CENTRUM ESTETICKÉ DERMATOLOGIE, LÉČBY AKNÉ A OBLIČEJOVÝCH DERMATÓZ SYNCAR E PLUS, s.r.o.

Non-State Medical Facility, REG: 605/200 8 JMK

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Comprehensive Test Report on Performed Epicutaneous Tests of Cosmetic Products

for Determining the Compatibility of the Cosmetic Product According to the Methodology of Cosmetics Europe (formerly COLI PA Product Test Guidelines for the Assessment of Human Skin Compatibility from 1997)

DEVELOPED BY TEST LEADER:	doc. MUDr. Jarmila Rulcová, CSc.
ETHICS COMMITTEE APPROVAL NUMBER:	22/2016, approval verified by: Dr. Jaromír Houzar, Chairman of EC
PROTOCOL NUMBER:	108/2023
TEST PERFORMED ON:	11.09.2023 - 27.09.2023

TEST CONTRACTOR:	**Madario Company s.r.o** **IČO: 06321313** **Bryksova 947/21, Černý Most, 198 00 Prague 9**

EVALUATED AGENTS:		
Sample Number	**Order Number**	**Name of the tested sample**
257	9/2023	**Puella Queenka Laundry Perfume**
258	9/2023	**Puella JaLu Laundry Perfume**
259	9/2023	**Puella First Dream Laundry Perfume**
260	9/2023	**Puella EmiJa Laundry Perfume**
261	9/2023	**Puella Frayo Laundry Perfume**
262	9/2023	**Puella Double Y Laundry Perfume**

A) Overview - Input Data

A-1) Method Used and Test Objective:

The test was conducted according to the Cosmetics Europe (formerly known as Cosmetic Product Test Guidelines for the assessment of human skin compatibility, Colipa, Brussels 1997). The aim of the study was to accurately assess the safety of cosmetic products for dermal tolerance - to ensure they meet the intended use.

A-2) Demographic Information of Study Participants:

Subject number	Subject initials	Gender (M- male, F-female)	Age (years)
1	KL	F	50
2	TM	F	64
3	KK	F	38
4	FJ	F	61
5	SM	M	58
6	SV	F	27
7	MJ	M	41
8	PJ	F	66
9	DM	F	49
10	MM	M	59

A-3) Tested Products:

Sample number	Sample name	Type of study
257	**Puella Queenka Laundry Perfume**	JOT
258	**Puella Jalu Laundry Perfume**	JOT
259	**Puella First Laundry Perfume**	JOT
260	**Puella EmiJa Laundry Perfume**	JOT
261	**Puella Frayo Laundry Perfume**	JOT
262	**Puella Double Laundry Perfume**	JOT

Key: *JUT - simple closed patch epicutaneous test with occlusion*

JOT - simple application open epicutaneous test

B) Study Methodology and Initial Testing Criteria

8-1) Description of the test method used:

a) Description of the JUT Method - Simple closed patch epicutaneous test with occlusion:

This testing method is used for products that can be left on the skin after application without subsequent washing. The test substance with an occlusive patch is applied to the chosen test site - the volar side of the forearm, arm, or back - in an amount of 0.10 ml. Subjects are instructed to keep the test area dry throughout the entire test duration. After a maximum of 24 hours, the patch is removed from the subjects, and any remaining test substance is wiped off with clean water. Skin reactions are assessed at 30 minutes, 24, 48, and 78 hours. Subsequently, the Primary Skin Irritation Index IKi• is determined.

b) Description of the JOT Method - Simple application open epicutaneous test:

This method is used for products that, after application, do not remain on the skin and are rinsed off. The substance can be applied in diluted or concentrated form using a swab to the chosen test site - the volar side of the forearm, arm, or back - in an amount of 0.10 ml. The application duration is 15 - 30 minutes. Then, the unabsorbed portion of the substance is rinsed off with water or lightly wiped off with a damp cotton pad. Evaluation is done immediately after removing the substance, and then after 24 and 48 hours. Subsequently, the Primary Skin Irritation Index IKi• is determinedi

B-2) Volunteer Selection Criteria:

1. Male or female between the ages of 18 and 68
2. Psychosomatic ability to undergo the test
3. Signed informed consent

B-3) Exclusion Criteria:

1. Pregnancy, breastfeeding, or attempting to conceive with the assistance of medication
2. Any obvious illness
3. Use of topical or systemic medications that may interfere with the test
4. Positive medical history of skin or allergic diseases
5. Participation in any other test conducted on the same skin area in the past 1 month
6. Concurrent participation in any other test
7. Skin irritation at the intended test site
8. Family member (partner, descendant, sibling, descendant of siblings), employee of the company conducting the testing

B-4) Informed Consent:

All test subjects received an informed consent form describing the goals and methods of the test. Informed consents are stored electronically by the test leader. In case of any significant irritation during the ongoing test, subjects were instructed to remove the patch, rinse the area with clean water, and visit the study leader.

