Title: *Tests for Acute Systemic Toxicity of Finished Products* *Report Form*

Customer Code: M0230030201017/01
Form Code: F03-P45

Identification

Applicant	Rasa Salamat Arvand		
Address	National Institute of Genetic Engineering and Biotechnology, Pajoohesh Blvd.		
Product Name	Revaheal Sept		
Batch Number	02032331		
Date of Receipt	1403.02.01	**Test Duration**	5-10 Days
Date of Test	1403.06.01	**Number of Received items**	1 Sample
Date of Report	1403.06.04	**Number of Tested animals**	20 Repeats

Animal Management

Species	Mouse	**Body Weight Range**	25-30 g
Strain/Type	NMRI	**Number of Treated Animals (polar & non-polar extract)**	10
Sex	Male	**Number of Control Animals (polar & non-polar solvent)**	10

Dosage condition & Route of Administration

Examination type	Acute☒ Subacute ☐ Subchronic ☐ Chronic ☐
Administration site	Gavage☐ Subcutaneous ☐ Intramuscular☐ Intraperitoneal(non-polar)☒ Intravenous(polar) ☒
Dosage volumes (ml/kg)	(Intraperitoneal & Intravenous) 50
Rate of injection	1 ml/min
Study Design	Single Dose Administration

Animal Husbendary

Food	Standard pellet provided from the authorized supplier, and unlimited supply of drinking water
Housing	Healthy animals were acclimatized to the laboratory conditions before the treatment, and then they were housed in spatial cages identified by a card indicating the requiered data
Humidity	at least 30% and preferably not exceed 70%
Lighting	12 hours light, 12 hours dark
Temperature	22 ± 3^0C

Sample preparation

Extraction Time (hours)	72±2 h
Extraction Temperature (℃)	37±1 ℃
Extraction solvent	solution sodium chloride 0.9% (polar) & corn oil (non-polar)
Solvent type	Polar & Non-polar
Extraction method	Dynamic incubation
pH of the test sample	>2 & <11.5 (It was within the acceptable range.)
Negative control	sodium chloride 0.9% & corn oil
Carrier arrival	Hansen connector

021 91 30 9890 0992 60 62 450
Nikopharmed@gmail.com Nikopharmed.com

Description of product

Material composition: Material composition: This Wound antiseptic solution provides effective disifection while promoting tissue regeneration and accelerating the healing process. It contains silver, Known for its strong antibacterial properties, and chitosan, which enhances wound healing and tissue repair.

Procedure (Sample preparation)

- **Sample preparation was done based on the ISO 10993-12 standard method.**

Extraction vehicles:
Polar: sodium chloride 0.9%
Non-polar: Corn oil

Extraction: The extraction procedure was performed at extraction ratios of 0.2 g/ml.
For the extraction of the internal fiber of the product, and taking into account the absorbent nature of the test material, the water absorption capacity of the sample was first determined. Based on the calculated absorption, the extraction medium was applied at a ratio of 0.2 g/ml.

Extraction temperature and time: 37 ± 1 °C for 72±2 hours

- **Extraction method:** Dynamic incubation in an incubator
- **Extraction container:** Chemically inert, sterile, and tightly sealed
- **Post-extraction handling:** Extracts were collected aseptically and used directly for acute toxicity testing according to ISO 10993-11.

Procedure (Acute Toxicity test)

- **The in vivo tests for systemic toxicity was performed based on the ISO 10993-11 standard method.**

Evaluation criteria

1) If during the observation period of an acute systemic toxicity test none of the animals treated with the test sample shows a significantly greater biological reactivity than animals treated with the vehicle control, the sample meets the requirements of this test.
2) Using five animals, if two or more animals die, or if behavior such as convulsions or prostration occurs in two or more animals, or if a final (end of study) body weight loss greater than 10 % occurs in three or more animals, the sample does not meet the requirements of the test. Any transitory body weight loss should be critically evaluated along with other clinical observations in the assessment of systemic toxicity.
3) If any animals treated with the sample show only slight signs of biological reactivity, and not more than one animal shows gross symptoms of biological reactivity or dies, repeat the testing using groups of 10 animals.
4) On the repeat test, if all 10 animals treated with the sample show no scientifically meaningful biological reactivity above the vehicle control animals during the observation period, the sample meets the requirements of this test.

