MEDICERT
TESTING LABORATORY
ISO/IEC 17025:2017
IAKS-TL-1018

ISO 10993-11:2018
Subacute Systemic Toxicity

IAKS ACCREDITED

Company Name Firma Adı	**AVAS KOZMETİK SANAYİ VE TİCARET LİMİTED ŞİRKETİ**
Company Address Firma Adresi	**GÖKEVLER MAH. 2331 SK. PASİAD NO. 1 D ESENYURT - İSTANBUL / TÜRKİYE**
Test Name Testin Adı	**Subacute Systemic Toxicity**
Test Standard Test Standardı	**TS EN ISO 10993-11:2018**
Commercial Brand (If You Have) Ticari Marka (Varsa)	**Felix Filler**
Description of the Sample Numunenin Adı ve Tarifi	**1 cc Felix Filler Dolgu**
Lot Number Lot Numarası	**AVAS012023-1**
Sample Registration Number Numune Kayıt Numarası	**FLXFHA/202302**
Sample Acceptance Date Numune Kabul Tarihi	**27.02.2023**
Report Number Rapor Numarası	**2023-04/BIYO/1532HA-SAST**
Date of Report Rapor Tarihi	**03.04.2023**
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SUMMARY

Subacute Systemic Toxicity test was performed on 1 cc Felix Filler Dolgu sample with lot number **AVAS012023-1** according to TS EN ISO 10993-11. The samples were prepared in SF for 37°C - 72 hours by weighing equal amounts under sterile conditions according to TS EN ISO 10993-12 Biological evaluation of medical devices - Part 12: Sample preparation and reference materials standard. Clinical observations, hematological and biochemical values were recorded after the surgical application of the samples. The negative control was also analyzed at the same time. **As a result, it was determined that the sample did not cause any toxic effect with subacute application.**

1. INTRODUCTION

Purpose : The report described below evaluated the potential of a single sample variety for subacute systemic toxicity testing.

Test Guide: This study was carried out according to the requirements of the International Organization for Standardization. 10993: Biological Evaluation of Medical Devices, Part 11: Tests for Systemic Toxicity

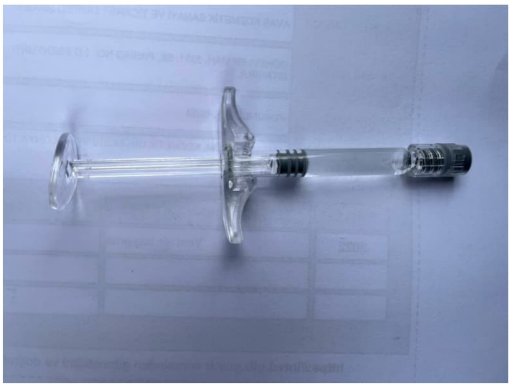
Dates

Sample Acceptance Date : 27.02.2023
Test Date : 03.03.2023
Observation Date : 30.03.2023

2. SAMPLE INFORMATION

Company Name :	Avas Kozmetik
Date of the Sample Acceptance :	27.02.2023 13.05
Sample Record Number :	FLXFHA/202302
Sample Lot Number :	AVAS012023-1
Number of Sample :	4
Packaging Infırmation :	CLOSED PACKED
Sample Delivery Method :	CARGO
Expiration Date of the Sample :	17.01.2028
Production Date of the Sample :	17.01.2023
Description of the Sample :	Bir şırınga 1 cc Felix Filler dolgu maddesi içermektedir.
Characteristics of the Sample Use/Application :	Cross linked hyaluronic acid

Sample Image :



3. TEST SYSTEM

Animal used in the test :	MOUSE
Strain :	CD1
Source :	Burdur Mehmet Akif Ersoy University Experimental Animals Production and Research Center
Gender :	Male - Female
Weight :	27 - 35 GR
Acclimation time :	5 Days
Number of the animals :	20

4. ANIMAL MANAGEMENT

Animal Care :	The animals used in the experiments are performed in accordance with the standards of Biological Evaluation of Medical Devices - Part 2: Requirements for Animal Welfare.
Food :	OPTİMA experimental animal feed is given.
Water :	Water is supplied as ad-libitum in suitable drinkers.
Cage System :	Each animal was identified and placed in appropriate cages.
Environmental Conditions :	12 hours night and 12 hours day environment is provided; 50-70% humidity and 18-21℃ environment are provided. Temperature and humidity are controlled daily. For 5 days after the experiment, the animals were taken individually into conventional euro type 1 cages.
Personnel :	Tests are carried out by trained and suitably qualified personnel.
Selection of the animal :	Animals that are healthy, free of any disease, and not pregnant when female animals are used, were selected under the supervision of veterinarians.
Veterinary Care :	This study was carried out under the supervision of a veterinarian.

