HAMILTON CROATIA

COSMETIC PRODUCT SAFETY REPORT

Mystery kozmetika / Srijemuš LaVita - Mast Od Liofiliziranog Srijemuša

100mL

Report: C221037

17/02/2022

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List of abbreviations

BMD	*Benchmark Dose*
CAS	*Chemical Abstracts Service*
CIR	*Cosmetic Ingredients Review*
EINECS	*European Inventory of Existing Chemical Substances*
ELINCS	*European List of Notified Chemical Substances*
FDA	*Food and Drug Administration*
GRAS	*Generally Recognized as Safe*
HMT	*Human Maximisation Test*
HRIPT	*Human Repeated Insult Patch Test*
IFRA	*International Fragrance Association*
INCI	*International Nomenclature of Cosmetic Ingredients*
INN	*International Nonproprietary Name*
ISO 11930	*Guidelines for the preservative effectiveness test (Challenge Test)*
ISO 22716	*Guidelines for the production, control, storage and shipment of cosmetic products (Good Manufacturing Practices)*
ISO 29621	*Guidelines for the risk assessment and identification of microbiologically low-risk products*
IUPAC	*International Union of Pure and Applied Chemistry*
LD50	*Median Lethal Dose 50%*
LOAEL	*Lowest Observed Adverse Effect Level*
MoE	*Margin of Exposure*
MoS	*Margin of Safety*
NOAEL	*No Observed Adverse Effect Level*
OECD	*Organisation for Economic Co-operation and Development*
PAO	*Period After Opening*
REACH	*Registration, Evaluation, Authorisation and Restriction of Chemicals*
SCCS	*Scientific Committee on Consumer Safety*
SED	*Systemic Exposure Dose*
TTC	*Threshold of Toxicological Concern*
VSD	*Virtually Safe Dose*
POD	*Point of Departure*

Legal framework

This Safety Assessment Report relates to Mystery kozmetika / Srijemuš LaVita - Mast Od Liofiliziranog Srijemuša. It was prepared according to the Regulation (CE) No 1223/2009 of the European Parliament and the Council of 30 of November of 2009 on cosmetic products (1).

Applicant: **Mystery kozmetika**

Address: **Cara Dušana 123, 78252 Trn - Laktaši, Bosnia and Herzegovina**

Contact Number: **+38665018500**

Safety Assessor: **Luka Beretin**

(Proof of qualification of safety assessor attached).

Revisions log

Version nº	Comments	Author	Date
1	This product is considered safe for use, however, caution is advised due to high content of highly bioactive natural compounds and extracts.	Luka Beretin	17/02/2022

PART A | COSMETIC PRODUCT SAFETY INFORMATION

1 Product's name and description

Mystery kozmetika / Srijemuš LaVita - Mast Od Liofiliziranog Srijemuša is a leave-on product intended to be used as face and body cream. It belongs to the skin care category in accordance with the guidelines published by the *Scientific Committee on Consumer Safety* (SCCS). This classification is based on the final purpose intended for the product.

This product has the following presentation:

Jar (100mL)

The toxicological evaluation of the cosmetic product will be performed taking into account its toxicological profile and classification, according to the criteria established by the SCCS.

2 Qualitative and quantitative composition of the product

The ingredients present in Mystery kozmetika / Srijemuš LaVita - Mast Od Liofiliziranog Srijemuša are identified in table 1 as well as their concentration and individual purpose.

Raw material	RM Conc. % (w/w)	INCI	EINECS/ ELINCS	CAS	Ing. Conc. % (w/w)	Function
Allium Ursinum Extract	25.0 - 50.0	ALLIUM URSINUM EXTRACT	291-059-3	90320-33-5	25.0 - 50.0	Skin conditioning
Calendula Officinalis Flower Extract	25.0 - 50.0	CALENDULA OFFICINALIS FLOWER EXTRACT	283-949-5	84776-23-8	25.0 - 50.0	Fragrance / Perfuming / Skin conditioning
Cera Alba	5.0 - 10.0	CERA ALBA	232-383-7	8012-89-3	5.0 - 10.0	Surfactant - emulsifying / Film forming / Perfuming / Skin conditioning - emollient
Citrus Limon Peel Oil	0.1 - 1.0	CITRUS LIMON PEEL OIL	- / 284-515-8	8008-56-8 / 84929-31-7	0.1 - 1.0	Fragrance / Perfuming / Skin conditioning
		LIMONENE	205-341-0/931-893-3	138-86-3	0.01 - 0.9	
Cupressus Sempervirens Leaf Oil	0.1 - 1.0	CUPRESSUS SEMPERVIRENS LEAF OIL	283-626-9	84696-07-1	0.1 - 1.0	Fragrance / Skin conditioning - miscellaneous
Mentha Piperita Oil	0.1 - 1.0	MENTHA PIPERITA OIL	- / 282-015-4	8006-90-4 / 84082-70-2	0.1 - 1.0	Fragrance / Perfuming / Refreshing / Tonic
Phenoxyethanol	0.1 - 1.0	PHENOXYETHANOL	204-589-7	122-99-6	0.1 - 1.0	Antimicrobial / Preservative
Menthol	0.1	MENTHOL	216-074-4 / 218-690-9 / 201-939-0 / 239-388-3	1490-04-6 / 2216-51-5 / 89-78-1 / 15356-70-4	0.1	Denaturant / Fragrance / Refreshing / Soothing
Rosmarinus Officinalis Leaf Oil	0.1	ROSMARINUS OFFICINALIS LEAF OIL	283-291-9	84604-14-8 / 8000-25-7	0.1	Fragrance / Skin conditioning

Table 1 - Qualitative and quantitative composition of the product Mystery kozmetika / Srijemuš LaVita - Mast Od Liofiliziranog Srijemuša

3 Physical and chemical characteristics and stability of the cosmetic product

Mystery kozmetika / Srijemuš LaVita - Mast Od Liofiliziranog Srijemuša formulation was designed taking into account its final purpose. Each raw material has specific physical/chemical characteristics and unique properties. Together, the mixture of raw materials confers the desired functional properties to the finished product and ensures its stability during its minimum durability time.

3.1 Physical and chemical characteristics of the raw materials

The raw materials' selection was made accordingly to their safety profile for application in cosmetics as well as the desired functionality of the finished product. The raw materials used in this formulation are biocompatible, have well documented safety data and a long history of use in cosmetic products. There were no toxic solvents or other toxic ingredients used in the formulation.

Allium Ursinum Extract

Composition	Conc. (%)	CAS	EINECS
ALLIUM URSINUM EXTRACT	100.0	90320-33-5	291-059-3

Table 2 - Qualitative and quantitative composition of the raw material Allium Ursinum Extract

IUPAC/Description (ALLIUM URSINUM EXTRACT)	Allium Ursinum Extract is an extract of the plant Ramsons (wild garlic), Allium ursinum L., Liliaceae

Table 3 – Physical and chemical properties of the raw material Allium Ursinum Extract and its constituents

Comments

Allium sativum L. (Liliacea) is a perennial bulb with a tall, erect flowering stem that grows to between 2 and 3 feets. The plant produces pink to purple flowers that bloom from July to September and the bulb is odifereous (McMahon and Vargas, 1993). The name A. sativum L. comes from the Celtic word "all" meaning burning or smarting. The bulb of the plant has been used in many parts of the world as a stimulant, carminative, antiseptic, anthelmintic, expectorant and diuretic.[1]

Regulatory restrictions

According to Regulation (EC) No 1223/2009 of 30 of November 2009 there are no restrictions concerning ALLIUM URSINUM EXTRACT.

