TÜBİTAK
MAM

THE SCIENTIFIC AND TECHNOLOGICAL RESEARCH COUNCIL OF TURKEY
MARMARA RESEARCH CENTER
GENETIC ENGINEERING AND BIOTECHNOLOGY INSTITUTE
P.O. Box 21, 41470 GEBZE – KOCAELİ / TURKEY
T +90 262 677 20 00 F +90 262 641 23 09
http://www.mam.gov.tr

ANALYSIS REPORT

(Industrial Services)

Report no : B.14.2.TBT.5.01.13.00-181.06.03-345/**11847**

Report date : **12.07.2012**

Requested by : MODE MEDİKAL SAN. TİC. LTD. ŞTİ.

Address : ABDİ İPEKÇİ CAD. NO:58, BAYRAMPAŞA/İSTANBUL

Subject : CYTOTOXICITY, IRRITATION, ACUTE SYSTEMIC TOXICITY, SUBCHRONIC SYSTEMIC TOXICITY, SENSITIZASTION, IMPLANTATION AND GENOTOXICITY TESTS OF "DENTAL IMPLANT" 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 29 pages.

TÜBİTAK
MAM

Report no	: B.14.2.TBT.5.01.13.00-181.06.03-345/**11847**
Requested by	: MODE MEDİKAL SAN. TİC. LTD. ŞTİ.
Address	: ABDİ İPEKÇİ CAD. NO:58, BAYRAMPAŞA/İSTANBUL

Sample	: Single type sample	**Expiry date**	: --
Number of samples	: 85 pieces	**Institute sample register no**	: 12/1194-GMBE
Sample handling	: Packaged in different numbers and are provided in non-sterile form.	**Reception date and time**	: 29.05.2012
Condition of sample at reception: Packaged in different numbers and are provided in non-sterile form.		**Date of the analysis**	: 01.06.2012-11.07.2012

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

1- Samples

According to the biocompability test procedures cytotoxicity, irritation, acute systemic toxicity, subchronic systemic toxicity, sensitization, implantation and genotoxicity tests were carried out on 85 sterile samples which is defined as "Dental Implant".

Table 1. Tested product.

Sample	Sample properties	Number of samples
Dental Implant	The product looks like a screw form and it has matt color. It is inserted into the patient's jawbone with surgical operation to remove the missing teeth and remains in the body for prolonged periods.	85

Notes:

Authorized Signatures:

53192

P.O. Box 21, 41470 GEBZE – KOCAELİ / TURKEY
T +90 262 677 20 00 F +90 262 641 23 09
http://www.mam.gov.tr

Report no : B.14.2.TBT.5.01.13.00-181.06.03-345/**11847**

2- Test for irritation

The irritation test was carried out considering "ISO 10993-10:2010 Tests for irritation and delayed-type hypersensitivity", "ISO 10993-2: 2006 Animal welfare requirements" and "ISO-10993-12:2012 Sample preparation and reference materials" standart protocols.

Three healty young adult female New Zelland albino rabbits were used for irritation test. All three animals were heavier than 2 kg. As described in the ISO 10993-10:2010 standart protocol, the tested product was directly applied to the application sites as shown in Figure 1.

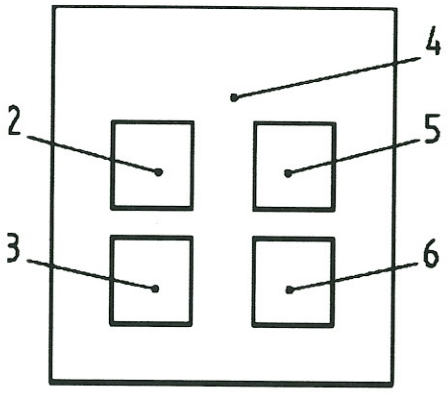

Figure 1. 2; test sample site, 3; negative conrol site, 5; test sample site, 6; positive control site, 4; cranial end

Positive Control

90% lactic acid was used as positive control (6). Its appropriate or reactive response was previously shown in the test system.

Negative Control

Physiological saline was used as negative control (3). Its appropriate negative or nonreactive response was previously shown in the test system.

Notes:

Authorized Signatures: **53301**

P.O. Box 21, 41470 GEBZE – KOCAELİ / TURKEY
T +90 262 677 20 00 F +90 262 641 23 09
http://www.mam.gov.tr

Report no : B.14.2.TBT.5.01.13.00-181.06.03-345/**11847**

Test Procedure

The backs of the animals (a sufficient distance on both sides of the spine) were shaved to obtain enough application area. The test and control samples were applied as shown in Figure 1. As explained in ISO 10993-10:2010 standart protocol, the application sites were covered with absorbent gauze patch and then wraped with an elastic bandage for 4 hours. At the end of contact time, the dressings were removed and the positions of the sites were marked. After that, the appearance of each application site was observed and recorded at 1h, 24h, 48h and 72h following removal of the patches. Observations were scored as described in Table 2. The results of evaluation based on the observation scores are presented in Table 3.

Table 2. Scoring system for skin reaction.

Reaction	Primary Irritation Score
Erythema and eschar formation	
No erythema	0
Very slight erythema (barely perceptible)	1
Well-defined erythema	2
Moderate erythema	3
Severe erythema (beet-redness) to eschar formation preventing grading of erythema	4
Oedema formation	
No oedema	0
Very slight oedema (barely perceptible)	1
Well-defined oedema (edges of area well-defined by definite raising)	2
Moderate oedema (raised approximately 1mm)	3
Severe oedema (raised more than 1 mm and extending beyond exposure area)	4
Total possible score for irritation	8
Other adverse changes at the skin shall be recorded and reported.	

Table 3. Irritation response categories in rabbit.

Mean Score	Response Category
0 - 0,4	Negligible
0,5 - 1,9	Slight
2 - 4,9	Moderate
5 - 8	Severe

Notes:

Authorized Signatures: 53301

P.O. Box 21, 41470 GEBZE – KOCAELİ / TURKEY
T +90 262 677 20 00 F +90 262 641 23 09
http://www.mam.gov.tr

Report no : B.14.2.TBT.5.01.13.00-181.06.03-345/**11847**

Evaluation of results

The application sites were examined with Binocular Loupes (3X). The observation scores and mean scores for samples were represented in Table 4 and Table 5, respectively.

