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ANALYSIS REPORT

(Industrial Services)

Report no : 16563500-125.05- 106 / **3622**

Report date : **17.07.2020**

Requested by : HAYAT KİMYA SAN. A. Ş.

Address : SEPETLİPINAR MAH. HAYAT CAD. NO:1, BAŞİSKELE / KOCAELİ

Subject : SENSITIZATION AND CYTOTOXICITY TESTS OF "GOODCARE EARLOOP SURGICAL MASK" ACCORDING TO ISO 10993: BIOLOGICAL EVALUATION OF MEDICAL DEVICES TEST PROTOCOLS

The results in this report are valid only for the analyzed samples.

Approved by

Assoc. Prof. Fatıma YÜCEL
Head of GEBI Industrial Services

This report is prepared as two originals (one for the customer, one for the institute archives) and contains 9 pages.

TÜBİTAK
MAM

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Sample	: Single type sample
Number of samples	: 100 pieces
Sample handling	: by Cargo
Condition of sample at reception: Non-sterile samples were received in original packages.	

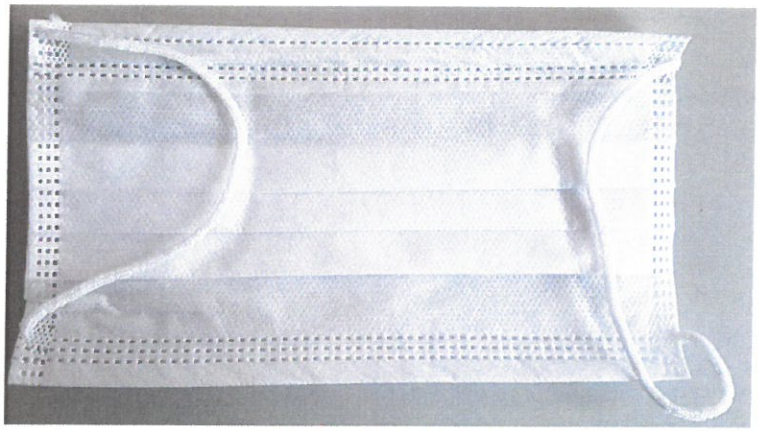
Expiry date	: --
Institute sample register no:	20/90-GMBE
Reception date and time	: 05/06/2020
Date of the analysis	: 22.06.2020-17.07.2020

Information on retention samples:
( ) Sample returned to the customer (x) Retention sample available ( ) Retention sample is not taken

1- Sample Information

According to the application of HAYAT KİMYA SAN. A. Ş. with the reference no 2224 and dated 05/06/2020 sensitization and cytotoxicity tests were carried out on one type of sample which is defined as "Goodcare Earloop Surgical Mask".

Table 1. Test Item

Örnek	Özellik	Adet
Goodcare Earloop Surgical Mask		100

Notes: The information describing the test item has been declared by the company.

Authorized Signatures:

53192

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2- Skin Sensitization Test

Sensitization test was applied according to "ISO 10993-10: 2010 Tests for irritation and delayed-type hypersensitivity" standart protocol. Extracts were prepared by incubation at 37°C for 24 hours and extraction ratio was defined as 6 cm^2/ml surface area/volume. The extraction protocol was detremined concerning of the clinical usage of the test item and PBS (Dulbecco's Phosphate Buffer Saline, Sigma-Aldrich, D8537-500ml) was used for extraction.

There was no color change or particles in the extraction solvent (pre- and post extraction). Extracts was used at the end of the extraction process. They were not stored before the administration. Sensitization tests of samples were carried out using healthy adult nulliparous and not pregnant female guinea pigs (Cavia porcellus), weighing 300 g to 500 g. As explained in the ISO 10993-10: 2010 standart protocol, experiments were carried out by injecting intradermally 0,1 ml of tested material per site. Animals are pretreated with 10 % sodium dodecyl sulfate 24 (±2) hours before the intradermal induction phase. Topical application was carried on non-injected site of the animals at 7 day after completion of the intradermal induction phase. Challenge phase was performed at 14 day after completion of the topical induction phase. Test item was applied topically to sites that were not treated during the induction stage. The experimental procedure applied on animals was shown in Figure 1.

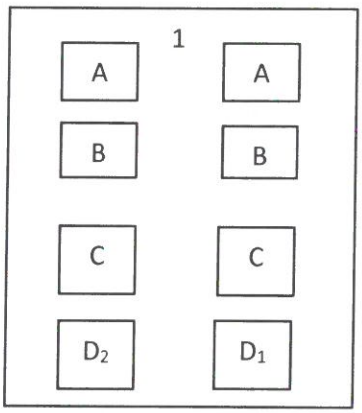

Figure 1.

1- Cranial end of animal.

A- 50:50 (volume ratio) stable emulsion of Freund's complete adjuvant (FCA) mixed with the physiological saline applied test sites.

B- Only test material applied test sites.

C- 50:50 (volume ratio) stable emulsion of the sample used at Site A mixed with test sample used at Site B applied test sites.

D- Topical induction at intrascapular region using 0,3 ml of test material.