Calendula Officinalis Flower Extract

Composition	Conc. (%)	CAS	EINECS
CALENDULA OFFICINALIS FLOWER EXTRACT	100.0	84776-23-8	283-949-5

Table 4 - Qualitative and quantitative composition of the raw material Calendula Officinalis Flower Extract

IUPAC/Description (CALENDULA OFFICINALIS FLOWER EXTRACT)	Calendula Officinalis Flower Extract is an extract obrained from the flowers of the Calendula, Calendula officinalis L., Compositae

Table 5 – Physical and chemical properties of the raw material Calendula Officinalis Flower Extract and its constituents

Comments

The CIR Expert Panel concluded that C. officinalis extract, C. officinalis flower,C. officinalis flower extract, C. officinalis flower oil, and C. officinalis seed oil are safe for use in cosmetics in the practices of use and concentration given in this amended safety assessment.[2]

Regulatory restrictions

According to Regulation (EC) No 1223/2009 of 30 of November 2009 there are no restrictions concerning CALENDULA OFFICINALIS FLOWER EXTRACT.

Cera Alba

Composition	Conc. (%)	CAS	EINECS
CERA ALBA	100.0	8012-89-3	232-383-7

Table 6 - Qualitative and quantitative composition of the raw material Cera Alba

Odour	Odourless
Colour	White
Appearance	Solid

IUPAC/Description (CERA ALBA)	Beeswax. The wax obtained from the honeycomb of the bee. It consists primarily of myricyl palmitate, cerotic acid and esters and some high-carbon paraffins

Table 7 – Physical and chemical properties of the raw material Cera Alba and its constituents

Comments

Beeswax is a complex mixture of saturated and unsaturated linear and complex monoesters, hydrocarbons, free fatty acids, free fatty alcohols, and other minor substances produced by the worker honeybee.[3]

Regulatory restrictions

According to Regulation (EC) No 1223/2009 of 30 of November 2009 there are no restrictions concerning CERA ALBA.

Citrus Limon Peel Oil

Composition	Conc. (%)	CAS	EINECS
CITRUS LIMON PEEL OIL	100.0	8008-56-8 / 84929-31-7	- / 284-515-8
LIMONENE	10.0 - 90.0	138-86-3	205-341-0/931-893-3

Table 8 - Qualitative and quantitative composition of the raw material Citrus Limon Peel Oil

IUPAC/Description (CITRUS LIMON PEEL OIL)	Citrus Limon Peel Oil is the volatile oil obtained from the fresh peel of the Lemon, Citrus limon (L.), Rutaceae
Density	0.8527 at 20ºC

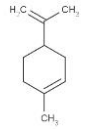
Log Pow	4.83
IUPAC/Description (LIMONENE)	1-Methyl-4-Isopropenylcyclohexene; dipentene
Chemical Structure	
Molecular formula	$C_{10}H_{16}$
Molecular weight	136.23 Da

Table 9 – Physical and chemical properties of the raw material Citrus Limon Peel Oil and its constituents

Comments

Essential oil of lemon obtained from the peel of Citrus limonum (Rutaceae) by expression and/or distillation, including cold pressed, distilled, terpenes and essence qualities.[4]

Limonene is a monocyclic monounsaturated terpene. It occurs as the d and l isomers, and as the racemic mixture dl-limonene known as dipentene. Limonene, like other monoterpenes, occurs naturally in certain trees and bushes, being found in peel from citrus fruits, in dill, caraway, fennel, and celery, and in turpentine. It is used globally as a flavour and fragrance additive in consumer products such as household cleaning products, and in beverages and food. In cosmetic products, it is used in the formulation of aftershave lotions, bath products, bubble baths, cleansing products, eye shadows, hair products, lipsticks, mascara, moisturizers, perfumes and colognes, shampoos, skincare products and suntan products. This ingredient is used in cosmetics as a deodorant, perfuming and solvent agent.

Regulatory restrictions

This ingredient is a possible source of Furocoumarines. Fourocoumarines are a prohibited substance (Annex II/358, e.g. trioxysalen (INN), 8 -methoxypsoralen, 5-methoxypsoralen) except for normal content in natural essences used. In sun protection and in bronzing products, furocoumarines shall be below 1 mg/kg.

According to the Regulation (EC) No 1223/2009 of 30 November 2009 Limonene is included in the list of substances (Annex III) which cosmetic products must not contain except subject to the restrictions laid down:

- Peroxide value less than 20 mmoles/L (this limit applies to the substance and not to the finished cosmetic product).

The presence of Limonene must be indicated in the list of ingredients referred to in Article 19(1)(g) when its concentration exceeds: - 0.001% in leave-on products - 0.01% in rinse-off products.

Cupressus Sempervirens Leaf Oil

Composition	Conc. (%)	CAS	EINECS
CUPRESSUS SEMPERVIRENS LEAF OIL	100.0	84696-07-1	283-626-9

Table 10 - Qualitative and quantitative composition of the raw material Cupressus Sempervirens Leaf Oil

IUPAC/Description (CUPRESSUS SEMPERVIRENS LEAF OIL)	Cupressus Sempervirens Leaf Oil is the volatile oil obtained from the leaves of Cupressus sempervirens.
Density	0.869 ± 0.001 g/mL at 20 °C

Table 11 – Physical and chemical properties of the raw material Cupressus Sempervirens Leaf Oil and its constituents

Comments

Cypress oil is a pale yellow, clear, mobile, liquid at ambient temperature; it doesn't undergo freezing down to -20°C, and distillates from ca 177°C. It is expected to float over water, because of its very low relative density (0.87), and slight solubility of its major components (ca 2 to 4 mg/L). Some of them may also exhibit moderate volatility (up to ca 630 Pa) and potential for bioaccumulation (log K > 4). By analogy, no surface-active properties are anticipated. Based on its flash point of ca 36°C, Cypress oil will be classified as a Flammable liquid (cat.3, according to CLP criteria). It is not regarded as hazardous with regard to other physical hazard classes (explosivity, self-reactive nor oxidising properties). It should be noted that, as a Natural Complex Substance, the above properties of Cypress oil may exhibit some variations.[5]

Regulatory restrictions

Annex III/123: Peroxide value less than 10 mmoles/L (15)

Mentha Piperita Oil

Composition	Conc. (%)	CAS	EINECS
MENTHA PIPERITA OIL	100.0	8006-90-4 / 84082-70-2	- / 282-015-4

Table 12 - Qualitative and quantitative composition of the raw material Mentha Piperita Oil

IUPAC/Description (MENTHA PIPERITA OIL)	Mentha Piperita Oil is the volatile oil obtained from the whole plant of the Peppermint, Mentha piperita (L.), Labiatae

Table 13 – Physical and chemical properties of the raw material Mentha Piperita Oil and its constituents

Comments

Mentha Piperita Oil is the volatile oil obtained from the whole plant of the Peppermint, Mentha piperita (L.), Labiatae. The major constituents of Mentha Piperita Oil are terpenes, namely menthol (30-55%) and menthone (14-32%). Mentha Piperita Oil is distilled with steam from the fresh, above-ground parts of the flowering plant Mentha piperita, rectified by distillation and not dementholized. The ingredient Mentha Piperita Oil is used in cosmetic products due to its fragrance, perfuming, refreshing and tonic characteristics.

