ISO 10993-11:2018
Subchronic Systemic Toxicity

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	**Subchronic 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-SKST**
Date of Report Rapor Tarihi	**14.04.2023**
Date of Test Deney Tarihi	**03.03.2023 - 11.04.2023**
Report Total Page Raporun Sayfa Sayısı	**12 Page / *Sayfa***

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SUMMARY

Subchronic Systemic Toxicity test was performed on the 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 -72 hours at 37°C 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 subchronic application for 40 days.**

1. INTRODUCTION

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

Test Guide: TS EN ISO 10993: Biological Evaluation of Medical Devices, Part 11: Tests for Systemic Toxicity - 6. Subchronic Systemic Toxicity

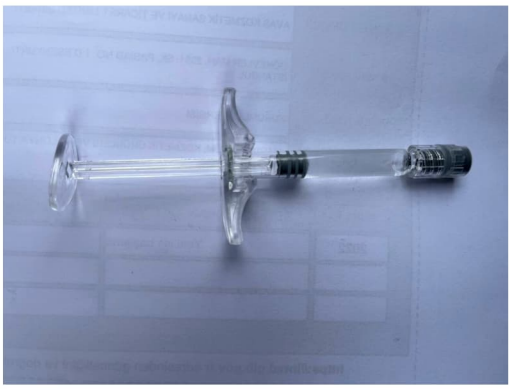
Dates

Sample Acceptance Date : 27.02.2023
Test Date : 03.03.2023
Observation Date : 11.04.2023 (Application and observation were made for 40 days.)

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 Infirmation :	KAPALI PAKET
Sample Delivery Method :	KARGO
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 :	RAT
Strain :	SPRAGUE DAWLEY
Source :	Burdur Mehmet Akif Ersoy University Experimental Animals Production and Research Center
Gender :	Male - Female
Weight :	160 - 220 GR
Acclimation time :	5 Days
Number of the animals :	25

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; 55% humidity and 18 - 21°C environment are provided. Temperature and humidity are controlled daily.
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

Experimental animals were placed under general anesthesia by IP administration of 80 mg/kg Ketasol and 5 mg/kg Rompun. The hind leg gluteal muscle area was shaved and cleaned with skin Femalenfectant. The skin was incised. The test substance was placed in the gluteal muscle of each animal in the test group, parallel to the muscle fibers. Control substance was placed in the gluteal muscle of each animal in the control group. The skin incision line was sutured with 6/0 absorbable surgical suture. Wound dressing was done twice, morning and evening, for 5 days. On day 40, animals were euthanized by cervical dislocation method.

Table 1: Test Animal Information

Group	Animal No.	Type	Strain	Date of Birth	Gender
Working Group	1	Rat	Sprague Dawley	24.11.2022	Male
	2	Rat	Sprague Dawley	24.11.2022	Male
	3	Rat	Sprague Dawley	24.11.2022	Male
	4	Rat	Sprague Dawley	24.11.2022	Male
	5	Rat	Sprague Dawley	24.11.2022	Male
	6	Rat	Sprague Dawley	24.11.2022	Male
	7	Rat	Sprague Dawley	24.11.2022	Male
	8	Rat	Sprague Dawley	24.11.2022	Male
	9	Rat	Sprague Dawley	24.11.2022	Male
	10	Rat	Sprague Dawley	24.11.2022	Male
	11	Rat	Sprague Dawley	24.11.2022	Female
	12	Rat	Sprague Dawley	24.11.2022	Female
	13	Rat	Sprague Dawley	24.11.2022	Female
	14	Rat	Sprague Dawley	24.11.2022	Female
	15	Rat	Sprague Dawley	24.11.2022	Female
	16	Rat	Sprague Dawley	24.11.2022	Female
	17	Rat	Sprague Dawley	24.11.2022	Female
	18	Rat	Sprague Dawley	24.11.2022	Female
	19	Rat	Sprague Dawley	24.11.2022	Female
	20	Rat	Sprague Dawley	24.11.2022	Female
Control Group	1	Rat	Sprague Dawley	24.11.2022	Female
	2	Rat	Sprague Dawley	24.11.2022	Female
	3	Rat	Sprague Dawley	24.11.2022	Male
	4	Rat	Sprague Dawley	24.11.2022	Male
	5	Rat	Sprague Dawley	24.11.2022	Male

Table 2: Deney başlangıcındaki hayvan ağırlıkları

Group	Animal No.	Body Weight
Working Group	1	195
	2	205
	3	212
	4	198
	5	201
	6	214
	7	199
	8	189
	9	206
	10	194
	11	170
	12	165
	13	159
	14	168
	15	174
	16	170
	17	165
	18	179
	19	155
	20	164
Control Group	1	178
	2	171
	3	158
	4	162
	5	165