5. METHOD

The application dose and route of administration are given in Table B1 in the ISO 10993-11:2018 standard test protocol. Weight and surface area, physical, chemical and biological properties of test animals were taken into account in determining appropriate dose rates (ml/kg body weight). According to Table B1 in the standard protocol, intraperitoneal administration was done at 50 ml/kg. In line with the directives in ISO 10993-1 and ISO 108993-12, clinical observations were made according to the information in Table 1 and systemic effects were determined. No clinical signs were observed in individuals in all groups.

Table 1: General Clinical signs and observations

Clinical Observation	Observation	Included Systems
Dyspnoea	Dyspnoea	Dyspnoea
Apnea, Cyanosis, Tachypnea, Runny Nose, Central Nervous System, Lung, Cardiac	Apnea, Cyanosis, Tachypnea, Runny Nose, Central Nervous System, Lung, Cardiac	Apnea, Cyanosis, Tachypnea, Runny Nose, Central Nervous System, Lung, Cardiac
Motor Activities	Motor Activities	Motor Activities
Increase/Decrease Sleep, Deafness	Increase/Decrease Sleep, Deafness	Increase/Decrease Sleep, Deafness
Anesthesia, Catalepsy, Ataxia, Abnormal	Anesthesia, Catalepsy, Ataxia, Abnormal	Anesthesia, Catalepsy, Ataxia, Abnormal
Movement, Prostration, Tremor, Fasciculation	Movement, Prostration, Tremor, Fasciculation	Movement, Prostration, Tremor, Fasciculation
Central Nervous System, Soma Motor, Sensory	Central Nervous System, Soma Motor, Sensory	Central Nervous System, Soma Motor, Sensory
Neuromuscular, Autonomous	Neuromuscular, Autonomous	Neuromuscular, Autonomous
Convulsion Clonic, Tonic, Tonic-Clonic, Asphyxia	Convulsion Clonic, Tonic, Tonic-Clonic, Asphyxia	Convulsion Clonic, Tonic, Tonic-Clonic, Asphyxia
Opistotonus	Opistotonus	Opistotonus
Central Nervous System, Neuromuscular, Autonomous	Central Nervous System, Neuromuscular, Autonomous	Central Nervous System, Neuromuscular, Autonomous
Respiratory	Respiratory	Respiratory

6. EVALUATION

Twenty 8-12 weeks old adult CD1 mice (10 male and 5 naive and 5 non-pregnant female) were used. Phosphate saline buffer was used as a negative sample. 5 randomly selected female and 5 male mice were used as negative controls.

Table 2. Change in body weight of animals

Animal No.	Initial body weight (g)	Body weight at the end of the experiment (g)	Liver weight (g)	% change of body weight*	Liver weight ratio index (%)**
1	27.6	28.0	1.35	1.45	4.82
2	27.8	28.2	1.39	1.44	4.93
3	27.4	28.6	1.64	4.38	5.73
4	27.0	27.4	1.38	1.48	5.04
5	27.0	27.8	1.41	2.96	5.07
6	27.6	29.2	1.58	5.80	5.41
7	27.6	28.8	1.38	4.35	4.79
8	27.8	30.2	1.54	8.63	5.10
9	27.8	30.6	1.56	10.07	5.10
10	26.6	28.8	1.43	8.27	4.97
11	31.6	31.4	1.80	-0.63	5.73
12	31.2	30.2	1.58	-3.21	5.23
13	31.4	36.4	2.00	15.92	5.49
14	31.4	33.8	1.74	7.64	5.15
15	31.6	34.8	1.61	10.13	4.63
16	32.4	36.0	2.24	11.11	6.22
17	32.4	28.2	1.29	-12.96	4.57
18	32.2	32.8	1.84	1.86	5.61
19	32.2	32.2	1.64	0.00	5.09
20	32.6	32.4	1.65	-0.61	5.09

* Weight loss ≥ 10% is considered a clinical sign.
** Liver weight index should be between 4-6%.

Food and water consumption was similar in the control and study groups. An abnormal change in body weight was observed in both test and control animals, with the exception of the number 17 mouse. No statistical difference was observed in the liver index data of the test and control group animals, except for the animal number 16.

6.1. Pathological Findings

As a result of 28 days of observation, a general pathological evaluation was made and the findings are given in the table below:

Female Test 2	Emphysema in both lobes of the right lung Small area of emphysema in the left lobe of the lung
Female Test 5	Necrosis in the small lobe of the liver

6.2. Hematology

Hematological measurements were obtained using a veterinary hematology analyzer and the following 6 parameters were evaluated:

1- RBC: Red blood cell
2- WBC: White blood cell
3- HGB: Hemoglobin
4- HCT: Hematocrit
5- MCHC: Average Particle Hemoglobin Concentration
6- PLT: Platelet