Regulatory restrictions

According to Regulation (EC) No 1223/2009 of 30 of November 2009 there are no restrictions concerning MENTHA PIPERITA OIL.

Phenoxyethanol

Composition	Conc. (%)	CAS	EINECS
PHENOXYETHANOL	100.0	122-99-6	204-589-7

Table 14 - Qualitative and quantitative composition of the raw material Phenoxyethanol

IUPAC/Description (PHENOXYETHANOL)	2-Phenoxyethanol

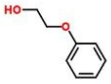
Chemical Structure	
Molecular formula	$C_8H_{10}O_2$
Molecular weight	138.16 Da

Table 15 – Physical and chemical properties of the raw material Phenoxyethanol and its constituents

Comments

Phenoxyethanol, also known as 2-phenoxyethanol, is an aromatic ether with an alcohol moiety; It is used in cosmetics as a preservative and as a fixative for perfumes; It can be found in skincare products, eye makeup, fragrances, blushers, foundations and makeup bases, lipstick, cuticle softeners, bath soaps and detergents, baby products, suntan and sunscreen products, and face, body and foot powders; It is also used as an antiseptic, tissue preservative, and solvent, and in the synthesis of plastics and pharmaceuticals, and as a component of adhesives.[6] This ingredient is used in cosmetics as a preservative agent.

Regulatory restrictions

According to Regulation (EC) No 1223/2009 of 30 of November 2009, Phenoxyethanol is included in the list of preservatives allowed in cosmetic products (Annex V) provided it meets the following restrictions:

- Maximum concentration in ready for use preparation: 1.0%.

Menthol

Composition	Conc. (%)	CAS	EINECS
MENTHOL	100.0	1490-04-6 / 2216-51-5 / 89-78-1 / 15356-70-4	216-074-4 / 218-690-9 / 201-939-0 / 239-388-3

Table 16 - Qualitative and quantitative composition of the raw material Menthol

IUPAC/Description (MENTHOL)	Cyclohexanol, 5-methyl-2-(1-methylethyl)-

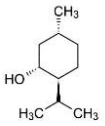
Chemical Structure	
Molecular formula	$C_{10}H_{20}O$
Molecular weight	156.269 g/mol
Density	0.890 g/cm3
Log Pow	Log Pow of 3.4 for L- and DL-Menthol
Melting point	30 - 32 °C

Table 17 – Physical and chemical properties of the raw material Menthol and its constituents

Comments

Menthol is an organic compound made synthetically or obtained from the oils of corn mint, peppermint, or other mints. It is a waxy, crystalline substance, clear or white in color, which is solid at room temperature and melts slightly above. The main form of menthol occurring in nature is (−)-menthol, which is assigned the (1R,2S,5R) configuration. Menthol has local anesthetic and counterirritant qualities, and it is widely used to relieve minor throat irritation. Menthol also acts as a weak κ-opioid receptor agonist.

Regulatory restrictions

According to Regulation (EC) No 1223/2009 of 30 of November 2009 there are no restrictions concerning MENTHOL.

Rosmarinus Officinalis Leaf Oil

Composition	Conc. (%)	CAS	EINECS
ROSMARINUS OFFICINALIS LEAF OIL	100.0	84604-14-8 / 8000-25-7	283-291-9

Table 18 - Qualitative and quantitative composition of the raw material Rosmarinus Officinalis Leaf Oil

Appearance	Liquid

IUPAC/Description (ROSMARINUS OFFICINALIS LEAF OIL)	Rosmarinus Officinalis Leaf Oil is the essential oil obtained from the flowering tops and leaves of the Rosemary, Rosmarinus officinalis L., Lamiaceae
Density	0.907 - 0.92
Log Pow	2.85 - 6.30
Melting point	-20 °C

Table 19 – Physical and chemical properties of the raw material Rosmarinus Officinalis Leaf Oil and its constituents

Comments

According to the classification provided by companies to ECHA in REACH registrations this substance may be fatal if swallowed and enters airways, is toxic to aquatic life with long lasting effects, is a flammable liquid and vapour, causes serious eye irritation, may cause damage to organs, causes skin irritation and may cause an allergic skin reaction.[7]

Regulatory restrictions

According to Regulation (EC) No 1223/2009 of 30 of November 2009 there are no restrictions concerning ROSMARINUS OFFICINALIS LEAF OIL.

3.2 Physical and chemical characteristics of the product

The product should comply with the following chemical/physical specifications established by the manufacturer:

Parameter	Specifications
Appearance	Cream
Colour	Green, opaque
Odour	Strong herbal odour

Table 20 - Physical and chemical specifications of the product Mystery kozmetika / Srijemuš LaVita - Mast Od Liofiliziranog Srijemuša

3.3 Cosmetic product's stability

A Stability Study was performed for this product. The study protocol and corresponding results can be found on Stability Annex.

A date of minimum durability of 24 months was assigned to the product. The finished product was assigned a PAO of 9 months.

4 Microbiological quality

4.1 Microbiological characteristics of the raw materials

The raw materials do not have microbiological specifications.

4.2 Microbiological characteristics of the product

The microbiological quality of a cosmetic product is directly related to its capacity to enable the development of microorganisms. The risk of product's contamination will depend directly on its composition, the preservative content, the compliance with hygienic practices during the manufacturing process, the packaging material and the transport and storage conditions.

The evaluation of a product's microbiological quality aims to ensure that the product is free of microorganisms that may affect its quality and consumer's health. At the same time, this study intends to ensure that microorganisms that may contaminate the product during its period of use will not affect its safety and effectiveness.

Parameter	Specifications
Total microbial count	< 10 cfu/mL
Moulds & yeast	< 10 cfu/mL
Pseudomonas aeruginosa	Not present in 1mL
Staphylococcus aureus	Not present in 1mL
Escherichia coli	Not present in 1mL
Candida albicans	Not present in 1mL
Aspergilus brasiliensis	< 10 cfu/mL

Table 21 - Microbiological specifications of the product Mystery kozmetika / Srijemuš LaVita - Mast Od Liofiliziranog Srijemuša

4.3 Challenge test

A Challenge Test was performed for this product. The study protocol and corresponding results can be found on Challenge Test Annex.

5 Impurities, traces, information about the packaging material

In accordance with Article 17 of Regulation (CE) 1223/2009, it is permitted in the cosmetic product the "non-intended presence of a small quantity of a prohibited substance, stemming from impurities of natural or synthetic ingredients, the manufacturing process, storage or migration from packaging, provided it is technically unavoidable in good manufacturing practice and does not endanger the safety of the product".

5.1 Impurities of the raw materials

The impurities present in the raw materials are inherent to the manufacturing process and are found in trace concentrations in the finished product.

5.2 Traces of prohibited substances on the cosmetic product

Based on the available information, no traces were identified in the finished product.