Table 4. Scores of observations.

Animal ID	Samples	Application Sites	Observations (h)							
			Erythema				Oedema			
			1.	24.	48.	72.	1.	24.	48.	72.
1	*Dental Implat*	*Left Front Site*	0	0	0	0	0	0	0	0
		Right Front Site	0	0	0	0	0	0	0	0
	Positive Control	*Right Back Site*	3	3	3	4	3	4	4	4
	Negative Control	*Left Back Site*	0	0	0	0	0	0	0	0
2	*Dental Implant*	*Left Front Site*	0	0	0	0	0	0	0	0
		Right Front Site	0	0	0	0	0	0	0	0
	Positive Control	*Right Back Site*	3	3	3	3	3	3	3	3
	Negative Control	*Left Back Site*	0	0	0	0	0	0	0	0
3	*Dental Implant*	*Left Front Site*	0	0	0	0	0	0	0	0
		Right Front Site	0	0	0	0	0	0	0	0
	Positive Control	*Right Back Site*	4	4	4	4	3	3	3	3
	Negative Control	*Left Back Site*	0	0	0	0	0	0	0	0

Table 5. Scores for test and control samples.

Samples	Application Site	Erythema	Oedema	Total
Dental Implant	Left Front Site	0	0	0
	Right Front Site	0	0	0
Positive Control	Right Back Site	3,41	3,25	6,66
Negative Control	Left Back Site	0	0	0

Notes:

Authorized Signatures: **53301**

P.O. Box 21, 41470 GEBZE – KOCAELİ / TURKEY
T +90 262 677 20 00 F +90 262 641 23 09
http://www.mam.gov.tr

Report no : B.14.2.TBT.5.01.13.00-181.06.03-345/**11847**

Conclusion

As mentioned, after the observations at the four time points for the two criterions (Table 4), *average score* was ocalculated (Table 5). In the observations of the tested material, in any application sites, erythema and oedema formations were not observed. According to data obtained from observations and the evaluation criterias defined in the ISO 10993-10:2010, **the tested sample defined as "Dental Implant" has no skin irritation effect.**

Notes:

Authorized Signatures: **53301**

P.O. Box 21, 41470 GEBZE – KOCAELİ / TURKEY
T +90 262 677 20 00 F +90 262 641 23 09
http://www.mam.gov.tr

Report no: B.14.2.TBT.5.01.13.00-181.06.03-345/**11847**

3- Acute Systemic Toxicity

Subchronic systemic toxicity test was carried out taking into account the following standards; "ISO 10993-11: 2006 Biological evaluation of medical device: Systemic toxicology", "ISO 10993-2: 2006 Animal welfare requirements" and "ISO 10993-12: 2012 Sample preparation and reference materials".

Acute systemic toxicity test in an proposed animal model provides information about single or multiple exposure in 24 hours to potential harmful effects of the medical devices, materials and / or their extracts.

Sample Preparation

According to the "ISO 10993-12: 2012 Biological evaluation of medical devices: sample preparation" protocol, extract was prepared due to the properties of the test sample and its solid form. As explained in Section 10.3.1 (ISO 10993-12: 2012) extract was prepared at 37°C for 72 hours incubation and extraction volume was defined as 0,2 g/ml weight/volume ratio (ISO 10993-12: 2012, Section 10.3.3).

Experimental Animals

Species	Mouse	
Race	CD1	
Gender	Male	
Number of Animals	Test	5
	Control	5
Age	8-10 weeks	
Housing	individual housing in conventional euro type-1 cage	

Initial Weight (g)

ID	Weight (g)		ID	Weight (g)
Test 1	20,0		**Control 1**	21,2
Test 2	21,2		**Control 2**	22,0
Test 3	22,2		**Control 3**	22,0
Test 4	18,6		**Control 4**	21,4
Test 5	23,2		**Control 5**	20,8

Notes:

Authorized Signatures:

53301

P.O. Box 21, 41470 GEBZE – KOCAELİ / TURKEY
T +90 262 677 20 00 F +90 262 641 23 09
http://www.mam.gov.tr

Report no: B.14.2.TBT.5.01.13.00-181.06.03-345/**11847**

Test Procedure

Dose	50 ml/kg	
Route of administration	Oral gavage	
Housing Conditions	Temperature	22 ± 3 °C
	Humidity	%40-70
	Photoperiod	12:12h light-dark
Feeding	Standardized commercial laboratory diet was used with an unlimited supply of drinking water.	

Test Results

Test	Initial Weight (g)	Final Weight (g)	Weight Cahnge (%)*
Test 1	20,0	20,1	0,50
Test 2	21,2	21,4	0,94
Test 3	22,2	22,4	0,90
Test 4	18,6	20,0	7,53
Test 5	23,2	23,8	2,59
Control 1	21,2	22,6	6,60
Control 2	22,0	22,6	2,73
Control 3	22,0	24,4	10,91
Control 4	21,4	21,6	0,93
Control 5	20,8	19,8	-4,81

* Weight loss is evaluated as clinical sign if it is ≥%10.

- Feed and water consumption of all groups were normal.
- According to the observetions, abnormal weight changes were not recorded for the test and control mice (over than %10).

Clinical Observations and Evaluation

Systemic effects were followed according to clinical observation criteria in Table 6 as defined in the ISO 10993-11 and ISO 10993-12 protocols. No clinical sign was detected in individuals of all groups.

Notes:

Authorized Signatures:

53301

P.O. Box 21, 41470 GEBZE – KOCAELİ / TURKEY
T +90 262 677 20 00 F +90 262 641 23 09
http://www.mam.gov.tr

Report no: B.14.2.TBT.5.01.13.00-181.06.03-345/**11847**

Table 6. Observation and evaluation criteria.