- The items required for performing acute systemic toxicity testing are shown in Table 3.

021 91 30 9890 0992 60 62 450
Nikopharmed@gmail.com Nikopharmed.com

Observation	Acute	Subacute/subchronic	Chronic [a]
Body weight change	+	+	+
Clinical observations	+	+	+
Clinical pathology	b	a, b	+
Gross pathology	b	+	+
Organ weights	b	+	+
Histopathology	b	a, b	+

\+ Data should be provided.

[a] Chronic systemic toxicity testing is generally a time extension of subacute/subchronic testing, justified by the human exposure period. Many of the same parameters are recorded and reported. Group sizes may be increased to include satellite groups for which some, or all, of these observations may be made.

[b] Consideration should be given to these measurements when clinically indicated or if longer exposure testing is not anticipated.

Table 3. Summary of observations

Test Results

Polar Extract											
Description	Acceptable limit	Animals No. & Groups									
		Treatments					**Controls**				
		1	2	3	4	5	1	2	3	4	5
A) Body Weight Changes											
Beginning of Test (g)	**25-30 (g)**	26.6	25.4	27.4	27.3	25.4	25.6	25.4	27.3	29.2	25.2
Day 1 (g)		27.1	26.6	28.0	28.2	26.3	27.1	26.5	27.8	28.5	25.7
Day 2 (g)		27.9	26.9	28.9	28.5	28.4	26.3	26.7	28.5	28.1	26.2
End of Test (Day 3 (g))		28.8	28.2	29.5	29.4	28.8	27.9	27.3	29.2	29.4	27.1
Mean	**±20%**	27.68					27.25				
± SD		1.18					1.27				
Description	Acceptable limit	Animals No. & Groups									
		Treatments					**Controls**				
		1	2	3	4	5	1	2	3	4	5
B) Clinical Observations											
First Day											
Respiratory	N*	N	N	N	N	N	N	N	N	N	N
Motor activities	N*	N	N	N	N	N	N	N	N	N	N
Ocular signs	N*	N	N	N	N	N	N	N	N	N	N
Cardiovascular signs	N*	N	N	N	N	N	N	N	N	N	N
Analgesia	N*	N	N	N	N	N	N	N	N	N	N
Gastrointestinal (Diarrhea, etc.)	N*	N	N	N	N	N	N	N	N	N	N
Convulsion	N*	N	N	N	N	N	N	N	N	N	N
Reflexes	N*	N	N	N	N	N	N	N	N	N	N
Salivation	N*	N	N	N	N	N	N	N	N	N	N

021 91 30 9890 0992 60 62 450
Nikopharmed@gmail.com Nikopharmed.com

The Third Day											
Respiratory	N*	N	N	N	N	N	N	N	N	N	N
Motor activities	N*	N	N	N	N	N	N	N	N	N	N
Ocular signs	N*	N	N	N	N	N	N	N	N	N	N
Cardiovascular signs	N*	N	N	N	N	N	N	N	N	N	N
Analgesia	N*	N	N	N	N	N	N	N	N	N	N
Gastrointestinal (Diarrhea, etc.)	N*	N	N	N	N	N	N	N	N	N	N
Convulsion	N*	N	N	N	N	N	N	N	N	N	N
Reflexes	N*	N	N	N	N	N	N	N	N	N	N
Salivation	N*	N	N	N	N	N	N	N	N	N	N
Piloerection	N*	N	N	N	N	N	N	N	N	N	N
Muscle tone	N*	N	N	N	N	N	N	N	N	N	N
Skin	N*	N	N	N	N	N	N	N	N	N	N
Death	-	-	-	-	-	-	-	-	-	-	-
N: No signs of Clinical Observations, **N.M**: Not Measured											
Piloerection	N*	N	N	N	N	N	N	N	N	N	N
Muscle tone	N*	N	N	N	N	N	N	N	N	N	N
Skin	N*	N	N	N	N	N	N	N	N	N	N
Death	-	-	-	-	-	-	-	-	-	-	-
N: No signs of Clinical Observations, **N.M**: Not Measured											
Second Day											
Respiratory	N*	N	N	N	N	N	N	N	N	N	N
Motor activities	N*	N	N	N	N	N	N	N	N	N	N
Ocular signs	N*	N	N	N	N	N	N	N	N	N	N
Cardiovascular signs	N*	N	N	N	N	N	N	N	N	N	N
Analgesia	N*	N	N	N	N	N	N	N	N	N	N
Gastrointestinal (Diarrhea, etc.)	N*	N	N	N	N	N	N	N	N	N	N
Convulsion	N*	N	N	N	N	N	N	N	N	N	N
Reflexes	N*	N	N	N	N	N	N	N	N	N	N
Salivation	N*	N	N	N	N	N	N	N	N	N	N
Piloerection	N*	N	N	N	N	N	N	N	N	N	N
Muscle tone	N*	N	N	N	N	N	N	N	N	N	N
Skin	N*	N	N	N	N	N	N	N	N	N	N
Death	-	-	-	-	-	-	-	-	-	-	-
N: No signs of Clinical Observations, **N.M**: Not Measured											