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A pair of 0,1 ml intradermal injections into each animal (right and left sites) at the A, B, C injection sites were administrated.
At D site; the test materials were applied at day 7 left topical site (D_1), and at day 14 right topical site (D_2).

Negative control was carried out comparatively at 2 different sites at 2 different applications (Figure 2).

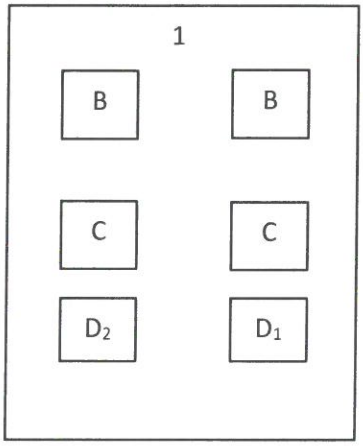

Figure 2.
1- Cranial end of the experimental animal.
B- 0,1 ml of serum physiologic.
C- (FCA) and serum physiologic mixed at 50:50 (volume ratio) was applied.
D- 0,3 ml of serum physiologic was applied on topical sites.

The fur of experimental animals were shaved to obtain enough application sites and a day after shaving, test materials were applied as shown in Figure 2 and in control animals as shown in Figure 3. All injections were carried out intradermally using 0,1 ml test samples. After application, no dressing was applied to the sites. In topical application, test material in experimental animals and 0,3 ml of serum physiologic in control animals were administered onto skin and absorbent gauze patch was applied and wrapped by an elastic bandage. The bandages were removed after 48 hours and the skin reactions were visualized. The second topical induction was performed 7 days after that and same experimental procedures were followed.

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Applied Test Materials
In the application, 10 animals for test sample and 5 animals as control were used. A total of 15 animals were used for one test material.

Test Material: Goodcare Earloop Surgical Mask

Table 2. Evaluation criteria and grading.

Reaction	Grading scale
No visible change	0
Discrete or patchy erythema	1
Moderate and confluent erythema	2
Intense erythema and swelling	3

Table 3. Observation values.

Groups	ID	Topical Induction Phase	Challenge Phase	Mean	Group Means	Score
Male Test	1	1	0	0.5	0.4	0.4
	2	1	0	0.5		
	3	1	0	0.5		
	4	0	0	0		
	5	1	0	0.5		
Female Test	1	0	0	0	0.4	
	2	1	1	1.0		
	3	1	0	0.5		
	4	1	0	0.5		
	5	0	0	0		
Negative Control	1	0	0	0	0.4	0.4
	2	1	0	0.5		
	3	0	0	0		
	4	1	1	1.0		
	5	0	1	0.5		

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Mean Scores

Table 4. Mean score values.

Sample	Result
Goodcare Earloop Surgical Mask	0.4
Negative control	0.4

Results

In the experiment carried out for test and control samples. the observations were graded according to the evaluation and grading criteria defined in Table 2. In the evaluation of "Goodcare Earloop Surgical Mask" extraction applied group of animals. no visible skin reaction was detected and sensitization score was obtained as 0.4 (Table 4).There was no discrete weight loss in the tested animals. There was also no visible change in the overall health situation of the tested animals.
According to the results of observations and the evaluation criterions defined in the ISO 10993-10: 2010 international standard protocol; **the tested product does not have a sensitization (against material) effect.**

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3- Cytotoxicity Test

Cytotoxicity Test of samples was performed by following the Biological Evaluation of Medical Devices - Part 5: Tests for in Vitro Cytotoxicity (ISO 10993-5: 2009).

Start date of analysis: 08.06.2020
End date of analysis: 12.06.2020
Definition of Samples: Sample of "Goodcare Earloop Surgical Mask" was defined as in Section 1. Test Item was supplied by "HAYAT KİMYA SAN. A. Ş.".
Cell line. justification of the choice and cell source: L929 (NCTC clone 929) mouse cell line was used as the test subject. This cell line is one of the recommended ISO 10993-5: 2009 cell lines and it is suitable to represent the mammalian system under study.

Name of company and batch of medium. serum and antibiotics. when added: Minimum Essential Medium (MEM. Sigma Cat # D0547. lot # SLBH5487) was used as medium supplemented by %10 Fetal bovine serum (FBS. heat inactivated. Gibco. #10500-064) + 1% antibiotic-antimycotic solüsyon (Gibco. #15240-062). 1% Non-Essential Amino Acids. (NEAA. Gibco. #11140050). 1% GlutaMAX™ (Gibco. #35050061). 1% Sodium Pyruvate (Gibco. #11360070).

Assay Method: Indirect Contact - Extraction method

Rationale: To be able to measure the cytotoxicity from the "Goodcare Earloop Surgical Mask" as a result of soluble toxic substances.

Extraction Method: The "Goodcare Earloop Surgical Mask" was supplied non-sterile and sterilized under the UV irradiation. The surface area of the test items was calculated with the digital caliper. The extraction volume was calculated using 6 cm^2/ml ratio (<0.5 mm tickness) based on the ISO 10993-12: 2012 test standard. The samples were extracted with medium including serum at 120 rpm. 37°C for 24 h. There was no color change or particles in the extraction solvent (pre- and post extraction). Extracts was used at the end of the extraction process. They were not stored before the administration.