Clinical Observation	Observed Sign	Involved Systems
Respiratory	Dyspnea (abdominal breathing, gasping), apnoea, cyanosis, tachypnea	Central nervous system (CNS), pulmonary, cardiac
Motor activities	Decrease/increase somnolence, anaesthesia, ataxia, unusual locomotion, tremors	CNS, somatomotor, sensory, neuromuscular, autonomic
Convulsion	Clonic, tonic, tonic-clonic, asphyxial, opisthotonos	CNS, neuromuscular, autonomic, respiratory
Reflexes	Corneal, righting, myotact, light, startle reflex	CNS, sensory, autonomic, neuromuscular
Ocular signs	Lacrimation, miosis, mydriasis, exophthalmos, ptosis, opacity, iritis, conjunctivitis, chromodacryorrhea	Autonomic, irritation
Cardiovascular signs	Bradycardia, tachycardia, arrhythmia, vasodilation, vasoconstriction	CNS, autonomic, cardiac, pulmonary
Salivation	Excessive	Autonomic
Piloerection	Rough hair	Autonomic
Analgesia	Decrease reaction	CNS, sensory
Muscle tone	Hypotonia, hypertonia	Autonomic
Gastrointestinal	Soft stool, diaorrhea, emesis, diuresis, rhinorrhea	CNS, autonomic, sensory, GI motility, kidney
Skin	Edema, Erythema	Tissue damage, irritation

Conclusion

Acute systemic toxicity test was carried out by using the extract of the test material. After 72 hours observation period, test was terminated and animals were evaluated by different biological parameters. The results of evaluations including clinical observation and gross pathology showed that the tested product which is named as "**Dental Implant**" **has no acute systemic toxicity effect according to the ISO 10993-11: 2006 standart test protocol.**

Notes:

Authorized Signatures:

53301

P.O. Box 21, 41470 GEBZE – KOCAELİ / TURKEY
T +90 262 677 20 00 F +90 262 641 23 09
http://www.mam.gov.tr

Report no: B.14.2.TBT.5.01.13.00-181.06.03-345/**11847**

4- Subchronic Systemic Toxicity

Subchronic systemic toxicity test was carried out taking into account the following standards; "ISO 10993-11:2006 Biological evaluation of medical device: Systemic toxicology", "ISO 10993-2:2006 Animal welfare requirements" and "ISO 10993-12:2012 Sample preparation and reference materials".

Repeated exposure subacute systemic toxicity test provides information on health hazards likely to arise from a prolonged exposure. It might also provide information on the mode of toxic action, toxic effects, target organs, reversibility or other effects of a substance by the intended clinical exposure route.

Sample Preparation

According to the "ISO 10993-12:2012 Biological evaluation of medical devices: sample preparation" protocol, extract was prepared due to the properties of the test sample and its solid form. As explained in Section 10.3.1 (ISO 10993-12) extract was prepared at 37°C for 72 hours incubation and extraction volume was defined as 1,25 cm^2/ml surface area/volume ratio (ISO 10993-12, Section 10.3.3. Table 1).

Experimental Animals

Species	Mouse			
Race	CD1			
Number of Animals	Female		Male	
	Test:10	Control:5	Test:10	Control:5
Age	8-10 weeks			
Housing Conditions	individual housing in conventional euro type-1 cage			
	Temperature		22 ± 3 °C	
	Humidity		%40-70	
	Photoperiod		12:12h light-dark	
Feeding	Standardized commercial laboratory diet was used with an unlimited supply of drinking water.			

Notes:

Authorized Signatures:

53067 53605 53006 53301

P.O. Box 21, 41470 GEBZE – KOCAELİ / TURKEY
T +90 262 677 20 00 F +90 262 641 23 09
http://www.mam.gov.tr

Report no: B.14.2.TBT.5.01.13.00-181.06.03-345/**11847**

Test Procedure

Dose	50 ml/kg	
Route of administration	Oral gavage	
Housing Conditions	Temperature	22 ± 3 °C
	Humidity	%40-70
	Photoperiod	12:12h light-dark
Feeding	Standardized commercial laboratory diet was used with an unlimited supply of drinking water.	

4.1. Clinical Observations and Evaluation

Systemic effects were followed according to clinical observation criteria in Table 7 as defined in the ISO 10993-11:2006 and ISO 10993-12:2012 protocols. No clinical sign was detected in individuals of all groups.

Table 7. Common clinical signs and observations.

Clinical Observation	Observation	Involved system(s)
Respiratory Dyspnea (abdominal breathing, gasping),	Respiratory Dyspnea (abdominal breathing, gasping),	Respiratory Dyspnea (abdominal breathing, gasping),
apnoea, cyanosis, tachypnea, nostril discharges CNS, pulmonary, cardiac	apnoea, cyanosis, tachypnea, nostril discharges CNS, pulmonary, cardiac	apnoea, cyanosis, tachypnea, nostril discharges CNS, pulmonary, cardiac
Motor activities	Motor activities	Motor activities
Decrease/increase somnolence, loss of righting,	Decrease/increase somnolence, loss of righting,	Decrease/increase somnolence, loss of righting,
anaesthesia, catalepsy, ataxia, unusual	anaesthesia, catalepsy, ataxia, unusual	anaesthesia, catalepsy, ataxia, unusual
locomotion, prostration, tremors, fasciculation	locomotion, prostration, tremors, fasciculation	locomotion, prostration, tremors, fasciculation
CNS, somatomotor, sensory,	CNS, somatomotor, sensory,	CNS, somatomotor, sensory,
neuromuscular, autonomic	neuromuscular, autonomic	neuromuscular, autonomic
Convulsion Clonic, tonic, tonic-clonic, asphyxial,	Convulsion Clonic, tonic, tonic-clonic, asphyxial,	Convulsion Clonic, tonic, tonic-clonic, asphyxial,
opisthotonos	opisthotonos	opisthotonos
CNS, neuromuscular, autonomic,	CNS, neuromuscular, autonomic,	CNS, neuromuscular, autonomic,
respiratory	respiratory	respiratory

Notes:

Authorized Signatures:

53067 **53605** **53006** **53301**

The marked (*) analyses are accredited.
Analysis reports without authorized signature are not legitimate.