5.3 Specifications of the packaging material

The relevant characteristics of the packaging material that are in direct contact with the final product are important for the safety of the cosmetic product. The information on the relevant characteristics of the packaging material in direct contact with the product must make it possible to estimate the potential risks. The relevant characteristics may include, for example, the following:

- composition of the packaging material including technical materials, as additives;

- technically unavoidable impurities;

- the possible migration of substances from the packaging.

It was not possible to address the relevant characteristics of the packaging material, since no information on packaging material was available at the time of this assessment.

6 Normal and reasonably foreseeable use

The Mystery kozmetika / Srijemuš LaVita - Mast Od Liofiliziranog Srijemuša is intended to be used as face and body cream, applied on the face and body without subsequent washing after application.

7 Exposure to the cosmetic product

The Mystery kozmetika / Srijemuš LaVita - Mast Od Liofiliziranog Srijemuša is intended to be applied on face and body by dermal exposure route without subsequent washing after application (leave-on product).

7.1 Normal and reasonably foreseeable exposure route(s)

Dermal.

7.2 Exposure levels to the cosmetic product

- ➔ Surface area of application: 16235.0 cm^2.
- ➔ Amount of cosmetic product applied (per application): 1.0 g.
- ➔ Duration and frequency of application: 2.28 times/day.
- ➔ Retention factor: 1.0.
- ➔ Weight: 60.0 kg.

The reference values presented are in accordance with the SCCS's Notes of Guidance for the Testing of Cosmetic Ingredients and their Safety Evaluation, for face cream (1.54 g/d) plus body lotion (7.82 g/d), for a total area of 16235 cm^2 (565 cm^2 ½ area head + 15670 cm^2 area body-area head).

7.3 Targeted population

The cosmetic product is formulated to be used by general (adults).

8 Exposure to the ingredients

To each product category is associated a certain quantity of substance that may be ingested, inhaled or absorbed through the skin. The calculation of the Systemic Exposure Dose (SED) is an important tool that allows the estimation of exposure values of an organism to a particular chemical substance, taking into account:

- ➔ $E_{product}$ (mg/kg bw/day) – Estimated daily exposure to a cosmetic product per kg (body weight) based on the applied quantity and frequency of application;
- ➔ C (%) – Concentration of the substance in the finished cosmetic product;
- ➔ DA_p (%) – Dermal Absorption as a percentage (since the reference values are not available, the maximum percentage is assigned to dermal absorption, as stipulated by the OECD).

$$\mathbf{SED} = \mathbf{E_{product}} \times \frac{\mathbf{C}}{\mathbf{100}} \times \frac{\mathbf{DA_p}}{\mathbf{100}}$$

Based on this formula, the values of the *Systemic Exposure Dose* to the formulation's ingredients were calculated as follows:

Ingredients (INCI)	**Estimated daily exposure** (mg/kg bw/day)	**Concentration in the finished product** (%)	**Dermal absorption** (%)	**SED** (mg/kg bw/day)
ALLIUM URSINUM EXTRACT	38.0	50.0	100.0	19.0
CALENDULA OFFICINALIS FLOWER EXTRACT	38.0	50.0	100.0	19.0
CERA ALBA	38.0	10.0	100.0	3.8
CITRUS LIMON PEEL OIL	38.0	1.0	100.0	0.38
CUPRESSUS SEMPERVIRENS LEAF OIL	38.0	1.0	100.0	0.38
MENTHA PIPERITA OIL	38.0	1.0	100.0	0.38
PHENOXYETHANOL	269.0	1.0	100.0	2.69
LIMONENE	38.0	0.9	100.0	0.342
MENTHOL	38.0	0.1	100.0	0.038
ROSMARINUS OFFICINALIS LEAF OIL	38.0	0.1	100.0	0.038

Table 22 - Systemic exposure data of the ingredients

9 Toxicological profile of the substances

The toxicological evaluation of the product is performed based on the assessment of the individual toxicological profile of each substance in the formulation. It is established a safety profile for each ingredient taking into account the reference values of acute toxicity by different routes of administration, the potential of skin and mucous membranes reactivity, the sensitizing potential of the ingredient (in the concentrations and vehicles to be incorporated in the formulation) and safety data on mutagenicity and carcinogenicity.

INCI	Acute toxicity	Dermal irritation	Eye irritation	Sensitization
ALLIUM URSINUM EXTRACT	LD50 (Rats): 3034mg/kg	No relevant data	No relevant data	No relevant data
CALENDULA OFFICINALIS FLOWER EXTRACT	LD50 (Rats) > 4.64 g/kg	Non-irritant	Minimal ocular irritant	Non-senzitizing
CERA ALBA	Non-toxic	Non-irritant	Non-irritant	Non-sensitizing
CITRUS LIMON PEEL OIL	Acute oral toxicity: LD50>5000 mg/kg bw Acute dermal toxicity: LD50>10000 mg/kg bw	Irritating	Non-iritating	Sensitizing
CUPRESSUS SEMPERVIRENS LEAF OIL	LD50 (rat) > 5000 mg/kg bw	Irritating	Non-irritating	Sensitizing
MENTHA PIPERITA OIL[8] [9] [10]	Mice (Oral) LD50 = 2410 mg/kg	Irritating (Rabbits) Non-irritating (20%, HRIPT)	Non-irritating (60%, Rabbits)	Non-sensitizing (8%, Humans) Non-sensitizing (20%, HRIPT)
PHENOXYETHANOL[6] [11] [12] [13]	Rat (Oral) LD50 = 1300 mg/kg bw Rat (Dermal) LD50 > 2000 mg/kg bw	Non-irritating (Rabbits) (OECD Guideline 404) Non- to Slightly-irritating (Guinea pigs) Non-irritating (10%, Humans) Mildly-irritating (Humans)	Non- to Strongly-irritating (Rabbits)	Non-sensitizing (GPMT) (OECD Guideline 406) Possibly sensitizing (Humans)
LIMONENE[14] [15] [16]	Rats (Oral) LD50 = 4400 mg/kg bw Rabbits (Dermal) LD50 > 5000 mg/kg bw	Irritating (Rabbits) (OECD Guideline 404) Irritating (Humans)	Irritating (Rabbits)	Sensitizing (Humans)
MENTHOL	LD50(Rats) = 3180 mg/kg bw	Irritating	Irritating	Non-sensitizing
ROSMARINUS OFFICINALIS LEAF OIL	Acute oral toxicity : LD50 = 5000 mg/kg bw Acute dermal toxicity : LD50 = 8000 mg/kg bw Acute inhalation toxicity : 13000 mg/m3.	Skin irritation/corrosion: irritating	Eye irritation: irritating	Skin sensitisation: Skin sensitiser 1B

Table 23 - Summary of the toxicological profile of the ingredients

These studies were selected based on the similarity of the substances to the analysed ingredients, their concentration in the formulation and the compliance of the studies with Good Laboratory Practices and OECD Guidelines.

