TEST FACILITY

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STUDY TITLE

Cytotoxicity Study Using the ISO Elution Method (1X MEM Extract)

TEST ARTICLE NAME

Black Tecanyl MT XRO

TEST ARTICLE IDENTIFICATION

Lot: 17940

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Summary

An *in vitro* biocompatibility study, based on the International Organization for Standardization 10993: Biological Evaluation of Medical Devices, Part 5: Tests for Cytotoxicity: *in vitro* Methods guidelines, was conducted on the test article, Black Tecanyl MT XRO, Lot: 17940, to determine the potential for cytotoxicity. A single extract of the test article was prepared using single strength Minimum Essential Medium supplemented with 5% serum and 2% antibiotics (1X MEM). This test extract was placed onto three separate monolayers of L-929 mouse fibroblast cells propagated in 5% CO_2. Three separate monolayers were prepared for the reagent control, negative control and for the positive control. All monolayers were incubated at 37°C in the presence of 5% CO_2 for 48 hours. The monolayer in the test, reagent control, negative control and positive control wells was examined microscopically at 48 hours to determine any change in cell morphology.

Under the conditions of this study, the 1X MEM test extract showed no evidence of causing cell lysis or toxicity. The 1X MEM test extract met the requirements of the test since the grade was less than a grade 2 (mild reactivity). The reagent control, negative control and the positive control performed as anticipated.

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1. Introduction

Purpose
The test article identified below was extracted, and the extract was subjected to an *in vitro* cytotoxicity study for biocompatibility based on the requirements of the International Organization for Standardization 10993: Biological Evaluation of Medical Devices, Part 5: Tests for Cytotoxicity: *in vitro* Methods. The test was performed to determine whether leachables extracted from the material would cause cytotoxicity.

Dates
The test article was received on October 11, 2007. The cells were first exposed to the extract October 19, 2007, and the observations were concluded on October 21, 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
Extraction Vehicle:	Single strength Minimum Essential Medium supplemented with 5% serum and 2% antibiotics (1X MEM)
Test Article Preparation:	Based on the USP ratio of 4 g:20 ml, a 3.6 g portion of the test article was covered with 18 ml of 1X MEM. A single preparation was extracted with agitation at 37°C for 24 hours. Following extraction the test extract was centrifuged at 3500 rpm for 10 minutes prior to testing.
Negative Control Preparation:	High density polyethylene was used as the negative control. Based on the USP ratio of 60 cm^2:20 ml, a single 30.8 cm^2 portion of the control material was covered with 10 ml of 1X MEM. The preparation was subjected to the extraction conditions previously described for the test article.
Reagent Control Preparation:	A single aliquot of 1X MEM without test material was subjected to the same extraction conditions as described for the test article.
Positive Control Preparation:	The current NAMSA positive control, tin stabilized polyvinylchloride, was used. Based on the USP ratio of 60 cm^2:20 ml, a single 60.8 cm^2 portion of the control material was covered with 20 ml of 1X MEM and extracted with agitation at 37°C for 24 hours.
Condition of Extracts:	Test: clear with particulates Reagent Control: clear Negative Control: clear Positive Control: clear

3. Test System

Test System Management
L-929, mouse fibroblast cells, (ATCC CCL 1, NCTC Clone 929, of strain L, or equivalent source) were propagated and maintained in open wells containing single strength Minimum Essential Medium supplemented with 5% serum and 2% antibiotics (1X MEM) in a gaseous environment of 5% carbon dioxide (CO_2). For this study, 10 cm^2 wells were seeded, labeled with passage number and date, and incubated at 37°C in 5% CO_2 to obtain sub-confluent monolayers of cells prior to use. Aseptic procedures were used in the handling of the cell cultures following approved NAMSA Standard Operating Procedures.

4. Method

Triplicate culture wells were selected which contained a sub-confluent cell monolayer. The growth medium contained in triplicate cultures was replaced with 2 ml of the test extract. Similarly, triplicate cultures were replaced with 2 ml of the reagent control, negative control and the positive control. Each well was labeled with the corresponding lab number, replicate number and the dosing date. The wells were incubated at 37°C in 5% CO_2 for 48 hours.

Following incubation, the cultures were examined microscopically (100X) to evaluate cellular characteristics and percent lysis. The color of the test medium was observed. A color shift toward yellow was associated with an acidic pH range and a color shift toward magenta to purple was associated with an alkaline pH range.

Each culture well was evaluated for percent lysis and cellular characteristics using the following table (direct excerpt from USP):

Grade	Reactivity	Conditions of all Cultures
0	None	Discrete intracytoplasmic granules; no cell lysis
1	Slight	Not more than 20% of the cells are round, loosely attached, and without intracytoplasmic granules; occasional lysed cells are present
2	Mild	Not more than 50% of the cells are round and devoid of intracytoplasmic granules; no extensive cell lysis and empty areas between cells
3	Moderate	Not more than 70% of the cell layers contain rounded cells or are lysed
4	Severe	Nearly complete destruction of the cell layers

For the test to be valid, the reagent control and the negative control must have had a reactivity of none (grade 0) and the positive control must have been a grade 3 or 4. The test sample met the requirements of the test if the biological response was less than or equal to grade 2 (mild). The test would have been repeated if the controls did not perform as anticipated and/or if all three test wells did not yield the same conclusion.

5. Results

See Appendix 1 for results.

pH Observation: No pH shift observed at 48 hours.

6. Conclusion

Under the conditions of this study, the 1X MEM test extract showed no evidence of causing cell lysis or toxicity. The 1X MEM test extract met the requirements of the test since the grade was less than a grade 2 (mild reactivity). The reagent control, negative control and the positive control performed as anticipated.

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.

7. Records

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

8. References

ISO 10993-5 (1999) Biological evaluation of medical devices – Part 5: Tests for cytotoxicity, *in vitro* methods.

USP 30-NF 25, General Chapter <87> BIOLOGICAL REACTIVITY TESTS, IN VITRO.

Wilsnack, R. E., "Quantitative Cell Culture Biocompatibility Testing of Medical Devices and Correlation to Animal Tests," *Biomaterials, Medical Devices and Artificial Organs* 4 (1976): 235-261.

Wilsnack, R. E., F. J. Meyer and J. G. Smith, "Human Cell Culture Toxicity Testing of Medical Devices and Correlation to Animal Tests," *Biomaterials, Medical Devices and Artificial Organs* 1 (1973): 543-562.

Appendix 1 - Reactivity Grades For Elution Testing

Well	Percent Rounding	Percent Cells Without Intracytoplasmic Granules	Percent Lysis	Grade	Reactivity
Test (1A)	0	0	0	0	None
Test (1B)	0	0	0	0	None
Test (1C)	0	0	0	0	None
Negative Control (1A)	0	0	0	0	None
Negative Control (1B)	0	0	0	0	None
Negative Control (1C)	0	0	0	0	None
Reagent Control (1A)	0	0	0	0	None
Reagent Control (1B)	0	0	0	0	None
Reagent Control (1C)	0	0	0	0	None
Positive Control (1A)	100	100	100	4	Severe
Positive Control (1B)	100	100	100	4	Severe
Positive Control (1C)	100	100	100	4	Severe