P.O. Box 21, 41470 GEBZE – KOCAELİ / TURKEY
T +90 262 677 20 00 F +90 262 641 23 09
http://www.mam.gov.tr

TÜBİTAK
MAM

Report no: B.14.2.TBT.5.01.13.00-181.06.03-345/**11847**

Initial Weight (g)

Male			
Test 1	25,2	**Control 1**	20,0
Test 2	24,4	**Control 2**	25,0
Test 3	22,6	**Control 3**	25,4
Test 4	26,4	**Control 4**	26,2
Test 5	25,6	**Control 5**	27,8
Test 6	23,2		
Test 7	26,2		
Test 8	25,4		
Test 9	25,2		
Test 10	25,8		

Female			
Test 1	20,0	**Control 1**	21,2
Test 2	21,2	**Control 2**	22,0
Test 3	22,2	**Control 3**	22,0
Test 4	18,6	**Control 4**	21,4
Test 5	23,2	**Control 5**	20,8
Test 6	25,2		
Test 7	19,6		
Test 8	23,0		
Test 9	19,8		
Test 10	23,4		

Notes:

Authorized Signatures:

53067 **53605** **53006** **53301**

The marked (*) analyses are accredited.
Analysis reports without authorized signature are not legitimate.

This report is prepared as two originals (one for the customer, one for the institute archives) and contains 29 pages.

P.O. Box 21, 41470 GEBZE – KOCAELİ / TURKEY
T +90 262 677 20 00 F +90 262 641 23 09
http://www.mam.gov.tr

Report no: B.14.2.TBT.5.01.13.00-181.06.03-345/**11847**

Daily Body Weight Records

Table 8. Body weight records of animals.

Group	Test	Initial Weight (g)	Final Weight (g)	Weight Change Rate (%)*	Liver Weight (g)	Liver Weight Rate Index** (%)
Male	Test 1	25,2	28,6	13,49%	1,48	5,17
	Test 2	24,4	24,2	-0,82%	1,28	5,29
	Test 3	22,6	27,8	23,01%	1,56	5,61
	Test 4	26,4	29,0	9,85%	1,64	5,66
	Test 5	25,6	24,4	-4,69%	1,25	5,12
	Test 6	23,2	33,0	42,24%	1,92	5,82
	Test 7	26,2	29,2	11,45%	1,32	4,52
	Test 8	25,4	28,0	10,24%	1,21	4,32
	Test 9	25,2	24,4	-3,17%	1,26	5,16
	Test 10	25,8	26,2	1,55%	1,44	5,50
	Control 1	20,0	25,0	25,00%	1,19	4,76
	Control 2	25,0	33,2	32,80%	1,79	5,39
	Control 3	25,4	25,0	-1,57%	1,2	4,80
	Control 4	26,2	33,8	29,01%	1,72	5,09
	Control 5	27,8	31,4	12,95%	1,61	5,13
Female	Test 1	20,0	25,6	28,00%	1,28	5,00
	Test 2	21,2	23,2	9,43%	1,16	5,00
	Test 3	22,2	25,6	15,32%	1,14	4,45
	Test 4	18,6	24,6	32,26%	1,16	4,72
	Test 5	23,2	24,2	4,31%	1,2	4,96
	Test 6	25,2	27,4	8,73%	1,23	4,49
	Test 7	19,6	25,0	27,55%	1,27	5,08
	Test 8	23,0	24,0	4,35%	1,34	5,58
	Test 9	19,8	25,4	28,28%	1,39	5,47
	Test 10	23,4	19,2	**-17,95%**	0,97	5,05
	Control 1	21,2	23,0	8,49%	0,94	4,09
	Control 2	22,0	27,2	23,64%	1,32	4,85
	Control 3	22,0	28,4	29,09%	1,38	4,86
	Control 4	21,4	26,4	23,36%	1,11	4,20
	Control 5	20,8	24,8	19,23%	1,12	4,52

Notes:

Authorized Signatures:

53067 53605 53006 53301

The marked (*) analyses are accredited.
Analysis reports without authorized signature are not legitimate.

P.O. Box 21, 41470 GEBZE – KOCAELİ / TURKEY
T +90 262 677 20 00 F +90 262 641 23 09
http://www.mam.gov.tr

* ≥ 10% weight loss is considered as clinical sign.
** liver weight body mass index should be between 4-6%.

Weight Assesments:
Body weight changes were within normal limits except DT10 individual, normal liver weight rate index was observed in all groups.

- Feed and water consumption of all groups were normal.
- Weight changes were within normal limits except DT10 individual.

Gross Pathology:
At the end of the observation period, gross pathology examination was carried out and observations were reported as follows;

Female Test 5	Emphysema in the lungs and atrophy in spleen.
Female Test 6	Anemia in lung, yellow spot due to inflammation in a small lobe of liver.
Male Test 9	Atrophy, edema and inflammation in right kidney.
Male Test 10	Emphysema in a lobe of lung.

4.2. Haematology
Haematological measurements were achieved by using veterinary haematology analyzer (Mindray BC2800 Vet). 30 (20 test and 10 control) mice were tested for following six parameters;

1) *RBC: Red Blood Cell*
2) *WBC: White Blood Cell*
3) *HGB: Haemoglobin*
4) *HCT: Haematocrit*
5) *MCHC: Mean Corpuscular Hemoglobin Concentration*
6) *PLT: Platelet*

There were no significantly differences between test and control groups for RBC, WBC, HGB, HCT, MCHC and PLT parameters ($p<0.05$). When male and female mice were separately analyzed, significant differences were recorded between male control and male test groups for red blood cell concentration (RBC), hemoglobin (HGB) and hematocrit (HCT) parameters.