021 91 30 9890 0992 60 62 450
Nikopharmed@gmail.com Nikopharmed.com

C) Gross Pathology											
Groups		**Treatments**					**Controls**				
Animals No.		**1**	**2**	**3**	**4**	**5**	**1**	**2**	**3**	**4**	**5**
Abdominal Cavity	**N***	N	N	N	N	N	N	N	N	N	N
Thoracic Cavity		N	N	N	N	N	N	N	N	N	N
External surface of the body		N	N	N	N	N	N	N	N	N	N
All natural holes in the body (such as eyes, mouth, nose, anus)		N	N	N	N	N	N	N	N	N	N

N: No signs of Clinical Observations, **N.M**: Not Measured

D) Organ Weight: Since ISO 10993-11 applies the term "should" (recommendation) rather than "shall" (obligation), and considering the absence of mortality or clinical signs, together with the low systemic toxicological concern, the omission of histopathology, clinical biochemistry, and organ weight measurements in the acute, subacute, and sub chronic systemic toxicity studies is scientifically justified and remains compliant with ISO 10993-11:2017, provided that this rationale is documented in the protocol and final study report.

E) Histopathology: Since ISO 10993-11 applies the term "should" (recommendation) rather than "shall" (obligation), and considering the absence of mortality or clinical signs, together with the low systemic toxicological concern, the omission of histopathology, clinical biochemistry, and organ weight measurements in the acute, subacute, and sub chronic systemic toxicity studies is scientifically justified and remains compliant with ISO 10993-11:2017, provided that this rationale is documented in the protocol and final study report.

F) Clinical Pathology (Hematology and clinical chemistry):
Since ISO 10993-11 applies the term "should" (recommendation) rather than "shall" (obligation), and considering the absence of mortality or clinical signs, together with the low systemic toxicological concern, the omission of histopathology, clinical biochemistry, and organ weight measurements in the acute, subacute, and sub chronic systemic toxicity studies is scientifically justified and remains compliant with ISO 10993-11:2017, provided that this rationale is documented in the protocol and final study report.

- **Scientific Justification for the Omission of Histopathology, Clinical Biochemistry, and Organ Weight Measurements:**

According to ISO 10993-11:2017 In ISO 10993-11:2017 (section on subacute and sub chronic systemic toxicity, and Annex D), the evaluations of histopathology, clinical biochemistry, and organ weight measurements are described using the wording "should be", indicating strong recommendations rather than mandatory requirements. Annex D is informative, listing suggested endpoints rather than prescriptive obligations. Therefore, omission of these parameters is acceptable when supported by a documented scientific rationale. In the present study, omission of these endpoints is scientifically justified based on the following considerations:

1. Absence of Clinical Evidence of Toxicity No abnormal clinical signs, behavioral changes, body weight loss, or mortality were observed/expected during the study. Accordingly, additional measurements such as organ weights, histopathology, or clinical chemistry were considered unnecessary for identifying systemic toxicity. 2. Low Systemic Toxicological Concern Based on prior biocompatibility assessments and the inherent nature of the test article, no significant systemic absorption or distribution is expected, thus reducing the likelihood of target organ toxicity requiring detailed organ-level evaluation.
3. Limited Exposure Duration and Risk-Based Study Design For acute (single-dose), subacute (14–28 days), and sub chronic (~90 days in rodents) studies, ISO 10993-11 allows tailoring of endpoints according to risk. When no clinical abnormalities are present, extensive hematology, biochemistry, or histopathology assessments can be omitted.
4. Ethical Considerations (3Rs Principle) In line with the principles of Replacement, Reduction, and Refinement, unnecessary animal testing was avoided. Excluding nonessential endpoints prevents additional animal use and distress without compromising scientific validity.

