 20, 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 3.0 g portion of the test article was covered with 15 ml of the vehicle. The test article was extracted in SC, and SO at 121°C for 1 hour. The extraction vehicles without test article were similarly prepared to serve as controls.

		Test	Control
Condition of Extracts:	SC:	clear with black particles	clear
	SO:	clear with black particles	clear

3. Test System

Test System

Species:	Rabbit (*Oryctolagus cuniculus*)
Breed:	New Zealand White
Source:	Myrtle's Rabbitry, Inc.
Sex:	male
Body Weight Range:	2.2 kg to 2.4 kg at selection
Age:	Young adult
Acclimation Period:	Minimum 5 days
Number of Animals:	Two
Identification Method:	Ear tag

Justification of Test System
The intracutaneous injection test in rabbits is specified in the current ISO testing standards and has been used historically to evaluate biomaterial extracts.

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 rabbit 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 individually housed in stainless steel suspended cages identified by a card indicating the lab number, animal number, test code, sex, and date dosed.
Environment:	The room temperature was monitored daily. The temperature range for the room was within a range of 61-72°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, thin-skinned animals free of mechanical irritation or trauma that could interfere with the test 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

Within a 4 to 18 hour period before treatment, each rabbit was clipped free of fur from the back and both sides of the spinal column to yield a sufficient injection area. Two rabbits were prepared per pair of extracts. A 0.2 ml dose of the appropriate test article extract was injected by the intracutaneous route into five separate sites on the right side of the back of each rabbit. Similarly, the corresponding control was injected on the left side of the back of each rabbit. Injections were spaced approximately 2 cm apart. The appearance of each injection site was noted immediately after injection. The animals were returned to their respective cages following the procedure.

Observations for erythema and edema were conducted at 24, 48, and 72 hours after injection. Reactions were scored on a 0 to 4 basis. Any reaction at the injection sites was also noted. The reactions were evaluated according to the following subjective rating scale:

SCORE	ERYTHEMA (ER)	EDEMA (ED)
0	No erythema	No edema
1	Very slight erythema (barely perceptible)	Very slight edema (barely perceptible)
2	Well-defined erythema	Well-defined edema (edges of area well-defined by definite raising)
3	Moderate erythema	Moderate edema (raised approximately 1 mm)
4	Severe erythema (beet redness) to eschar formation preventing grading of erythema	Severe edema (raised more than 1 mm, and extending beyond exposure area)

6. Evaluation and Statistical Analysis

The mean erythema and edema scores for the test and control extracts for each animal at each scoring interval were calculated. All mean erythema and edema scores for the test and control extracts were totaled and divided by 12 (2 animals x 3 grading periods x 2 grading categories) to determine the overall mean score for the test extract and corresponding control. The difference between the overall mean score of the test and corresponding control extracts was calculated by subtracting the overall mean score for the control from the overall mean score for the test extract.

The requirements of the test were met if the difference between the test extract mean score and corresponding control mean score was 1.0 or less.

7. Results

All animals appeared normal throughout the study. Results of scores for individual rabbits appear in Appendix 1. All injection sites appeared normal immediately following injection. The overall mean difference for the extracts are summarized below:

Extract	Overall Test Group Mean	Overall Control Group Mean	Overall Mean Difference (Test – Control)
SC	0.0	0.0	0.0
SO	1.0	1.0	0.0

8. Conclusion

Under the conditions of this study, there was no erythema or edema from the SC extract injected intracutaneously into rabbits. There was very slight erythema and very slight edema from the SO extract injected intracutaneously into rabbits. The test article extracts met the requirements of the test since the difference between the test extracts and corresponding control mean score was 1.0 or less/greater than 1.0.

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 and certified to ISO 13485:2003.

9. Records

All raw data pertaining to this study and a copy of the final report are to be 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.

OLAW, Public Health Service Policy on Humane Care and Use of Laboratory Animals.

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

United States Pharmacopeia (USP), General Chapter <88> Biological Reactivity Tests, In Vivo.

Appendix 1 - ISO Intracutaneous Observations

Rabbit Number/ Gender	Body Weight (kg)	Extract	Scoring Interval											
			24 Hours				48 Hours				72 Hours			
			Test		Control		Test		Control		Test		Control	
			ER	ED	ER	ED	ER	ED	ER	ED	ER	ED	ER	ED
56453 Male	2.2	SC	0	0	0	0	0	0	0	0	0	0	0	0
			0	0	0	0	0	0	0	0	0	0	0	0
			0	0	0	0	0	0	0	0	0	0	0	0
			0	0	0	0	0	0	0	0	0	0	0	0
			0	0	0	0	0	0	0	0	0	0	0	0
Mean Score			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
56454 Male	2.4	SC	0	0	0	0	0	0	0	0	0	0	0	0
			0	0	0	0	0	0	0	0	0	0	0	0
			0	0	0	0	0	0	0	0	0	0	0	0
			0	0	0	0	0	0	0	0	0	0	0	0
			0	0	0	0	0	0	0	0	0	0	0	0
Mean Score			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
56453 Male	2.2	SO	1	1	1	1	1	1	1	1	1	1	1	1
			1	1	1	1	1	1	1	1	1	1	1	1
			1	1	1	1	1	1	1	1	1	1	1	1
			1	1	1	1	1	1	1	1	1	1	1	1
			1	1	1	1	1	1	1	1	1	1	1	1
Mean Score			1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
56454 Male	2.4	SO	1	1	1	1	1	1	1	1	1	1	1	1
			1	1	1	1	1	1	1	1	1	1	1	1
			1	1	1	1	1	1	1	1	1	1	1	1
			1	1	1	1	1	1	1	1	1	1	1	1
			1	1	1	1	1	1	1	1	1	1	1	1
Mean Score			1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0

ER = Erythema
ED = Edema

SC = 0.9% sodium chloride USP solution
SO = sesame oil, NF