Notes:

Authorized Signatures:

53067	53605	53006	53301

P.O. Box 21, 41470 GEBZE – KOCAELİ / TURKEY
T +90 262 677 20 00 F +90 262 641 23 09
http://www.mam.gov.tr

In male test mice, RBC, HGB and HCT values were higher than male control mice (p<0,05). However these values were still within the reference limits for mouse (Loeb and Quimby, 1999). These differences were not recorded between female test and control animals. When all test and control animals were analyzed together, there were no significant deifferences for any parameters. Statistical analyses were performed in 95% confidence interval (p < 0.05 [p | 1 ≡ %100]) and using the ***IBM SPSS*** software.

4.3. Clinical Chemistry

Clinical biochemical determination on blood was achieved by using semi automatic clinical chemistry analyzer (Mindray BA-88A). 15 (10 test, 5 control mice) mice were tested for following four parameters;

1) Alanine Transaminase (ALT)
2) Aspartate Transaminase (AST)
3) LDH: *Laktat dehidrogenaz*
4) *ALT/AST Oranı*

There were no significantly differences between test and control groups for all investigated parameters (p<0.05). Statistical analyses were performed in 95% confidence interval (p < 0.05 [p | 1 ≡ %100]) and using the ***IBM SPSS*** software.

4.4. Urine Analysis

Urine analysis on urine samples was achieved by using URIT-50 urine analyzer. 24 (18 test, 6 control mice) mice were tested for following parameters; leukocyte, ketone, urobilinogen, bilirubin, specific gravity, blood, protein, glucose, nitrite and pH. Bacteriological examination, staining, or precipitation processes have not been applied. Microscopic examination was carried on directly on urine samples and some crystals, somatic cell structures have been identified. There were no significantly differences between test and control groups for all investigated parameters (p<0.05). Statistical analyses were performed in 95% confidence interval (p < 0.05 [p | 1 ≡ %100]) and using the ***IBM SPSS*** software.

Microscopic Examination: Urine samples collected on the sterile bacteriological petri dish for microscopic examination. After that, urine samples were applied to the slide-lamella and examined immediately. Sedimentation or centrifugation was not applied to the urine samples. In urine samples of the test and control mice, sodium urat, calcium sulfate and uric acid crystals were observed. As a result of microscopic examination any abnormal findigs were observed in the test and control animals.

Notes:

Authorized Signatures:

53067 **53605** **53006** **53301**

P.O. Box 21, 41470 GEBZE – KOCAELİ / TURKEY
T +90 262 677 20 00 F +90 262 641 23 09
http://www.mam.gov.tr

Report no: B.14.2.TBT.5.01.13.00-181.06.03-345/**11847**

4.5. Histopathological Evaluation

After the 28 days of the test period, lung, liver, kidney and spleen tissue samples were taken from both control and test animals. Tissue samples were fixed with 10% neutral formalin, dehydrated in increasing alcohol series (70, 80, 90 and 100%) and xylene, prior to embedding in paraffin wax. Sections (5 µm thick) were obtained using a microtome and stained with hematoxylin–eosin. Before staining, sections were deparaffinizated with xylene and rehydrated in a series of alcohol solutions of decreasing concentration. Sections were stained with hematoxylin for 5 minutes and then rinsed in running tap water. After washing; they stained with eosin for 5 minutes and rinsed in running tap water. The stained sections were dehydrated, cleared and mounted.

Analysis was done as explained in the ISO 10993-11:2006 test protocol. According to the results of histopathological examinations the male and female control mice showed normal morphology. Normal histopathological findings were observed for the female test animals, except for two mice. DT5 mouse has normal morphology for the liver, kidney and spleen. However, emphysema and increased lymphocyte on the lung were observed. Lymph nodes around the respiratory bronchioles or alveoli and structural abnormalities have been identified. Normal morphoogy was observed for the kidney and spleen of the DT6 mouse. However, emphysema, increased lymphocytes and structural deformation in hepatocytes were observed in a lobe of liver tissue. Normal morphology was observed in orther lobes of liver of DT6. Alveolar contraction, slight fibrosis, elevated lymphocytes, lymph nodes around the respiratory bronchioles were observed in the lung of the same mouse.

Normal histopathological findings were observed for the female test animals, except for two mice. Edema and structural abnormalities were observed on the right kidney of the ET9 mouse. Reduction in the number of glomeruli, structural abnormalities in cortex and increase in lymphocytes were identified for this animal. The number of glomeruli was normal in the left kidney. However, the number of proximal and distal tubules was decreased, intensive emphysema and increased number of lymphocytes in the medulla was determined. Alveolar contraction and fibrosis were observed in the lung tissue. Increased number of lymphocytes, emphysema and alveolar contraction were observed in the lung tissue of the ET10 mouse.

When the all findings are eveluated together; anomalies of the liver of DT6 mouse and the kidney tissues of ET10 mouse is thought that they arising from individual reasons due to they have not be seen in other animals. In 80 % of the male and females test animals lung tissues were normal.

Notes:

Authorized Signatures:

53067 **53605** **53006** **53301**

P.O. Box 21, 41470 GEBZE – KOCAELİ / TURKEY
T +90 262 677 20 00 F +90 262 641 23 09
http://www.mam.gov.tr

4.6. Conclusion

Repeated dose subacute systemic toxicity test was carried out by using the extract of the test material. After 28 day observation period, test was terminated and animals were evaluated by different biological parameters. The results of evaluations including clinical observation, haematology, clinical chemistry, gross pathology and histopathological examinations showed that the tested product which is named as **"Dental Implant" has no subchronic systemic toxicity effect according to the ISO 10993-11: 2006 standart test protocol.**

Notes:

Authorized Signatures:

53067 **53605** **53006** **53301**

P.O. Box 21, 41470 GEBZE – KOCAELİ / TURKEY
T +90 262 677 20 00 F +90 262 641 23 09
http://www.mam.gov.tr

Report no: B.14.2.TBT.5.01.13.00-181.06.03-345/**11847**

5- Implantation

Implantation test was performed according to "ISO 10993 Biological evaluation of medical device – Part 6: 2007 Tests for local effects after implantation", "ISO 10993-2 Biological evaluation of medical devices - Part 2: 2006 Animal welfare requirements" and "ISO 10993-12 Biological evaluation of medical devices - Part 12: 2012 Sample Preparation" standards.
The implantation test exhibits possible local effects of biomaterials after implantation and includes macroscopic and microscopic histological examinations.