9.1 Calculation of the Margin of Safety (MoS)

The Margin of Safety (MoS) is a measure of the probability of a substance to cause harm to the human body. To be considered safe, an ingredient must have a MoS value greater than 100. This value takes into account the inter-species (10) variability and intra-species variability (10) (multiplying these values, the value 100 for the MoS is obtained). For cosmetic ingredients, the MoS is usually calculated by dividing the internal (systemic) PODsys by the SED, requiring the Bioavailability to be considered in the calculations. The Margins of Safety were calculated for each substance contained in the product's formulation according to the following mathematical formula:

- ➔ SED (mg/kg bw/day) – Systemic Exposure Dose;
- ➔ POD (mg/kg bw/day) – Dose descriptor for the point of departure. In this equation it is a NOAEL or LOAEL value ;
- ➔ POD_{sys} (mg/kg bw/day) – Dose descriptor for the systemic exposure to a substance;
- ➔ Bioavailability (%) – The extent to which a cosmetic ingredient reaches its site of action or can enter a biological fluid.

$$\mathbf{MoS} = \frac{\mathbf{POD_{sys}}}{\mathbf{SED}} = \frac{\mathbf{POD}}{\mathbf{SED}} \times \frac{\mathbf{Bioavailability}}{\mathbf{100}}$$

INCI	POD (mg/kg bw/day)	SED (mg/kg bw/day)	Bioavailability (%)	MoS
ALLIUM URSINUM EXTRACT	2200.0[1]	19.0	100.0	115.79
CALENDULA OFFICINALIS FLOWER EXTRACT	5000.0[2]	19.0	100.0	263.16
CERA ALBA	1200.0[3]	3.8	100.0	315.79
CITRUS LIMON PEEL OIL	340.0[4]	0.38	100.0	894.74
CUPRESSUS SEMPERVIRENS LEAF OIL	(no data)	0.38	100.0	---
MENTHA PIPERITA OIL	40.0[8]	0.38	100.0	105.26

PHENOXYETHANOL	357.0	2.69	100.0	132.71
LIMONENE	250.0	0.342	100.0	730.99
MENTHOL	188.0[17]	0.038	100.0	>1000
ROSMARINUS OFFICINALIS LEAF OIL	300.0[7]	0.038	100.0	>1000

Table 24 - Margin of Safety calculation of the ingredients

NOAEL comments

ALLIUM URSINUM EXTRACT

There was neither death recorded nor discernible gross pathological lesion seen in animals dosed with 300, 600, 1200 and 2200 mg/kg of the aqueous extract, thus 2200 mg/kg was considered as maximum tolerated dose.[1]

CALENDULA OFFICINALIS FLOWER EXTRACT

Silva et al. conducted a study of the acute effects of a hydroalcohol extract of C. officinalis L. in rats and mice. The extract was prepared from dried flowers extracted by hydroalcohol percolation. The material was evaporated to dryness andsuspended in distilled water at 350 to 450 mg/mL. Adult male and female Wistar rats and albino Swiss mice were used in this study (10 animals per group). Animals were fasted 12 hours prior to treatment, then administered the extract at 0 (distilled water only), 0.625, 1.25, 2.5, and 5.0 g/kg. Animals were observed daily for general behavioral changes, morbidity, and mortality. No deaths or morbidity were found in the control group or the treatment groups.[2]

CERA ALBA

The Panel concluded that the use of beeswax as an additive for the existing food uses and the proposed new food use is not of safety concern. The Panel noted that NOAELs identified in the toxicological studies on the main constituents of beeswax and plant waxes showing chemical structural similarities were 10 to 50 times higher than the very conservative exposure estimate of 22 mg/kg bw/day and were generally the highest doses tested. The Panel considered such margins of safety to be adequate for the assessment of beeswax which consists of components poorly absorbed from the gastrointestinal tract, which if absorbed to any extent at all, would be metabolised to compounds also occurring endogenously.[3]

CITRUS LIMON PEEL OIL

The NOAEL in this study is 1.2 mL/kg bw/day (equivalent to 1000 mg/kg bw/day) in males and 0.4 mL/kg bw/day (equivalent to 340 mg/kg bw/day) in females on the basis of decreased body weight and protein casts observed in the renal tubule at the next dose level. Food consumption was also

decreased in the intermediate dose females.[4]

MENTHOL

In a valid 2 years oral feed study in rats the NOAELs were 375 mg/kg bw/d for male rats and 667 mg/kg bw/d for male and female mice. For female rats the NOAEL is 188 mg/kg based on slightly reduced body weight at 375 mg/kg bw. For repeated dermal and inhalative toxicity no valid studies are available.[17]

ROSMARINUS OFFICINALIS LEAF OIL

Rosemary oil is not listed in Table 3 (harmonised classification and labelling of hazardous substances) of Annex VI to the CLP Regulation (EC) No 1272/2008 on the classification, labelling and packaging of substances and mixtures (Annex VI to CLP_ATP10 - in force from 1 December 2018). Based on the available experimental data and C&L information on the main constituents, the substance is classified for:

- Flammable liquid (H226)

- Aspiration hazard (H304), based on >10% constituents with aspiration hazard in the UVCB

- Skin irritation (H315), mainly based on >10% skin irritating constituents in the UVCB

- Skin sensitisation (H317), based on >1% skin sensitising constituents in the UVCB

- Eye irritation (H319), mainly based on >10% eye irritating constituents in the UVCB

- STOT SE 2 (H371), based on the content of Camphor in the UVCB

- Aquatic chronic toxicity (H411)[7]

It was not possible to calculate the value of MoS for all ingredients due to the lack of accurate values for NO(A)EL, LO(A)EL, BMD and VSD or the existence of inappropriate values to this exposure scenario. The ingredients for which it was not possible to calculate the MoS have low acute oral/dermal toxicity values and/or a long history of use without reported serious adverse effects. Therefore, it is not expectable that they can affect the overall product's safety.

10 Undesirable effects and serious undesirable effects

At the time of preparation of this report there were no reported undesirable and serious undesirable effects resulting from the application of Mystery kozmetika / Srijemuš LaVita - Mast Od Liofiliziranog Srijemuša.

11 Information on the cosmetic product

No more considerations.

PART B | COSMETIC PRODUCT SAFETY ASSESSMENT

1 Assessment conclusion

A detailed assessment was performed for the available information on the cosmetic product Mystery kozmetika / Srijemuš LaVita - Mast Od Liofiliziranog Srijemuša.

This product is safe, with restrictions, for human health when used under normal or reasonably foreseeable conditions.

The information provided in this report was assembled from reliable sources and based on current scientific knowledge. It is believed to be accurate at the date of this report's preparation.

For some ingredients, it was not possible to provide toxicological values or safety reference values, due to the inexistence of scientific studies. However, even without this information, based on the available information nowadays, it is unlikely that the ingredients of this specific formula, would exhibit toxicity or pose a risk for consumer's health. The specific ingredients that have been chosen for the manufacturing of this product have been used for years, for same products, without any known toxicity problems, under reasonable and foreseeable conditions of use. According to the available scientific information, all the ingredients are safe at the concentrations used in the formulation. The interaction between the ingredients in the cosmetic product and between these and the packaging material is not expected.

This assessment is valid until:

- Update or amendment to the current regulatory requirements concerning cosmetic products;

- Alteration of the cosmetic product's qualitative and quantitative formula;

- Alteration of the manufacturing method;

- Alteration of the packaging material and/or labelling;

- Safety Assessor has not timely access to information that may impact the risk-benefit ratio of the cosmetic product, such as information on serious adverse effects.