021 91 30 9890 0992 60 62 450
Nikopharmed@gmail.com Nikopharmed.com

Non-Polar Extract											
Description	Acceptable limit	Animals No. & Groups									
		Treatments					**Controls**				
		1	2	3	4	5	1	2	3	4	5
A) Body Weight Changes											
Beginning of Test (g)	**25-30 (g)**	27.2	25.1	27.7	28.2	26.3	26.5	25.5	26.7	25.2	28.3
Day 1 (g)		28.9	26.2	28.5	27.4	27.4	26.9	26.4	27.5	25.4	29.1
Day 2 (g)		29.2	27.5	29.5	28.2	28.5	27.4	27.7	28.5	26.2	29.5
End of Test (Day 3 (g))		29.9	28.8	30.4	29.6	29.2	28.1	28.5	29.4	27.6	30.1
Mean	**±20%**	28.18					27.52				
± SD		1.32					1.39				
Description	Acceptable limit	Animals No. & Groups									
		Treatments					**Controls**				
		1	2	3	4	5	1	2	3	4	5
B) Clinical Observations											
First Day											
Respiratory	N*	N	N	N	N	N	N	N	N	N	N
Motor activities	N*	N	N	N	N	N	N	N	N	N	N
Ocular signs	N*	N	N	N	N	N	N	N	N	N	N
Cardiovascular signs	N*	N	N	N	N	N	N	N	N	N	N
Analgesia	N*	N	N	N	N	N	N	N	N	N	N
Gastrointestinal (Diarrhea, etc.)	N*	N	N	N	N	N	N	N	N	N	N
Convulsion	N*	N	N	N	N	N	N	N	N	N	N
Reflexes	N*	N	N	N	N	N	N	N	N	N	N
Salivation	N*	N	N	N	N	N	N	N	N	N	N
Piloerection	N*	N	N	N	N	N	N	N	N	N	N
Muscle tone	N*	N	N	N	N	N	N	N	N	N	N
Skin	N*	N	N	N	N	N	N	N	N	N	N
Death	-	-	-	-	-	-	-	-	-	-	-
N: No signs of Clinical Observations, **N.M**: Not Measured											
Second Day											

021 91 30 9890 0992 60 62 450
Nikopharmed@gmail.com Nikopharmed.com

Respiratory	**N***	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**
Motor activities	N*	N	N	N	N	N	N	N	N	N	N
Ocular signs	N*	N	N	N	N	N	N	N	N	N	N
Cardiovascular signs	N*	N	N	N	N	N	N	N	N	N	N
Analgesia	N*	N	N	N	N	N	N	N	N	N	N
Gastrointestinal (Diarrhea, etc.)	N*	N	N	N	N	N	N	N	N	N	N
Convulsion	N*	N	N	N	N	N	N	N	N	N	N
Reflexes	N*	N	N	N	N	N	N	N	N	N	N
Salivation	N*	N	N	N	N	N	N	N	N	N	N
Piloerection	N*	N	N	N	N	N	N	N	N	N	N
Muscle tone	N*	N	N	N	N	N	N	N	N	N	N
Skin	N*	N	N	N	N	N	N	N	N	N	N
Death	-	-	-	-	-	-	-	-	-	-	-
The Third Day											
N: No signs of Clinical Observations, **N.M**: Not Measured											
Respiratory	N*	N	N	N	N	N	N	N	N	N	N
Motor activities	N*	N	N	N	N	N	N	N	N	N	N
Ocular signs	N*	N	N	N	N	N	N	N	N	N	N
Cardiovascular signs	N*	N	N	N	N	N	N	N	N	N	N
Analgesia	N*	N	N	N	N	N	N	N	N	N	N
Gastrointestinal (Diarrhea, etc.)	N*	N	N	N	N	N	N	N	N	N	N
Convulsion	N*	N	N	N	N	N	N	N	N	N	N
Reflexes	N*	N	N	N	N	N	N	N	N	N	N
Salivation	N*	N	N	N	N	N	N	N	N	N	N
Piloerection	N*	N	N	N	N	N	N	N	N	N	N
Muscle tone	N*	N	N	N	N	N	N	N	N	N	N
Skin	N*	N	N	N	N	N	N	N	N	N	N
Death	-	-	-	-	-	-	-	-	-	-	-
N: No signs of Clinical Observations, **N.M**: Not Measured											