Test Sample

Test samples were used in the original size they are sent. They are sterilized under the UV light for 30 minutes before the application.

Negative Control

According to the ISO 10993-12:2012 standart test protocol silicone was used as negative control. Control samples were sterilized under the UV light for 30 minutes before the application.

Application

Animal Species/Strain: Rat/Sprague Dawley
Number of Animals: 3
Age/Gender: 12-16 week-old/young adult female
Weight: 250-300 gr
Application Site: 10 pieces test samples and 3 negative control were implanted on lumbodorsal region of 3 animals.

5.1- Histopathological Examination

After the 28 days of the test period, tissue samples from 10 implantation site, 10 test-control sites and 3 negative controls were collected. Tissue samples were fixed with 4% paraformaldehyde. After fixation, samples were washed under running tap water for 8 hours and dehydrated in increasing alcohol series (70, 80, 90 and 100%) and xylene, prior to embedding in paraffin wax. Sections (5 µm thick) were obtained using a microtome and stained with hematoxylin–eosin. Before staining, sections were deparaffinizated with xylene and rehydrated in a series of alcohol solutions of decreasing concentration. Sections were stained with hematoxylin for 5 minutes and then rinsed in running tap water. After washing; they stained with eosin for 5 minutes and rinsed in running tap water. The stained sections were dehydrated, cleared and mounted. Tissue examinations were performed and evaluated according to Table 9.

Notes:

Authorized Signatures:

53067 **53301**

P.O. Box 21, 41470 GEBZE – KOCAELİ / TURKEY
T +90 262 677 20 00 F +90 262 641 23 09
http://www.mam.gov.tr

Report no: B.14.2.TBT.5.01.13.00-181.06.03-345/**11847**

Table 9. Histological evaluation system – cell type/response.

Cell Type/Response	Score				
	0	**1**	**2**	**3**	**4**
Polymorphonuclear cells	0	Rare,1-5/phf*	5-10/phf	Heavy infiltrate	Packed
Lymphocytes	0	Rare,1-5/phf	5-10/phf	Heavy infiltrate	Packed
Plasma cells	0	Rare, 1-5/phf	5-10/phf	Heavy infiltrate	Packed
Macrophages	0	Rare, 1-5/phf	5-10/phf	Heavy infiltrate	Packed
Giant cells	0	Rare,1-5/phf	5-10/phf	Heavy infiltrate	Packed
Necrosis	0	Minimal	Mild	Moderate	Severe
Neovascularisation	0	Minimal capillary proliferation, focal, 1-3 buds	Groups of 4-7 capillaries with supporting fibroblastic structures	Broad band of capillaries with supporting structurea	Extensize band of capillaries with supporting fibroblastic structures
Fibrosis	0	Narrow band	Moderat ely thick band	Thick band	Extensive band
Fatty infiltrate	0	Minimal amount of fat associated with fibrosis	Several layers of fat and fibrosis	Elongated and broad accumulation of fat cells about the implant site	Extensive fat completely surrounding the implant

* phf= per high powered (400x) field.

Notes:

Authorized Signatures:

53067 **53301**

P.O. Box 21, 41470 GEBZE – KOCAELİ / TURKEY
T +90 262 677 20 00 F +90 262 641 23 09
http://www.mam.gov.tr

Report no: B.14.2.TBT.5.01.13.00-181.06.03-345/**11847**

Table 10. Scoring system.

	Test Sample			Control Sample		
Animal Number	**1**	**2**	**3**	**1**	**2**	**3**
Inflammation	0	0	0	0	0	0
Polymorphonuclear	0	0	0	0	0	0
Lymphocytes	1	1	1	1	0	1
Plasma cells	0	0	0	0	0	0
Macrophages	0	0	0	0	0	0
Giant cells	0	0	0	0	0	0
Necrosis	0	0	0	0	0	0
SUB_TOTAL (x 2)	**1**	**1**	**1**	**1**	**0**	**1**
Neovascularisation	0	0	0	0	0	0
Fibrosis	0	0	1	0	0	0
Fatty infiltrate	0	0	0	0	0	0
SUB_TOTAL	0	0	1	0	0	0
TOTAL	**1**	**1**	**2**	**1**	**0**	**1**
GROUP TOTAL	**1.30**			**0.67**		
AVERAGE [a]			TEST (-) CONTROL =			
Traumatic necrosis	0	0	0	0	0	0
Foreign debris	0	0	0	0	0	0
No. sites examined	**3**	**3**	**4**	**3**	**3**	**4**

[a] Used to determine irritant ranking shown below as the conclusion. A negative difference is recorded as zero

Under the conditions of this study, the test sample was considered a
non- irritant (0,0 - 2,9)
slight irritant (3,0 - 8,9)
moderate irritant (9,0 - 15,0)
severe irrtiant (> 15)
to the tissue as compared to the negative control sample.

Notes:

Authorized Signatures:

53067 **53301**

The marked (*) analyses are accredited.
Analysis reports without authorized signature are not legitimate.

This report is prepared as two originals (one for the customer, one for the institute archives) and contains 29 pages.

P.O. Box 21, 41470 GEBZE – KOCAELİ / TURKEY
T +90 262 677 20 00 F +90 262 641 23 09
http://www.mam.gov.tr

Gross Pathology
Test and control animals were sacrificed by cervical dislocation at the end of the test procedure. Morfological abnormalities or necrotic regions were not observed in thorax and abdomen examinations.

Clinical Observations
No clinical sign was detected in test and control animals.

5.2- Results of Implantation Test
Clinical observations, gross pathology and histopatological examinations were performed for the evaluation of implantation test. The tested product was characterized according to observations and evaluation criteria. The irritation score of the application sites was recorded as "1.30" and according to ISO 10993-6: 2007 the tested product defined as **"Dental Implant" has not an irritation effect in implantation site.**

Notes:

Authorized Signatures:

53067 **53301**

The marked (*) analyses are accredited.
Analysis reports without authorized signature are not legitimate.