2 Labelled warnings and instructions of use

In accordance with the provisions of subparagraph d) of paragraph 1 of Article 19 of Regulation (EC) No 1223/2009 of November 30, 2009, it is not necessary to include any warning or instructions of use in the product's label.

3 Reasoning

Safe.

The product's safety assessment was based on the evaluation of the individual safety profile of each ingredient present in the formulation:

ALLIUM URSINUM EXTRACT

This profile is a read-across from Allium sativum:

The median lethal dose (LD50) was calculated to be 3034mg/kg (Table 4). According to the (Clarke and Clarke,1979), any substance whose LD50 is above 1000 mg/kg is regarded relatively safe. Similarly, WHO (1991) considers extract or agents with LD50 above 3000 mg/kg as essentially safe. The fact that in this study, high LD50 was obtained is an indication that this result is useful for the safety of persons, such as people who consume enough amounts of garlic or who handle or apply insecticide of garlic origin in agricultural crops or plantations.[1]

CALENDULA OFFICINALIS FLOWER EXTRACT

The Panel noted that animal safety test data on Calendulaextracts were available addressing acute, short-term, and sub-chronic toxicity, dermal irritation, sensitization, and photosensitization, ocular irritation, and genotoxicity. These data demonstrated an absence of adverse effects. Although limited carcinogenicity data were available in animal tests, one study suggested antitumor activity in vitro. Clinical testing demonstrated an absence of dermal irritation and infrequent sensitization reactions. Other safety test data of individual chemical components of Calendula (eg, lutein), likewise, did not suggest any adverse effects. There are no dermal reproductive or developmental toxicity data on Calendula extracts, but data on coriander oil, high in linalool and other terpenes, demonstrated that adverse effects occurred only at maternally toxic levels and did not occur at levels that were not maternally toxic.[2]

CERA ALBA

Experimental biochemical and toxicological studies on beeswax as such are lacking. However, the Panel considered that the safety of beeswax could be evaluated on the basis of published toxicological studies on its main constituents. The Panel considered that the data on beeswax itself were insufficient to establish an ADI, but concluded that the safety of beeswax could be assessed, based on available scientific literature on the main constituents of beeswax and plant waxes showing chemical structural similarities to beeswax, published since the last SCF evaluation. The Panel concluded that the use of beeswax as an additive for the existing food uses and the proposed new food use is not of safety concern. The Panel noted that NOAELs identified in the toxicological studies on the main constituents of beeswax and plant waxes showing chemical structural similarities were 10 to 50 times higher than the very conservative exposure estimate of 22 mg/kg bw/day and were generally the highest doses tested. The Panel considered such margins of safety to be adequate for the assessment of beeswax which consists of components poorly absorbed from the gastrointestinal tract, which if absorbed to any extent at all, would be metabolised to compounds also occurring endogenously. The Panel examined information on the presence of varroacides residues in beeswax and found that the active varroacides substances most often found in European samples of beeswax are fluvalinate, coumaphos and bromopropylate. The Panel noted that veterinary medicine residues and contaminants found in foodstuffs of animal origin are regulated by specific European legislation. The Panel noted that beeswax specifications for lead were set to 5 mg/kg in the European legislation and to 2 mg/kg by the Joint FAO/WHO Expert Committee on Food Additives (JECFA). The Panel considers that the specification for lead should be set as low as possible.[3]

CITRUS LIMON PEEL OIL

Lemon oil is a substance of Unknown or Variable composition, Complex reaction products or Biological material (UVCB substances), or more specifically an NCS (Natural Complex Substance). As such, lemon oil is part of the particular category of essential oils, extracts, fractions and distillation products of the citrus species of chapter 1.2, which are all variants of the botanical Rutacae family. A justification for read across within this category can be found in the “Reporting format for Natural Complexe Substances of the Citrus essential oils of the Rutacae family”. The other members of this category are orange oil, mandarin oil, lime oil, grapefruit oil, bitter orange oil, and tangerine oil. The category is based on the part of the plant from which the NCSs are produced (pericarb of the fruit), the common methods of production, the same dominant constituent (limonene), and the same and/or similar constituents.

The toxicokinetics assessment of the citrus oils will be based on the toxicokinetic assessment of dominant constituent limonene. For the other constituents, physical/chemical parameters will be used

to determine whether absorption via the oral, inhalation, and dermal route is expected. The major constituent of lemon oil, limonene, is expected to be readily absorbed by the oral and inhalation route. Dermal absorption is assumed to be lower, however, as no information is available on the amount of dermal absorption, it is assumed to be similar to oral absorption as a worst case. For the other constiuents of lemon oil, the absorption via the oral, inhalation and dermal route is expected to be moderate to high. The absorption via the oral, inhalation and dermal route is expected to be moderate to high for the other constituents of lemon oil. For risk assessment, a default absorption of 100% is assumed.[4]

CUPRESSUS SEMPERVIRENS LEAF OIL

In an acute oral toxicity study (limit test), ten rats were given a single oral dose of Cypress oil at 5000 mg/kg bw. Animals were observed for mortality and clinical signs for 14 days. 1/10 animal was died on Day 1. No clinical signs were observed. In this study, the oral LD50 of Cypress oil was higher than 5000 mg/kg bw in rats. Under the test conditions, oral LD50 of Cypress oil is higher than 5000 mg/kg bw in rats therefore it is not classified according to the Directive 67/548/EEC and the Regulation (EC) N° 1272-2008.[5]

MENTHA PIPERITA OIL

The Cosmetic Ingredient Review (CIR) Expert Panel has already assessed the safety of this ingredient; Also, the European Chemicals Agency (ECHA) has a registration dossier for Peppermint, ext. Mentha Piperita Oil is the volatile oil obtained from the whole plant of the Peppermint, Mentha piperita. Mentha Piperita Oil is a Generally Recognized as Safe (GRAS) ingredient for use in food for human consumption according to the U.S Food and Drug Administration (FDA); It has also been approved as an inactive ingredient in drug products. Dermal irritation was assessed in a study where 5 rabbits were injected intradermally with 0.05 ml Mentha Piperita Oil, between 5 and 10 times, and observed at 24 and 48 hours, at 1 and 2 weeks, and, in some cases, at 1 month after dosing; Moderate to severe reactions were observed. In a Human Repeated Insult Patch Test (HRIPT) on 20% Mentha Piperita Oil involving 104 subjects, results were negative for skin irritation and sensitization. In a human maximization test, 25 subjects received five applications of a 48-h occlusive induction patch containing 8% Mentha Piperita Oil; After a 10-day non-treatment period, the subjects were challenged with a 48-h patch and no evidence of sensitization was found. A multicentre study involving 13398 patients was also performed, whereby 71 patients were patch tested with Mentha Piperita Oil (2% in petrolatum); A positive reaction prevalence rate of 0.53% was reported. However, in another multicentre study, neither irritant nor allergic reactions were observed. Clinical cases of irritation and/or sensitization to Mentha Piperita Oil and/or its components have been reported; However, mostly positive patch test reactions to Mentha Piperita Oil and Mentha Piperita were reported on patients with diseased skin.[8] Ocular irritation was evaluated in a study where Mentha