6. EVALUATION

Table 3. Number of animals showing undesirable clinical signs and changes in body weight

Groups	Number of Animals at the Start of the Experiment	Number of animals showing undesirable clinical signs	Number of animals showing undesirable body weight change (more than 10% change)
Working Group	20	0	0
Control Group	5	0	0
Total	25	0	0

Table 4. Change in body weight of animals

Group	Animal No.	Day 7	Day 14	Day 21	Day 28	Day 35	Day 40	% Change
Working Group	1	195	198	201	202	204	205	
	2	205	209	210	214	215	216	
	3	212	212	216	219	224	224	
	4	198	205	207	209	210	211	
	5	201	204	205	211	211	215	
	6	214	219	220	221	223	228	
	7	199	205	208	209	214	216	
	8	189	189	191	197	198	200	
	9	206	209	210	212	216	217	
	10	194	195	200	205	205	207	
	11	170	172	178	185	185	190	
	12	165	169	170	174	174	179	
	13	159	162	170	174	174	177	
	14	168	171	172	176	178	179	
	15	174	176	176	179	180	182	
	16	170	171	174	175	179	173	
	17	165	165	165	168	169	171	
	18	179	180	183	184	185	186	
	19	155	159	161	163	164	166	
	20	164	166	168	170	172	175	
Control Group	1	178	180	182	183	184	184	
	2	171	173	174	176	179	180	
	3	158	160	162	165	169	170	
	4	162	164	166	170	176	180	
	5	165	169	170	174	178	181	

Table 5. Hematological and Biochemical Values

Group	Animal No.	ALB g/dL 2-4.7	ALKP u/L 44-118	ALT u/L 28-132	AST u/L 69-191	Ca mg/dL 9-12	PHOS mg/dL 6-13	CHOL mg/dL 63-174	CK mg/dL 0.5-0.8	Glu g/dL 106-278	TP g/dL 4.3-6.4	TRIG mg/dL 71-164	BUN mg/dL 19-34
Working Group	1	4.1	98	59	89	10	8	140	0.5	152	6.1	89	22
	2	4.3	76	47	84	9	10	89	0.5	144	6.0	112	30
	3	4.7	66	43	96	11	11	82	0.5	252	5.9	152	17
	4	3.9	81	51	92	14	12	91	0.8	290	5.5	89	33
	5	3.8	111	99	153	12	11	141	0.4	119	4.5	148	31
	6	4.0	102	85	178	11	13	103	0.9	135	4.8	153	36
	7	4.1	58	74	205	10	9	141	0.4	190	4.9	96	38
	8	2.5	65	92	135	9	8	60	0.5	205	5.0	107	20
	9	2.9	62	64	154	11	9	170	0.5	217	5.1	119	26
	10	2.7	69	32	160	13	10	59	0.5	190	6.2	135	31
	11	2.9	84	39	141	12	11	148	0.4	174	6.4	104	33
	12	2.4	77	58	180	11	13	152	0.6	126	6.5	116	25
	13	3.4	79	121	95	13	10	154	0.5	132	5.9	148	29
	14	3.6	82	106	108	9	9	107	0.6	198	5.8	152	34
	15	4.9	84	121	142	10	8	130	0.6	139	6.0	180	24
	16	4.1	88	86	165	9	8	96	0.5	152	5.4	165	25
	17	3.8	105	86	149	9	8	92	0.7	206	5.5	88	20
	18	3.9	56	52	94	10	7	83	0.6	269	5.2	78	22
	19	4.5	59	52	84	11	6	75	0.6	255	5.3	140	29
	20	4.1	92	29	157	9	13	77	0.7	211	6.2	153	24
Control Group	1	2.9	71	106	132	10	14	79	0.5	154	6.3	147	26
	2	3.6	84	55	153	11	10	86	0.4	212	6.7	156	28
	3	3.4	89	53	99	11	10	144	0.6	185	5.4	141	26
	4	4.7	92	96	123	10	9	95	0.7	147	5.9	100	24
	5	4.1	105	57	139	10	10	93	0.7	155	6.0	95	23