P.O. Box 21, 41470 GEBZE – KOCAELİ / TURKEY
T +90 262 677 20 00 F +90 262 641 23 09
http://www.mam.gov.tr

6- 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 72 hours and extraction ratio was defined as 0,2 g/ml weight/volume. Sensitization tests of samples were carried out using healthy young adult 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. Topical application was carried on non-injected site of the animal; left site of the animal at day 7 of test and right site of the animal at day 14 of test. The experimental procedure applied on animals was shown in Figure 2.

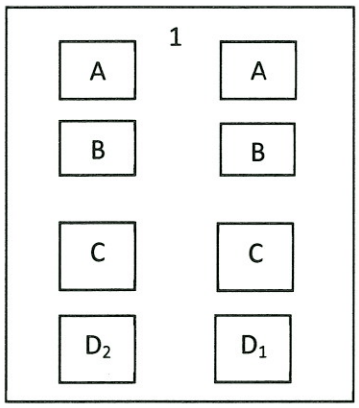

Figure 2.

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.

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).

Notes:

Authorized Signatures:

53006

53301

P.O. Box 21, 41470 GEBZE – KOCAELİ / TURKEY
T +90 262 677 20 00 F +90 262 641 23 09
http://www.mam.gov.tr

Negative control was carried out comparatively at 2 different sites at 2 different applications (Figure 3).

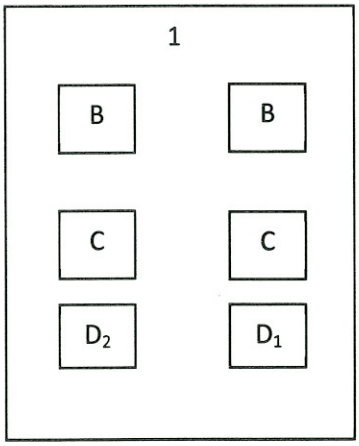

Figure 3.
1- Cranial end of the experimental animal.
B- 0,1 ml of serum physiologic.
C- (FCA) and serum physiologic mixed at 50:50 (volume ratio) was applied.
D- 0,3 ml of serum physiologic was applied on topical sites.

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 1 and in control animals as shown in Figure 2. 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.

Notes:

Authorized Signatures:

53006

53301

P.O. Box 21, 41470 GEBZE – KOCAELİ / TURKEY
T +90 262 677 20 00 F +90 262 641 23 09
http://www.mam.gov.tr

Report no: B.14.2.TBT.5.01.13.00-181.06.03-345/**11847**

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: Dental Implant

Table 11. 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

Mean Scores

Table 12. Mean score values.

Sample	Result
Dental Implant	0,5
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 11. In the evaluation of "Dental Implant" extraction applied group of animals, no visible skin reaction was detected and sensitization score was obtained as 0,5 (Table 12). Slightly hair loss was observed on the application site. 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.**

Notes:

Authorized Signatures:

53006 **53301**

P.O. Box 21, 41470 GEBZE – KOCAELİ / TURKEY
T +90 262 677 20 00 F +90 262 641 23 09
http://www.mam.gov.tr

Report no: B.14.2.TBT.5.01.13.00-181.06.03-345/**11847**

7- 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: 04-06-2012
End date of analysis: 08-06-2012

Definition of Samples: Sample of "Dental Implant" was defined as in section 1 and sample was supplied by "MODE MEDİKAL SAN. VE TİC.LTD.ŞTİ."

Cell line, justification of the choice and cell source: L929 mouse cell line was used as the test subject. This cell line is one of the recommended ISO 10993-5 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: DMEM/F12 (Gibco Cat # 32500-035, lot # 614238) was used as medium supplemented by %10 Fetal bovine serum (Biochrom AG Cat # S0115, lot # 0827H) + 100 U/100 µg/ml penicillin streptomycin (Biological Industries, Cat # 03-031-1C, lot # 655265).

Assay Method: Extraction Method was performed in sterile, chemically inert, closed containers by using aseptic techniques, in accordance with ISO 10993-12.

Rationale: To be able to analyze the cytotoxicity via dissociation of substances from tooth implant.

Extraction Method: "Tooth Implant" samples were extracted at 0.2 g/ml extraction ratio at 120 rpm, 37°C for overnight as required following ISO 10993-12 standards. The samples were sterilized by filter with 0.22 µm.

Cytotoxicity Method: The L929 cell line was seeded at a cell density of 1 X 10^4 cells/well into the 96-well plates and incubated at 37°C, %5 CO_2 for 24 hr. The following day, extracts of the samples and the controls were added to the 96-well plates and incubated at 37°C, %5 CO_2 for 24 hrs and then WST-1 was added into the each well at 10 % (v/v). After 1-3 hrs, the absorbance of the 96-well plate was measured at 450 nm by microplate reader.

Measure of Cytotoxicity: WST-1 Cell viability assay (Colorimetric)

Notes:

Authorized Signatures: **53687** **53618**

P.O. Box 21, 41470 GEBZE – KOCAELİ / TURKEY
T +90 262 677 20 00 F +90 262 641 23 09
http://www.mam.gov.tr

Report no: B.14.2.TBT.5.01.13.00-181.06.03-345/**11847**

Negative, positive and other controls

Controls:

Control 1: Culture medium incubated at %5 CO2, 37°C at 120 rpm 72 hrs under the same conditions of extraction.

Control 2: Fresh medium with no prior treatment. DMEM-F12 fresh.

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

Positive control: RAUMEDIC Tubings in silicone rubber grade RAUMEDIC PVC Org Sn

RESULTS

The results of the cell responses other observations were evaluated according to the Table 13. Qualitative morphological grading of cytotoxicity of 'Tooth Implant' extract.

