TEST FACILITY

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STUDY TITLE

ISO Systemic Toxicity Study – 2 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 sesame oil, NF. These extracts were evaluated for systemic toxicity in accordance with the guidelines of the current International Organization for Standardization 10993: Biological Evaluation of Medical Devices, Part 11: Tests for Systemic Toxicity (ISO).

A single dose of the appropriate test article extract was injected into each of five mice per extract by either the intravenous or intraperitoneal route. Similarly, five mice were dosed with each corresponding blank vehicle. The animals were observed immediately and at 4, 24, 48, and 72 hours after systemic injection.

Under the conditions of this study, there was no mortality or evidence of systemic toxicity from the extracts. Each test article extract met the test requirements.

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1. Introduction

Purpose
The test article identified below was extracted and the extracts were evaluated for biocompatibility in accordance with the guidelines of the current ISO. The purpose of the study was to determine whether leachables extracted from the material would cause acute systemic toxicity following injection into mice.

Dates
The test article was received on October 11, 2007. The animals were dosed 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 control blanks.

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

3. Test System

Test System

Species:	Mouse (*Mus musculus*)
Strain:	Hla®:(ICR)CVF®
Source:	Hilltop Lab Animals, Inc.
Sex:	Male
Body Weight Range:	18 grams to 20 grams at injection
Age:	Approximately 4 weeks of age at injection
Acclimation Period:	Minimum 1 day
Number of Animals:	Twenty
Identification Method:	Ear punch

Justification of Test System
Mice have historically been used to evaluate biomaterial extracts. The use of albino mice injected with a single intravenous (IV) or intraperitoneal (IP) dose of test article extract or control blank has been suggested by the current ISO standard for evaluation of medical plastics.

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 rodent 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 of five in stainless steel suspended cages or polycarbonate shoebox cages identified by a card indicating the lab number, animal numbers, test code, sex, animal code and date dosed.
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

Prior to dosing, the mice were identified and weighed. Five animals (per extract) were each injected with the test extract at a dose of 50 ml/kg. Five mice were similarly injected with the corresponding control. The SC was injected by the intravenous (IV) route while the SO was injected by the intraperitoneal (IP) route. The animals were then returned to their cages.

Mice were observed for adverse reactions immediately after dosing, and at 4, 24, 48, and 72 hours. The animals were weighed daily for three days after dosing

6. Evaluation and Statistical Analysis

If during the observation period, none of the mice treated with the individual test extract exhibited a significantly greater reaction than the corresponding control mice, the test extract met the requirements. If two or more mice died, or if abnormal behavior such as convulsions or prostration occurred in two or more mice, or if body weight loss greater than 2 grams occurred in three or more mice, the test sample did not meet the test requirements.

7. Results

Individual observations appear in Appendix 1.

Body Weight
Body weight data were acceptable.

Mortality
There was no mortality during the study.

Clinical Observations
The test and control animals injected with SO appeared ungroomed after dosing; this was considered an expected effect due to the unctuous nature of the extract. Otherwise, all animals appeared clinically normal throughout the study.

8. Conclusion

Under the conditions of this study, there was no mortality or evidence of systemic toxicity from the extracts. Each test article extract met the test requirements.

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 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 10993-11 (2006) Biological evaluation of medical devices – Part 11: Tests for systemic toxicity.

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

Appendix 1 - Systemic Toxicity Observations

Mortality And Body Weight Data

Extract	TEST EXTRACT						CONTROL BLANK					
	Animal Number	Weight (g)				#Dead/ #Tested	Animal Number	Weight (g)				#Dead/ #Tested
		Day 0	Day 1	Day 2	Day 3			Day 0	Day 1	Day 2	Day 3	
SC	91	20	21	22	23	0/5	81	19	21	23	24	0/5
	92	18	19	21	22		82	18	20	22	23	
	93	19	19	21	23		83	19	20	22	23	
	94	19	21	23	25		84	18	20	22	23	
	95	19	20	22	23		85	19	21	23	24	
SO	96	18	18	20	21	0/5	86	20	21	23	25	0/5
	97	18	19	20	21		87	19	20	22	23	
	98	19	21	23	24		88	20	21	23	24	
	99	19	21	22	24		89	20	23	25	26	
	100	19	20	21	23		90	19	21	23	24	

Clinical Observations

	TEST EXTRACT		CONTROL BLANK	
	SC	SO	SC	SO
Immediate	AN	AN	AN	AN
4 Hours	AN	U	AN	U
24 Hours	AN	AN	AN	AN
48 Hours	AN	AN	AN	AN
72 Hours	AN	AN	AN	AN

AN = Appeared Normal
U = Ungroomed