Group	Animal No.	WBC 10^3/uL 3-14	LYM % 65-87	RBC 10^3/uL 5.0-9.5	HGB 10^3/uL 10-16	HCT 10^3/uL	MCV fl 48-56	MCH pg 25-35	MCHC g/dL 25-35	PLT 10^3/uL 480-1992	MPV fl 5.2-13.1	PDW % 5.7-23.9
Working Group	1	3	81	6.5	13	52	49	14	25	900	7.5	10.5
	2	9	74	8.9	15	49	50	13	33	855	6.9	10.9
	3	5	75	8.5	17	48	51	14	32	711	6.0	8.9
	4	4	80	6.6	14	49	50	15	30	749	7.9	8.4
	5	8	81	7.0	13	65	48	10	33	859	4.6	8.6
	6	9	83	7.4	16	54	54	12	26	905	5.9	8.9
	7	11	81	8.0	14	52	52	16	28	658	10.0	7.5
	8	13	79	8.2	17	48	55	14	31	489	13.0	5.9
	9	11	81	9.1	18	41	56	15	29	694	11.0	6.0
	10	10	66	9.6	14	39	50	14	34	785	10.4	6.2
	11	8	70	8.9	15	58	48	15	34	985	9.8	21.4
	12	9	65	8.8	16	55	49	14	33	845	8.4	18.0
	13	11	85	8.4	12	62	55	14	36	862	9.6	15.5
	14	12	82	5.5	11	54	52	15	35	842	8.5	16.4
	15	8	83	6.5	10	58	51	16	32	805	8.6	14.7
	16	5	84	6.0	16	46	55	18	34	745	8.7	13.8
	17	8	82	9.0	15	45	54	14	29	843	8.0	10.9
	18	10	86	9.4	16	50	53	16	35	849	7.4	15.5
	19	11	79	9.3	15	52	52	14	34	822	12.1	14.3
	20	8	72	9.4	14	56	51	14	29	847	6.9	27.1
Control Group	1	9	77	8.7	15	54	54	14	29	852	11.5	13.4
	2	7	69	8.1	10	53	57	13	30	842	7.7	11.9
	3	5	74	8.0	13	58	52	12	31	904	9.1	21.7
	4	11	74	8.0	10	45	54	11	34	608	10.9	11.4
	5	9	79	6.9	12	56	55	15	33	588	8.8	15.7

Table 6. Animal Clinical Observations

Clinical Observation	Observed Symptoms
Respiratory	Normal
Motor Movements	Normal
Convulsion	No
Reflexes	Normal
Eye-Related Symptoms	The eye is normal in structure and movement.
Cardiovascular Symptoms	Not observed
Salivation	Not Available
Pyloerection	Not Occured
Analgesia	Not Occured
Muscle Tone	Normal
Gastrointestinal	Stool consistency and color are normal
Skin	Edema and erythema did not occur
Mortality	No

6.1. Histopathology Results

Materials and Methods: Sections of 5 micrometer thickness were taken from each organ block. In order to detect the lesions in the sections, deep sections of the blocks were taken. Routine Hematoxylin-Eosin Staining technique was first used for examinations under the light microscope. Afterwards, different staining techniques were used to confirm the histopathological evaluations. These techniques are Periodic Acid Schiff and Trichrom Masson techniques. Periodic Acid Schiff + Hematoxylin (PAS) technique shows the differentiation of the basement membrane, which provides the separation of epithelial tissue, which is one of the 4 basic tissues, and connective tissue histologically. It also provides the determination of polysaccharides in the tissue. These polysaccharides are found in heart muscle, kidney tissue, and testicles. Trichrom Masson, on the other hand, shows the differentiation of the fibrous structure in the connective tissue. While histopathological evaluation of organs was made under the light microscope, each organ was evaluated with different parameters and scoring.

6.1.1. Evaluation

As a result of the studies, no abnormal morphological structure was found in the brain, heart, kidney, liver, lungs and bronchi, trachea, spleen, eyebrow, border, skin, vein, bone marrow, esophagus stomach, cecum, colon, duodenum, ileum, jejunum, rectum, bladder, thymus, uterus, vagina, epididymis, gallbladder, testes, sternum, spinal cord, seminal sacs, salivary glands, prostate, pituitary gland, parathyroid, ovaries, lymph nodes, eyes and adrenal glands of the animals in the control and test groups.

6.1.2. Necropsy Findings

No negative findings were found in the necropsy performed in the test group and control group animals euthanized by cervical dislocation.

7. RESULT

In line with the results obtained, the product with lot number AVAS012023-1 was tested according to the method in the TS EN ISO 10993-11 Systemic Toxicity Tests - Subchronic Systemic Toxicity Test document and **it was determined that it did not cause any toxicity.**

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

Responsible for Laboratory *Laboratuvar Sorumlusu*	Laboratory Manager *Laboratuvar Müdürü*	Sample Acceptance Officer *Numune Kabul Sorumlusu*
Vet. Hek. Simge GARLI	Erol ÜSTÜN	Gonca AÇILMIŞ

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14.04.2023