Cytotoxicity Method: L929 cells were seeded at a cell density of 8×10^4 cells/well into the 96-well plates and incubated at 37°C. %5 CO_2 for 24 h. The sample extracts and controls were added (without modification or storage) to the 96-well plates and incubated at 37°C. %5 CO_2 for 24 h. Then WST-1 (Roche #11644807001) was added into each well at 10 % (v/v) concentration. After 2-3 hours. the change in color was measured at 450 nm with a reference wavelength of 650 nm by a microplate reader.

Measure of Cytotoxicity: WST-1 Cell viability assay (Colorimetric)
Rationale: Measurement of cell viability in precise and reproducible techniques.

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Negative. positive and other controls

Control 1: MEM fresh

Control 2: MEM extract incubated at 37°C. %5 CO_2 and 120 rpm for 24 h. under the same conditions of extraction.

Negative Control: RAUMEDIC Tubings in silicone rubber grade RAUMEDIC-SIK 8363.

Positive control*:* RAUMEDIC PVC Org Sn

RESULTS:

Table 5. Quantitative evaluation.

Sample	Degree	Positive and negative controls	Degree
Goodcare Earloop Surgical Mask	0	**Control 1.** MEM fresh	0
		Control 2. MEM ekstrakt	0
		Negative Control. RAUMEDIC-SIK 8363	0
		Positive Control. RAUMEDIC PVC Org Sn	4

Table 6. Qualitative morphological grading of cytotoxicity of extracts

Grade	Reactivity	Conditions of all cultures
0	None	Discrete intracytoplasmatic granules. no cell lysis. no reduction of cell growth.
1	Slight	Not more than 20 % of the cells are round. loosely attached and without intracytoplasmatic granules. or show changes in morphology; occasional lysed cells are present; only slight growth inhibition observable.
2	Mild	Not more than 50 % of the cells are round. devoid of intracytoplasmatic granules. no extensive cell lysis; not more than 50 % growth inhibition observable.
3	Moderate	Not more than 70 % of the cell layers contain rounded cells or are lysed; cell layers not completely destroyed. but more than 50 % growth inhibition observable.
4	Severe	Nearly complete or complete destruction of the cell layers.

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The quantitative results for the "Goodcare Earloop Surgical Mask" extracts on L929 cell culture over 24 h exposure. The cytotoxicity of samples on the cell viability relative to MEM Extract was found to be **97.61 ± 4.97 %**.

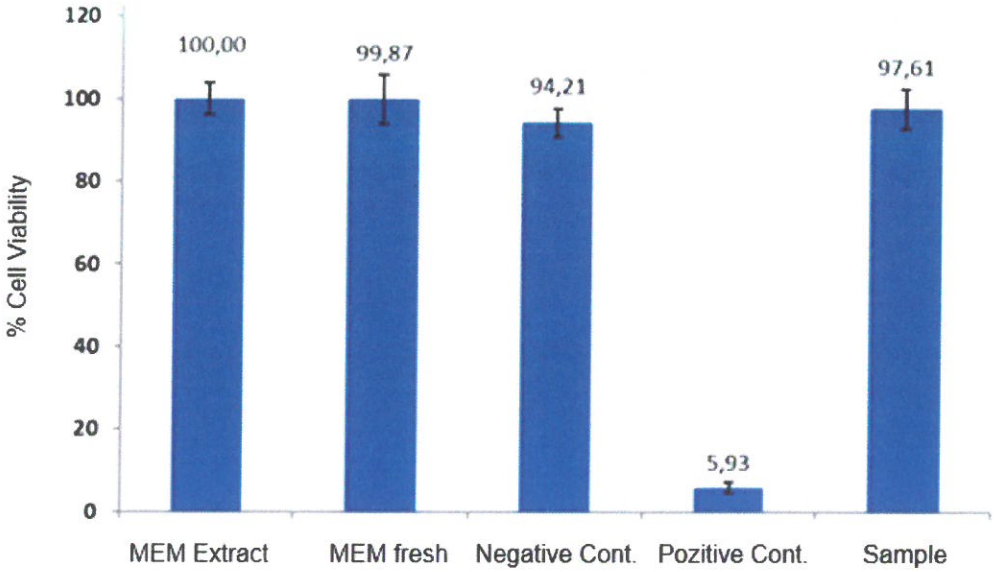

Figure 3. The percentage of cell viability relative to Control following exposure to "Mask" extracts for 24 h.

The cytotoxicity of "Goodcare Earloop Surgical Mask" samples was analyzed based on normalization to the % cell viability of Control. which was incubated at the same conditions with the samples without any treatment.

The results indicate the average of 3 random samples run in triplicate. The average value is indicated on the graph. Standard deviations are drawn based on values from 3 independent samples.

CONCLUSION:
"Goodcare Earloop Surgical Mask" extracts were analyzed following by WST-1 Cell viability assay (Colorimetric) and the sample **was found to be non-cytotoxic.**

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