021 91 30 9890 0992 60 62 450
Nikopharmed@gmail.com Nikopharmed.com

C) Gross Pathology											
Groups		**Treatments**					**Controls**				
Animals No.		**1**	**2**	**3**	**4**	**5**	**1**	**2**	**3**	**4**	**5**
Abdominal Cavity	**N***	N	N	N	N	N	N	N	N	N	N
Thoracic Cavity		N	N	N	N	N	N	N	N	N	N
External surface of the body		N	N	N	N	N	N	N	N	N	N
All natural holes in the body (such as eyes, mouth, nose, anus)		N	N	N	N	N	N	N	N	N	N

N: No signs of Clinical Observations, **N.M**: Not Measured

D) Organ Weight: Since ISO 10993-11 applies the term "should" (recommendation) rather than "shall" (obligation), and considering the absence of mortality or clinical signs, together with the low systemic toxicological concern, the omission of histopathology, clinical biochemistry, and organ weight measurements in the acute, subacute, and sub chronic systemic toxicity studies is scientifically justified and remains compliant with ISO 10993-11:2017, provided that this rationale is documented in the protocol and final study report.

E) Histopathology: Since ISO 10993-11 applies the term "should" (recommendation) rather than "shall" (obligation), and considering the absence of mortality or clinical signs, together with the low systemic toxicological concern, the omission of histopathology, clinical biochemistry, and organ weight measurements in the acute, subacute, and sub chronic systemic toxicity studies is scientifically justified and remains compliant with ISO 10993-11:2017, provided that this rationale is documented in the protocol and final study report.

F) Clinical Pathology (Hematology and clinical chemistry):
Since ISO 10993-11 applies the term "should" (recommendation) rather than "shall" (obligation), and considering the absence of mortality or clinical signs, together with the low systemic toxicological concern, the omission of histopathology, clinical biochemistry, and organ weight measurements in the acute, subacute, and sub chronic systemic toxicity studies is scientifically justified and remains compliant with ISO 10993-11:2017, provided that this rationale is documented in the protocol and final study report.

- **Scientific Justification for the Omission of Histopathology, Clinical Biochemistry, and Organ Weight Measurements:**

According to ISO 10993-11:2017 In ISO 10993-11:2017 (section on subacute and sub chronic systemic toxicity, and Annex D), the evaluations of histopathology, clinical biochemistry, and organ weight measurements are described using the wording "should be", indicating strong recommendations rather than mandatory requirements. Annex D is informative, listing suggested endpoints rather than prescriptive obligations. Therefore, omission of these parameters is acceptable when supported by a documented scientific rationale. In the present study, omission of these endpoints is scientifically justified based on the following considerations:

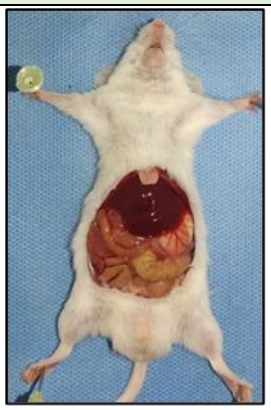
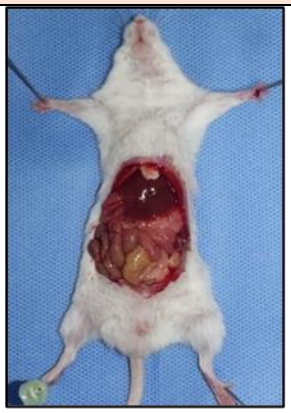
1. Absence of Clinical Evidence of Toxicity No abnormal clinical signs, behavioral changes, body weight loss, or mortality were observed/expected during the study. Accordingly, additional measurements such as organ weights, histopathology, or clinical chemistry were considered unnecessary for identifying systemic toxicity. 2. Low Systemic Toxicological Concern Based on prior biocompatibility assessments and the inherent nature of the test article, no significant systemic absorption or distribution is expected, thus reducing the likelihood of target organ toxicity requiring detailed organ-level evaluation.
3. Limited Exposure Duration and Risk-Based Study Design For acute (single-dose), subacute (14–28 days), and sub chronic (~90 days in rodents) studies, ISO 10993-11 allows tailoring of endpoints according to risk. When no clinical abnormalities are present, extensive hematology, biochemistry, or histopathology assessments can be omitted.