Piperita Oil was applied to the eyes of eight rabbits; Afterwards, the eyes of 4 animals were rinsed and those of the remaining 4 were not rinsed; No effects and no reactions were observed; Under these conditions, Mentha Piperita Oil up to 60% was not an eye irritant.[9] The mutagenic potential of Mentha Piperita Oil was investigated using the Salmonella/mammalian microsome test using Salmonella typhimurium strains TA1535, TA100, TA1537, and TA98; The test substance was not mutagenic.[8] Mentha Piperita Oil was also non-mutagenic in mammalian cells. Overall, based on the available information, it can be concluded that this ingredient does not have genotoxic properties at non-cytotoxic concentrations.[9] In a carcinogenicity study of toothpaste and its components, groups of 52 mice were dosed by gavage with 4 or 16 mg Mentha Piperita Oil/kg/day, 6 days per week, for 80 weeks; No apparent differences were noted between the treated and nontreated groups. Undiluted Mentha Piperita Oil was not phototoxic when applied to the skin of mice.[8] No information on reproductive toxicity is available for Mentha Piperita Oil, however, no effects on development and/or reproduction were observed for 70.6% of its constituents; For the remaining constituents also no effects on development or reproduction are expected, as most of them are structurally similar (terpenes, terpenoids); Based on the absence of effects on development and reproduction in the major and some minor constituent, Mentha Piperita Oil not expected to be toxic to the development or reproduction.[9] The CIR Panel expressed concern about pesticide residues, heavy metals, and other plant species that may be present in botanical ingredients and they stressed that the cosmetics industry should continue to use current good manufacturing practices to limit impurities. The issue of incidental inhalation exposure was also discussed by the Panel, as Mentha piperita-derived ingredients are used in products that could possibly be inhaled; For example, Mentha Piperita Oil is used in both pump hair sprays (maximum use concentrations up to 0.02%) and aerosol hair sprays (maximum use concentrations up to 0.017%) which may result in incidental inhalation exposure; The Panel noted that droplets/particles from spray cosmetic products would not be respirable to any appreciable amount; Furthermore, droplets/particles deposited in the nasopharyngeal or bronchial regions of the respiratory tract present no toxicological concerns based on the chemical and biological properties of these ingredients; Coupled with the small actual exposure in the breathing zone and the use concentrations, the available information indicates that incidental inhalation would not be a significant route of exposure that might lead to local respiratory or systemic effects. For Mentha piperita-derived ingredients, the Panel was also concerned about the presence of terpenes (e.g., limonene) and terpenoids (e.g. menthol), which could result in sensitization and, thus, a non-sensitizing caveat related to the avoidance of a cumulative effect of multiple botanicals, in a single formulation, that share one or more constituents in common, was agreed. The CIR Expert Panel, therefore, concluded that Mentha Piperita Oil is safe in cosmetics in the present practices of use and concentration when formulated to be non-sensitizing.[8] Taking into account the available toxicological information, Mentha Piperita Oil is acceptable for use in the cosmetic products under assessment.

PHENOXYETHANOL

The Cosmetic Ingredient Review (CIR) Expert Panel has already assessed the safety of Phenoxyethanol; Also, the European Chemicals Agency (ECHA) has a registration dossier for this ingredient. In addition, Australia's National Industrial Chemicals Notification and Assessment Scheme (NICNAS) has published an assessment report on this ingredient. Also, the Scientific Committee on Consumer Safety (SCCS) has published an opinion on Phenoxyethanol. A 90-day dermal toxicity study in rabbits with an adjusted NOAEL of 357 mg/kg bw/day was used as the key study for the Margin of Safety (MoS) calculation by the SCCS.[13] Phenoxyethanol is practically nontoxic to slightly toxic when orally administered to rats and mice and practically nontoxic when administered dermally to rats. Phenoxyethanol is reported to slightly irritate the skin in animal studies, particularly following repeated exposure. Diluted Phenoxyethanol was neither an irritant nor a sensitizer to guinea pig skin, while the undiluted chemical was slightly-irritating to guinea pig skin.[12] A mild primary irritation was observed in one rabbit 1 hour after application, which was reversible within 24 hours; However, Phenoxyethanol was considered to be non-irritating. Dermal irritation was also assessed in a study performed in accordance to OECD Guideline 404 (Acute Dermal Irritation/Corrosion), where Phenoxyethanol was applied to the skin of six rabbits for 4 hours, under occlusive conditions; No oedema, but very slight erythema were noted in 2 of 6 animals; All erythema were reversible within 48 hours; Under the test conditions, the test substance was also not irritating to the skin.[11] Furthermore, Phenoxyethanol was reported to be non-irritating to human skin up to concentrations of 10 % in several patch tests studies. However, mild reactions were observed in five out of 12 human volunteers following application of undiluted Phenoxyethanol to the skin. Undiluted Phenoxyethanol was strongly irritating to rabbit eyes, while diluted Phenoxyethanol was either non-irritating or mildly irritating. In an eye irritation study in rabbits, Phenoxyethanol was found to be irritating with well-defined conjunctival redness and slight corneal opacity observed at 24, 48 and 72 hours; The majority of effects were reversible within the 15-day observation period.[12] Sensitization was investigated in a Guinea Pig Maximization Test (GPMT) performed in accordance to OECD Guideline 406 (Skin Sensitisation), where undiluted Phenoxyethanol was used for the challenge after intradermal and epicutaneous induction; The observations at 24 h and 48 h after challenge exposition revealed no reactions in any animal; Under these conditions, Phenoxyethanol was considered to be non-sensitizing. Further guinea pig sensitization studies supported this result.[11] In humans, there is limited evidence of skin reactions consistent with allergic sensitisation in several patch test studies that have been conducted; Also, weak sensitisation responses have been observed in some patients with contact dermatitis; However, several negative patch test studies for Phenoxyethanol tested up to 10% have been reported.[12] Phenoxyethanol was evaluated for mutagenicity in an Ames test, using Salmonella typhimurium strains TA 1535, TA 1537, TA 1538, TA 98, and TA 100, in presence and absence of metabolic activation; The test substance was considered non-mutagenic. Phenoxyethanol was also non-mutagenic in a Micronucleus test. Two repeated dose studies (104 weeks) performed in accordance to OECD Guideline 451 (Carcinogenicity Studies) in rats and mice are available; The available data

indicate that Phenoxyethanol is not carcinogenic. Phenoxyethanol was also non-phototoxic. The Panel noted that Phenoxyethanol may contain free phenol (less than 1%), or the diethoxylate of Phenoxyethanol; However, cosmetic-grade Phenoxyethanol is generally 98% pure and is free of ethylene oxide (less than 1 ppm). In the EU, Phenoxyethanol is listed as an approved cosmetic preservative. The CIR Expert Panel concluded that Phenoxyethanol is safe as a cosmetic ingredient in the present practices of use and concentration. In 2007, as part of the scheduled re-evaluation of ingredients, the CIR Expert Panel considered available new data on Phenoxyethanol and reaffirmed the above conclusion.[6] The SCCS considers Phenoxyethanol safe for use as a preservative with a maximum concentration of 1.0%, taking into account the information provided.[13] Taking into account the available toxicological information, the ingredient Phenoxyethanol is acceptable for use in cosmetic products under assessment.

