REVISED REPORT

STUDY TITLE:

CYTOTOXICITY TEST
USING THE ISO AGAROSE OVERLAY METHOD
IN THE L-929 MOUSE FIBROBLAST CELL LINE

TEST ARTICLE:

Nylon Denture Material

IDENTIFICATION NO.:

Not Supplied

SPONSOR:

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SUMMARY

An in vitro biocompatibility test, based on the International Organization for Standardization (ISO 10993-5) guidelines, was conducted on the test article, Nylon Denture Material, in order to determine the potential for in vitro cytotoxicity. A 2.5 cm^2 portion of the test article was placed on triplicate agarose surfaces directly overlaying confluent monolayers of L-929 mouse fibroblast cells. Similarly, triplicate control flasks were prepared. Each control flask contained a negative and a positive control section. After incubating at 37°C for 24-26 hours, the cell cultures were examined macroscopically for cell decolorization around the test article and controls to determine the zone of cell lysis (if any). The cultures were then examined microscopically (100X) to verify any decolorized zones and to determine cell morphology in proximity to and beneath the test article.

The negative controls and the positive controls performed as anticipated. Under the conditions of this study, the test article showed no evidence of causing cell lysis or toxicity greater than a USP grade of 2 (mild reactivity). The test article was not cytotoxic and passes this ISO study.

Study and Supervisory Personnel:

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7-29-97
Date Completed

Comment: This report has been revised to correct the client contact's last name. The conclusion is not affected. This revision is authorized by signatures above.

/cj

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INTRODUCTION

The test article identified below was subjected to an in vitro cytotoxicity study for biocompatibility based on the ISO 10993-5 guidelines. The test was performed to determine the test article's potential for cytotoxicity. The test article was received on June 19, 1997. The cells were dosed on June 24, 1997, and the observations were concluded on June 25, 1997.

MATERIALS

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

Test Article:	Nylon Denture Material
Identification No.:	Not Supplied
Storage Conditions:	Room temperature
Test Article Preparation:	A 2.5 cm^2 portion of the test article was directly applied to each agarose surface.
Negative Control:	NAmSA Negative Control 2.5 cm piece, Lot UP-1
Positive Control:	Latex Material, approximate 2.5 cm^2 piece, Lot #018

METHODS

Test System Management:

L-929 mouse fibroblast cells (ATCC CCL1, NCTC Clone 929, Clone of strain L, or equivalent source), were propagated at 37°C in sealed flasks containing minimum essential medium (MEM) supplemented with calf serum and a 2% concentration of the antibiotics penicillin, streptomycin, and amphotericin B. For this study, triplicate 25 cm^2 flasks were seeded, labelled with passage number and date, and incubated at 37°C in order to obtain confluent monolayers of cells prior to use. Microbiological methods and culture conditions conformed to specifications of approved NAmSA standard operating procedures.

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Preparation of Agar Overlay:

Equal amounts of double strength minimum essential medium (2X MEM), supplemented with neutral red, and 2% agarose were combined to form an MEM-agarose mixture (final concentration 1% agarose, 1X MEM). Confluent monolayers of L-929 mouse fibroblast cell cultures were grown to confluency in culture flasks. The MEM-agarose mixture (5 ml) was then placed in the cell culture flasks and allowed to solidify over the cells to form the agarose overlay.

Experimental Procedure:

A 2.5 cm^2 portion of the test article was placed directly onto triplicate solidified overlay surfaces. Similarly, the controls used triplicate flasks, with a section of the negative control and the positive control in each flask (equidistant from each other on the agarose surface). Each flask was then sealed, labelled indicating its contents (lab number or control flask) and dosing date, and incubated at 37°C for 24-26 hours.

Following incubation, the cell cultures were examined macroscopically for cell decolorization around the test article and controls to determine the zone of cell lysis (if any). The cultures were then examined microscopically (100X) to verify any decolorized zones and to determine cell morphology in proximity to and beneath the test article.

Scoring for cytotoxicity was based on the following criteria:

Grade	Reactivity	Conditions of all Cultures
0	None	No detectable zone around or under specimen
1	Slight	Some malformed or degenerated cells under specimen
2	Mild	Zone limited to area under specimen
3	Moderate	Zone extends 5 - 10 mm beyond specimen
4	Severe	Zone extends greater than 10 mm beyond specimen, but does not involve entire flask

Note: This chart (direct excerpt from USP) fails to accommodate 1 mm - 4 mm zones and lysis of the entire flask. The USP was notified of this. They responded that 1 mm - 4 mm zones should be categorized as mild (2). Lysis of the entire flask should be categorized as greater than severe (4), after a retest has been done to verify the results.

For the suitability of the system to be confirmed, the negative controls must have been a USP grade of 0 (reactivity none) and the positive controls must have produced a zone of lysis. The test article passed if all three of the cell cultures exposed to the test article showed no greater than a USP grade of 2 (mild reactivity). The test would have been repeated if the controls did not perform as anticipated and/or if all three test flasks did not yield the same conclusion (e.g. one flask passed the other two flasks failed).

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RESULTS

The scores obtained were as follows:

TEST/CONTROL SAMPLES		ZONE OF LYSIS (mm)	GRADE	REACTIVITY
Test Article:	(a)	0	0	None
	(b)	0	0	None
	(c)	0	0	None
Negative Control:	(a)	0	0	None
	(b)	0	0	None
	(c)	0	0	None
NAmSA Positive Control:	(a)	5	3	Moderate
	(b)	5	3	Moderate
	(c)	5	3	Moderate

Results and conclusions apply only to the test article tested. No further evaluation of these results is made by NAmSA. Any extrapolation of these data to other samples is the responsibility of the sponsor.

CONCLUSION

The negative controls and the positive controls performed as anticipated. Under the conditions of this study, the test article showed no evidence of causing cell lysis or toxicity greater than a USP grade of 2 (mild reactivity). The test article was not cytotoxic and passes this ISO study.

RECORD STORAGE

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

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