021 91 30 9890 0992 60 62 450
Nikopharmed@gmail.com Nikopharmed.com

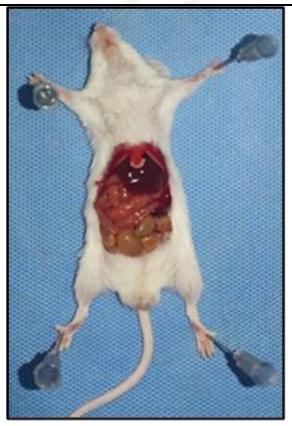
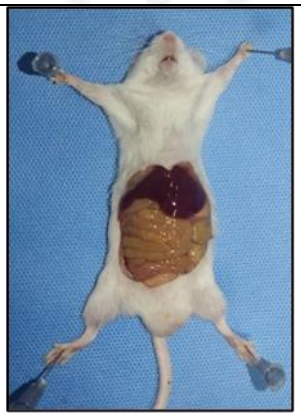
4. Ethical Considerations (3Rs Principle) In line with the principles of Replacement, Reduction, and Refinement, unnecessary animal testing was avoided. Excluding nonessential endpoints prevents additional animal use and distress without compromising scientific validity.

PHOTOGRAPH OF THE TEST ARTICLE

Macroscopic Examination

Gross Pathology (Polar)	
Control	**Sample**
	
Normal	**Normal**
Gross Pathology (Non-Polar)	
Control	**Sample**

021 91 30 9890 0992 60 62 450
Nikopharmed@gmail.com Nikopharmed.com

	
Normal	**Normal**

Conclusion

- None of the tested animals exhibited abnormal **Clinical Signs** in terms of toxicity during the test period.
- All the animals were **Alive** during the test duration.
- The **Body Weight** changes were within the acceptable range during the test.
- None of the animals exhibited abnormal **Gross Pathology** indicative of toxicity during the test period.

The results provide evidence to support that the examined product is **Non-Acute Toxicity.**

References

1. ISO 10993-11:2017, Biological evaluation of medical devices, Part 11: Tests for systemic toxicity
2. ISO 10993-12:2021, Biological evaluation of medical devices, Part 12: Sample preparation and reference materials.
3. ISO 10993-2: 2022, Biological evaluation of medical devices, Part 2: Animal welfare requirements.

Description Based on the customer request according to the F02-p12/05

Test Performer: *Z. Sayyahi*	**Lab. Manager:** *M. Daliri*	**CEO:** *M.Borjian*	**Nikopharmed laboratory sign**
Sign:	Sign:	Sign:	

- *Test results are only related to the tested products.*
- *Reproduction of test results without the permission of the laboratory is prohibited.*
- *Sampling has been done by the customer.*
- *This report is not valid without the seal and signature of the CEO.*
- *If the tests were performed by the contractor, the name of the contractor is given in the description section.*
- *Any objection to the issued results can be processed within 7 days after the date of issuance of the result.*
- *If the sample is stable, after the test, the sample will be stored in the laboratory for one month.*
- *Expanded uncertainty (CI: 95% , K=2) is calculated for quantitative tests and included upon customer request.*
- *Nikopharmed Arya Company, National Institute of Genetic Engineering and Biotechnology, Pajuhesh Boulevard, 17km Tehran-Karaj Highway, Tehran, Iran*

021 91 30 9890 0992 60 62 450
Nikopharmed@gmail.com Nikopharmed.com