HACETTEPE UNIVERSITY
Faculty of Pharmacy

Department of Pharmacology

CYTOTOXICITY TEST REPORT

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CYTOTOXICITY
TEST REPORT FORM

Contract Number: **BU-2020/49A**

Customer Name/Address: Narkonteks Tekstil İhr. İth. San. ve Tic. A.Ş.
Doğuş Cad. 3/19 Sok. No: 12 Begos Buca İZMİR

Test Sample Name: SS Laminated PP Fabric (55 gr/m^2)

Test Sample Description: Personal Protective Equipment Fabric

Test Sample Lot Number: 9040-9050-9080

Testing Facility: Hacettepe University Faculty of Pharmacy
Pharmacology Department
06100, Sıhhiye Ankara, Turkey

Arrival of the Test Sample: 15.09.2020

Date of Report: 12.10.2020

Attachment: Technical Information

RESULT

The test material "SS Laminated PP Fabric (55 gr/m^2) (Lot 9040-9050-9080)" **is not cytotoxic**.

ACTING COORDINATOR
ADMINISTRATIVE DIRECTOR

Professor Serdar Uma

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TECHNICAL INFORMATION

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GUIDELINES

TS EN ISO 10993:	Biological evaluation of medical devices
TS EN ISO 10993-1:2014	Evaluation and testing within a risk management process
TS EN ISO 10993-5:2010	Tests for *in vitro* cytotoxicity
TS EN ISO 10993-12:2013	Sample preparation and reference materials

DESIGN OF STUDYING

Test Sample Quantity: 1 piece

Start of Test: 07.10.2020

End of Test: 09.10.2020

Preparation of the Sample for the Test *:
The test was performed on the samplings that were provided by the customer. Preparation of the sample for the test was made according to the solid/gel or liquid form as specified in the table.

Solid/Gel Test Sample	x	Liquid Test Sample	-
Extraction method: The test sample extract is prepared according to table of "Standart surface areas and extract liquid volumes" in TS EN ISO 10993-12 "Sample preparation and reference materials" standart. Extract is obtained by incubation of sample with culture medium in oven with 5 % CO_2 (V/V) at 37 °C for 24 hours. The entire/parts of sample was used in preparing the exract of the test sample.		The test sample is used directly in accordance with TS EN ISO 10993-12 "Sample preparation and reference materials" and TS EN ISO 10993-5 "Tests for *in vitro* cytotoxicity" standarts.	
***SIGN THE SUITABLE CHOICE WITH "X", UNSUITABLE CHOICE WITH "-".**			

** Since the sample was not sterile / particle was observed in the extract, the extract was filtered through 0.22 µm injector filter and applied to the cells.

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Extraction ratios according to the Extract Method :

Thickness mm	Extraction ratio (surface area or mass/volume) ± % 10	Material formats
< 0,5	6 cm^2/mL	Film, sheet, pipe surface
0,5 - 1,0	3 cm^2/mL	Plate, small molded material
> 1,0	1,25 cm^2/mL	Wider molded material
Irregular shaped solid devices	0,2 g/mL	Powder, pellets, foam, non-absorbent material, molded material
Irregular shaped porous devices (low density material)	0,1 g/mL	Membranes
NOTE- While there are currently no standardized methods for testing absorbent materials and hydrocolloids, the following protocol has been proposed: The absorption capacity of the material is determined. For example, the amount of liquid absorbed per gram of material. The test sample should be 0.1 g / mL on the absorbent capacity of the material.		

Test Groups:

Negative Control: High Density Polyethylene (Extract of 0.2 g / ml in culture medium)
Positive Control: Natural Rubber Latex (Extract of 0.2 g / ml in culture medium)
Reactive Control (Blank): Culture Medium

Test Method: XTT Cytotoxicity Test

Cell Lines: L929 (Mouse Fibroblast Cell Line)
Culture Medium: Minimum Essential Medium (Biochrom GmbH, Lot 0883F) +
%10 FBS (Cegrogen biotech, Lot C1660) + 4 mM L-Glutamine (Biochrom AG, Lot 0812A)
+100 IU/ml-100 µg/ml Penicilin/Streptomicin (Biochrom AG, Lot 0616L)

The extraction ratio of the sample : Extract of 0.2 g / ml in culture medium.

Incubation time of cells with sample extract: 24 hours

XTT incubation time: 4 hours

Total incubation time: 28 hours

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TEST PROCESS CHART

1. Day	Detach of cultured cells in T25 flask to $1x10^5$ cells/ml and culture in 96-well plate ($1x10^4$ cells/well)
2. Day	Control of cells with a microscope Application of test sample extract/positive and negative control extracts to cells
3. Day	Morphological observation Spectrophotometric measurement

RESULTS

1. Morphological observation

X Cell morphology is normal.

☐ Cell morphology shows changes according to control.

2. Spectrophotometric measurement

XTT Cytotoxicity Test

XTT (2,3-bis(2-methoxy-4-nitro-5-sulfophenyl)-5-[(phenylamino(carbonyl]-2H-tetrazolium hydroxide) is metabolically reduced in viable cells to a water-soluble formazan product. The number of viable cells correlates to the colour intensity determined by photometric measurements.

A decrease in number of living cells results in a decrease in the overall activity of mitochondrial dehydrogenases in the sample. This decrease directly correlates to the amount of orange formazan formed, as monitared by the optical density at 450 nm. To calculate the reduction of viability compared to the blank, equation is used:

$$\text{Viability\%} = \frac{100 \times OD_{450e}}{OD_{450b}}$$

OD_{450e} : the mean value of the measured optical density of the 100% extracts of the test sample
OD_{450b} : the mean value of the measured optical density of the blanks

The lower the viability % value, the higher the cytotoxic potential of the item is.

If viability is reduced to < 70% of the blank, the test sample is considered to be cytotoxic.

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EVALUATION

Reactive control (Blank): At the end of 28 hours, **100**% cell viability was observed.

Negative control: At the end of 28 hours, **97**% cell viability was observed.

Positive control: At the end of 28 hours, **13**% cell viability was observed.

Sample: At the end of 28 hours, **110**% cell viability was observed.

CONCLUSION

The test sample with **BU-2020/49A** code **is not cytotoxic**.

CHIEF OF TEST DEPARTMENT

Professor Pelin Kelicen Uğur