LIMONENE

The Cosmetic Ingredient Review (CIR) Expert Panel hasn't already assessed the safety of this ingredient; However, the European Chemicals Agency (ECHA) has a registration dossier for d-Limonene. Limonene, is a monocyclic monounsaturated terpene and it occurs as the d and l isomers, and as the racemic mixture dl-limonene, known as dipentene. The Scientific Committee on Consumer Safety (SCCS) issued an opinion regarding allergens in fragrances, in which Limonene was included in the list of the 13 allergens which are less frequently reported and well-recognised as consumer allergens.[18] Limonene (d-limonene, l-limonene and dl-limonene) is considered to have fairly low toxicity. The skin irritancy of Limonene in guinea-pigs and rabbits is considered moderate and low, respectively. In an in vivo study of rabbit skin irritation, d-Limonene was ranked 3.5 of 8 on the basis of the primary irritation index; Effects were graded according to OECD Guideline 404 (Acute Dermal Irritation/Corrosion). In a study in rabbits, d-Limonene also caused irritation to the eyes. When Limonene was tested in four different sensitization assays with guinea-pigs (Open Epicutaneous Test, Maximization Test, Draize's Test, and a test with Freund's Complete Adjuvant), it was sensitizing in all but Draize's Test.[14] d-Limonene was also found to be a skin sensitizer in two Local Lymph Node Assays (LLNAs) conducted according to OECD Guideline 429 (Skin Sensitisation: Local Lymph Node Assay).[16] Although d-Limonene was once considered the main allergen in citrus fruits, data from studies in animals have revealed air-oxidized d-Limonene, rather than unoxidized d-Limonene, to be the sensitizing agent; Hydroperoxides and other oxidation products of d-Limonene formed on exposure to the air have proved to be potent contact allergens when tested with Freund's Complete Adjuvant in guinea-pigs, whereas unoxidized d-Limonene did not cause any sensitization. In a study in which the sensitivity of four patch testing systems was evaluated in human volunteers, d-Limonene (perfume-grade) reacted strongly in all types of patches within 10–15 minutes of exposure; Skin irritation was assessed before application, as well as immediately and 1, 24, 48, and 72 hours after removal of the patch, using a scoring system based broadly on that used for rabbit irritation studies, but modified to account for the nature of reactions on human skin; There was evidence of sensory

effects and urticarial responses on the removal of the patches; Significant irritation persisted for 24 hours, and these reactions persisted for 48 and 72 hours in many volunteers. On the basis of available data, there is no evidence that d-Limonene or its metabolites are genotoxic or mutagenic. Limonene and its epoxides were not mutagenic when tested in in vitro assays using different strains of Salmonella typhimurium, in the presence or absence of metabolic activation. No evidence of mutagenicity was reported in an in vivo spot test with mice, as well. The International Agency for Research on Cancer (IARC, 1993) has classified d-Limonene in Group 3 (not classifiable as to its carcinogenicity to humans) based on a lack of available data on carcinogenicity to humans and limited evidence for carcinogenicity in experimental animals. In its 41st meeting, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) withdrew the existing acceptable daily intake for d-Limonene. On the basis of the available data, the total daily intake of the chemical arising from its use at the levels necessary to achieve the desired effect and from its acceptable background levels in food did not, in the opinion of the Committee, represent a health hazard, and therefore the establishment of an acceptable daily intake expressed in numerical form was not deemed necessary. The restriction imposed by Regulation (EC) nº 1223/2009 of 30 November 2009 to the use of this ingredient in cosmetic products (peroxide value less than 20 mmoles/L) guarantees a reduction in oxidation products and consequently in sensitization potential.[14] [15] Taking into account the available toxicological information and the fulfilment of the restrictions to which it is subject, Limonene is acceptable for use in cosmetic products under assessment

MENTHOL

The available toxicity data indicate very similar toxicity profiles for D -, L-, D/L-menthol and the unspecified menthol isomer mixture. In mammalian species the low toxicity is manifested in LD50 values generally greater than 2000 mg/kg bw in acute studies, limited toxicity in repeated dose studies, and no effects in teratology evaluations. Irritation to skin and eyes was slight to moderate. The low hazard potential is not unexpected, since the FDA regulates menthol as a GRAS (generally recognized as safe) component and an acceptable daily intake (ADI) of 0-4 mg/kg bw for L- menthol and D/L-menthol was adopted in 1999 by the Joint FAO/WHO Committee.[17]

ROSMARINUS OFFICINALIS LEAF OIL

In an acute oral toxicity (limit test) study, a group of 5 rats/sex/dose were given a single oral dose of Rosemary oil at 5 mL/kg bw. Animals were then observed for mortality, clinical signs and body weight for 14 days and were all sacrificed for macroscopic examination.Four males were dead at 24 h and one male was sluggish and unthrifty in appearance. Facial grooming, increased spontaneous activity and salivation were noted in all females at 0.5 h with one exhibiting an apparent equilibrium problem as evidenced by head tilting. Ataxia and slight CNS depression were noted at 2 h, and rigidity and sluggishness at 4 h. Unthriftiness and sluggishness were noted at 24 h. All females appeared normal

at 48 h and thereafter. Facial grooming, salivation and increased spontaneous activity were noted in two males at 0.5 and 1 h. Slight ataxia and slight CNS depression were noted in 3 males at 2 h. Occassional tremors in one male and withdrawl in one male were also noted at 2 h. All males exhibited rigidity and sluggishness at 4 h. The surviving male appeared normal at 48 h and thereafter. Mean body weight gain of the females was 50 g and the body weight of surviving male was 43 g. Gross pathological findings of the four males that died consisted of gastric hyperemia. No gross changes were noted in animals necropsied after 14 day observation period. In this study, the oral LD50 of test item was 5 mL/kg bw in rats. Under the test conditions, the oral LD50 for Rosemary oil is 5000 mg/kg bw in rats. Based on this result, the substance is considered to be not acute toxic via the oral route.

Rosemary oil was tested in an acute dermal toxicity study similar to OECD TG 402. Four New Zealand White rabbits were exposed to the test substance via dermal application. 2 males and 2 females were exposed to 10 mL/kg bw undiluted test substance under occlusive conditions for 24 hours. The substance was applied on the back and flanks of the rabbits. The skin was clipped and intact in two rabbits (one male and one female) and clipped and abraded in the other two animals. Skin responses consisted of very slight to welldefined erythema and slight to very slight edema at 24 hours to moderate erythema and induration with skin sloughing by 14 days. After an observation period of 14 days animals were necropsied. Ataxia was noted in one rabbit for three days (animal with abraded skin). On day 4 this rabbit died. No toxic symptoms were observed in the remaining rabbits. Body weight gain of the three surviving animals averaged 0.3 kg. No gross pathological findings were noted in any of the animals. The LD50 was determined to be higher than 10 mL/kg bw corresponding with ca. 8000 mg/kg bw. Based on this result, the substance is considered to be not acute toxic via the dermal route.[7]

4 Assessor’s credentials and approval of part B

This report was prepared with information provided by the company Mystery kozmetika, as well as available scientific information about the ingredients used in this formulation.

The author of this report has the qualifications required in the pharmaceutical and toxicological areas, according to the defined in paragraph 2 of Article 10 of Regulation (EC) 1223/2009.

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ANNEX | TO THE COSMETIC PRODUCT SAFETY REPORT

Annexes of the Cosmetic Product Safety Report can be found on the following pages.

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