B-5) Criteria for Assessing Skin Reactions:

The tested area was visually assessed under standard lighting conditions by a qualified person. The scoring system takes into account various skin symptoms. Different numerical values were assigned to individual symptoms. Each reaction was further assigned a rating characterizing the severity of the symptom. The assessment of skin reactions and evaluation is carried out by a dermatologist.

Formation of edema:

Symptom	Abbreviation	Assessment
Vesicle	V	5
Severe edema (elevation of the application area by more than 1 mm)	E	4
Moderate edema (elevation of the application area up to 1 mm)	P	3
Mild edema (well-defined borders)	R	2
Barely noticeable edema	F	1
Without edema	D	0

Formation of erythema:

Assessment	Description of erythema
0	Without visible erythema
1	Minimal erythema formation (slightly visible reaction)
2	Clearly visible diffuse erythema
3	Erythema with edema formation
4	Erythema with edema and vesiculation (damage to depth)

The evaluation of skin reactions is performed using the Primary Skin Irritation Index IK1, which expresses the average of the sum of the reaction degrees for inflammation and edema in individual subtracting intervals for one subject, followed by calculating the average for all exposed subjects.

Result classifications:

Description	Assessment
Non-irritating	IKI < 0,5
Slightly irritating	IKI ≥ 0,5
Moderately irritating	IKI ≥ 3,0
Strongly irritating and even corrosive	IKI ≥ 5,0

C) Own Study

C-1) Description of the Method Used

The test substance was applied to the skin of volunteers and monitored at specified time intervals as determined by the selected testing method. The application site for the test sample was consistent among all volunteers. The test was conducted according to the chosen testing method, as indicated below. The test used for a specific sample is marked with a cross. The evaluation of the results was transparently processed into a table.

☐ **a) JUT - Simple closed patch epicutaneous test with occlusion**

An occlusive patch with the tested substance was applied in an amount of 0.10 ml. Subjects were instructed to keep the test area dry throughout the entire test. After 24 hours, the patch was removed from the subjects, and any remaining test material was wiped off with clean water. Skin reactions were assessed at 30 minutes, 24, 48, and 72 hours.

☒ **b) JOT - Simple open patch epicutaneous test**

Substance **257 - Puella Queenka laundry perfume; 258 - Puella Jalu laundry perfume; 259 - Puella First Dream laundry perfume; 260 - Puella EmiJa laundry perfume; 261 - Puella Frayo laundry perfume; 262 - Puella Double Y laundry perfume** was applied in diluted form (10% solution) using a swab to the left half of the back in an amount of 0.10 ml. The application duration was 30 minutes. Then, the unabsorbed portion of the substance was rinsed off with water or wiped with a damp cotton pad. Evaluation was performed immediately after removing the substance and then again after 24 and 48 hours.

C-2) Evaluation of the test results

The dermal tolerance skin test was completed by all test participants. The tested area of the skin, the test site, was visually assessed under standard lighting conditions by a qualified person at the required time intervals. The evaluation was performed using the **Primary Skin Irritation Index (IK1)**, which expresses the average of the sum of the reaction degrees for inflammation and edema in individual subtracting intervals for one subject, followed by calculating the average for all exposed subjects. The result evaluation was processed into a table for clarity. The final table of the **Primary Skin Irritation Index (IK)** assessment is attached at the end of this protocol.

D) Study Conclusion

The tested cosmetic products **257 - Puella Queenka laundry perfume; 258 - Puella Jalu laundry perfume; 259 - Puella First Dream laundry perfume; 260 - Puella EmiJa laundry perfume; 261 - Puella Frayo laundry perfume; 262 - Puella Double Y laundry perfume** were evaluated according to the tests mentioned above. Under the given testing conditions, no objective irritative reaction or any subjective negative observations were recorded among the volunteers.

The products can be recommended from the skin point of view for the intended use based on the assessment of dermal tolerance.

Doc. MUDr. Jarmila Rulcová, CSc.

Date of Issuance of the Test Report: 27.09.2023

Study Director:

Doc. MUDr. Jarmila Rulcová

Individual Skin Irritation Score

Date: 27.09.2023

Study Number: 108/2023

Agent:

subject	**257**-Puella Queenka Laundry Perfume;	**258**-Puella Jalu Laundry Perfume;	**259**-Puella First Dream Laundry Perfume;	**260**-Puella EmIJa Laundry Perfume;	**261**-Puella Frayo Laundry Perfume;	**262**-Puella Double Y Laundry Perfume;
1	o	o	o	o	o	o
2	o	o	o	o	o	o
3	o	o	o	o	o	o
4	o	o	o	o	o	o
5	o	o	o	o	o	o
6	o	o	o	o	o	o
7	o	o	o	o	o	o
8	o	o	o	o	o	o
9	o	o	o	o	o	o
10	o	o	o	o	o	o
11						
12						
13						
14						
15						
n	10	10	10	10	10	10
total score	0	0	0	0	0	0
average	0.0	0.0	0.0	0,0	0,0	0,0
standard deviation	0.0	0,0	0.0	0.0	0,0	0.0