Sample	Degree	Positive and negative controls	Degree
Dental Implant extract (72 hrs)	0	**Control 1,** DMEM-F12 fresh	0
		Control 2, DMEM-F12 ekstrakt	0
		Negative Control, RAUMEDIC-SIK 8363	0
		Positive Control, RAUMEDIC PVC Org Sn	4

Table 13. Qualitative morphological grading of cytotoxicity of dilutions

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.

Notes:

Authorized Signatures: **53687** **53618**

P.O. Box 21, 41470 GEBZE – KOCAELİ / TURKEY
T +90 262 677 20 00 F +90 262 641 23 09
http://www.mam.gov.tr

Report no : B.14.2.TBT.5.01.13.00-181.06.03-345/**11847**

The qualitative results showed that the potential toxicity of "Dental implant extracts" on L929 cell culture over 24 h exposure. The cytotoxicity of samples on the cell viability relative to control (DMEM-F12 extract) was found **102 ± 2,17%.**

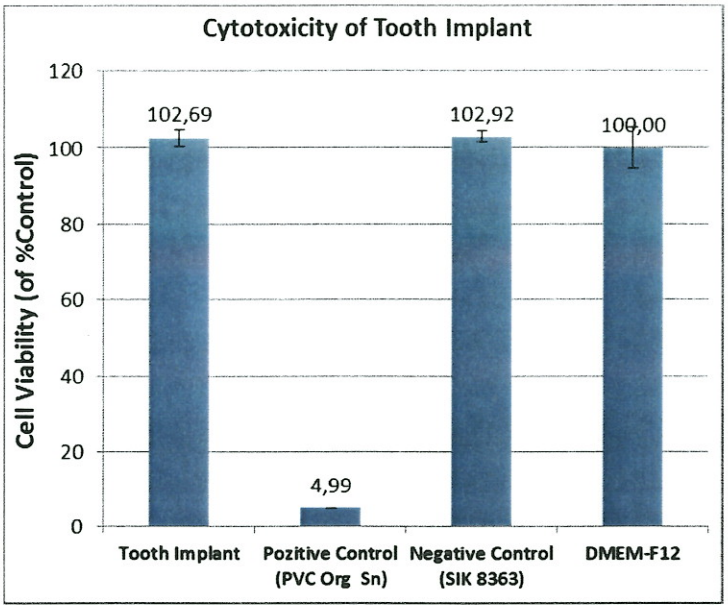

Figure 4. The percentage of cell viability relative to control over exposure for 24 hr to "dental implant extracts".

The cytotoxicity of 'dental implant extracts' were analyzed based on normalization to the % cell viability of control DMEM-F12 extract which was incubated at the same conditions with the samples without any treatment (Control 2).

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:
"Dental implant extracts" analyzed by following by WST-1 Cell viability assay (Colorimetric) **was found non-cytotoxic.**

Notes:

Authorized Signatures: **53687** **53618**

P.O. Box 21, 41470 GEBZE – KOCAELİ / TURKEY
T +90 262 677 20 00 F +90 262 641 23 09
http://www.mam.gov.tr

Report no: B.14.2.TBT.5.01.13.00-181.06.03-345/**11847**

8- Bacterial Reverse Mutation Test – AMES Test

AMES test was performed according to "ISO 10993-3 Biological evaluation of medical devices: Tests for genotoxicity (OECD 471), carcinogenicity and reproductive toxicity" and "ISO-10993-12 Sample preparation and reference materials" standards.

Positive Control

2-Aminoanthracene and **Sodium Azide** defined in the The MOLTOX Salmonella Mutagenicity Assay Kit were used as positive control.

Negative Control

PBS which was used in the sample preparation (extraction) was used as negative control.

Phenotypic Control of Strains

Phenotypic control of strains were performed using "Quad PC plate" provided in the kit. This plate has four different sectors:

Table 14. Expected Observations in the Sectors of Quad PC Plate

Sector	Expected Observation	Genotype
1	No growth (all strains)	*his-*
2	Zonal inhibition around CV disc (Rfa disc in the kit) (all strains)	*Rfa*
3	Growth (all strains)	pKM101
4	Only TA102 should grow	pAQ1(carries tetracycline resistance gene)

Each of the four sectors of Quad PC plate was inoculated with *S.typhimurium* TA100 strain using a "Z" inoculation pattern. After inoculation, CV disc was placed on the agar surface in sector 2 of the Quad PC plate. The plate was incubated at 37±1 °C for 48 hours.

Chemical Mutagenicity Control Test

LS9 rat liver extract was used in the metabolic activation of appropriate mutagens. LS9 (-) solvent sample, LS9 (+) solvent sample, LS9 (-) mutagen sample, LS9 (+) mutagen sample, LS9 (-) test sample and LS9 (+) test sample were prepared and poured onto the surface of minimal glucose plates. The plates were incubated at at 37±1 °C for 48 hours. Double plating was used.

Notes:

Authorized Signatures: **53644**

P.O. Box 21, 41470 GEBZE – KOCAELİ / TURKEY
T +90 262 677 20 00 F +90 262 641 23 09
http://www.mam.gov.tr

Report no: B.14.2.TBT.5.01.13.00-181.06.03-345/**11847**

Observation Results

Table 15. Observation results.

	LS9	Colony Number Plate #1	Colony Number Plate #2	Mean Number of Colonies
Sample	+	77	78	77
Sample	-	64	71	67
2-Aminoanthracene	+	584	320	452
2-Aminoanthracene	-	116	101	108
Sodium Azide	-	624	578	601
PBS	+	61	56	58
PBS	-	72	63	67

Conclusion

The number of colonies per plate was counted and the mean number of colonies was obtained (Table 5). According to the protocol and evaluation criteria defined in the ISO 10993-3 document and based on the test results, **the AMES test of the material known as "Dental Implant" gave negative (-) result. It was shown that test material is non-mutagenic in these tested conditions and in defined bacterial strain (*S.typhimurium* TA100).**

Notes:

Authorized Signatures: **53644**

P.O. Box 21, 41470 GEBZE – KOCAELİ / TURKEY
T +90 262 677 20 00 F +90 262 641 23 09
http://www.mam.gov.tr