Table 3. Hematological Values

Animal No	WBC (10^9 cell/L)	RBC (10^{12} cell/L)	HGB (g/dL)	MCHC (g/dL)	HCT (%)	PLT (10^9 cell/L)
1	5.4	9.38	13.0	30.3	45.8	386
2	12.1	9.05	13.9	29.4	42.8	152
3	9.7	8.69	12.6	30.6	41.7	810
4	5.7	8.34	12.8	30.9	38.5	607
5	4.9	8.06	11.9	30.8	37.0	492
6	9.8	8.44	11.4	30.8	40.8	727
7	3.9	8.81	12.6	30.5	38.3	789
8	4.9	9.07	11.7	30.4	43.4	639
9	5.5	8.33	13.2	30.4	38.8	877
10	5.9	8.77	11.8	30.8	39.2	751
11	2.7	8.30	12.1	29.9	35.7	773
12	5.1	8.42	10.7	31.2	37.8	702
13	6.7	8.85	11.8	30.0	41.9	147
14	4.1	7.50	12.6	30.1	34.2	842
15	7.0	9.42	10.3	30.8	40.5	980
16	4.4	9.66	12.5	29.4	45.9	628
17	16.6	8.88	13.5	29.9	42.7	888

Animal No	WBC (10^9 cell/L)	RBC (10^{12} cell/L)	HGB (g/dL)	MCHC (g/dL)	HCT (%)	PLT (10^9 cell/L)
18	9.2	7.61	12.8	29.4	43.1	754
19	5.5	7.09	13.6	30.1	38.8	741
20	6.9	8.51	12.4	30.2	39.6	623

There was no statistical difference between these obtained values ($p<0.05$). Statistical analyzes were performed using the IBM SPSS program at 95% confidence level.

6.3. Clinical Chemistry

Clinical biochemistry analysis in blood was performed with a semi-automatic clinical chemistry analyzer.

Table 4. Clinical Chemistry Results

Animal No	ALT U/L	AST U/L	ALP U/L	GLU mg/dL	UREA mg/dL	URIC ACID mg/dL	GGT U/L	T. Protein mg/dL	T. Cholesterol mg/dL	Calcium mg/dL
1	39.40	75.10	56.10	99.94	27.17	0.71	1.68	4.11	69.00	1.35
2	35.20	81.10	72.00	128.39	29.07	0.91	9.73	4.89	59.98	2.37
3	31.80	64.30	75.90	110.96	31.13	0.70	0.00	4.40	63.67	1.94
4	32.70	59.70	89.00	133.15	28.36	0.76	3.68	4.51	63.84	1.99
5	37.70	74.70	62.70	108.48	20.83	0.74	2.05	4.12	48.99	0.89
6	36.80	126.70	61.20	101.48	25.23	0.88	0.00	4.21	50.90	1.32
7	61.60	87.40	78.60	125.44	33.66	1.57	2.32	4.37	46.32	1.66
8	48.00	99.00	104.50	115.09	30.12	0.92	1.51	4.76	58.50	1.66
9	47.00	81.60	77.00	121.08	29.03	0.99	4.44	4.67	66.45	2.31
10	187.60	210.70	82.8	124.68	29.11	1.66	4.71	4.34	62.64	1.70
11	38.60	60.40	87.20	148.94	23.62	0.60	2.32	4.40	83.90	1.84
12	38.90	81.70	82.50	85.76	20.91	0.98	2.103	4.54	52.36	2.23
13	32.50	66.50	57.40	93.22	25.75	1.27	1.690	3.74	53.10	1.61
14	44.60	246.40	68.00	118.48	19.25	1.09	8.33	5.17	80.50	1.31
15	127.90	144.00	92.40	134.02	31.70	0.84	7.11	4.52	77.68	2.45
16	35.90	83.40	82.50	130.49	23.65	1.05	-	4.53	70.37	0.97
17	39.20	69.70	107.00	132.33	25.47	0.78	-	5.49	97.52	2.35
18	34.80	67.70	55.40	153.44	29.22	1.01	-	3.83	95.84	1.63
19	48.70	74.51	61.60	147.48	27.77	0.54	-	4.63	101.96	2.02
20	52.60	78.89	80.00	141.08	26.91	0.62	-	4.90	79.80	1.55

There was no statistical difference between these obtained values ($p<0.05$). Statistical analyzes were

performed using the IBM SPSS program at 95% confidence level.

7. RESULT

According to the results obtained, no statistical difference was found between the test and control groups. The product with lot number AVAS012023-1 was tested according to the method in the TS EN ISO 10993-11 Systemic Toxicity Tests - Subacute Systemic Toxicity Test document and **no effect of the sample product on the organism was detected**.

8. RECORD

All raw data and a copy of the final report are stored in the Medicert archive files.

9. REFERENCES

- Guide for The Care and Use of Laboratory Animals Eighth Edition National Research Council of The National Academies
- TS EN ISO 10993-1 Biological evaluation of Medical Devices - Chapter 1: Evaluation and experiment in a risk management process
- TS EN ISO 10993-2 Biological evaluation of Medical Devices - Chapter 2: Conditions for animal welfare
- TS EN ISO 10993-11 Biological evaluation of medical devices - Part 11: Tests for Subacute Systemic Toxicity
- TS EN ISO 10993-12 Biological evaluation of Medical Devices - Chapter 12: Sample preparation and reference materials

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