Technical Information

HyaCare® 50

The tight junction strengthening Hyaluronic Acid

Intended use

Active for skin care

Benefits at a glance

- 50 kDa hyaluronic acid
- Supplies hyaluronic acid into the skin
- Increases the content of hyaluronic acid
- Rejuvenates the skin by improving its viscoelastic properties
- Fills wrinkles from inside
- Reduces deep wrinkles significantly
- Reduces crow feet
- Usage concentration: 0.01 – 0.2%

INCI (PCPC name)

Hydrolyzed Hyaluronic Acid

Chemical and physical properties (not part of specifications)

Form	Powder
Molecular weight	20 – 70 kDa
Solubility in water	10 g/100 g

Hyaluronic acid (HA) is a non-sulfated glycosaminoglycan (GAG), naturally distributed in human body. Hyaluronic acid is the major component of the extra cellular matrix. It is found in high quantity in the skin where it is produced by fibroblasts and keratinocytes.

One of its main functions is to store water in the extracellular matrix of the connective tissue. This water-binding capacity contributes significantly to the elasticity of the skin. Hyaluronic acid plays the role of water pump maintaining the elasticity by acting as a water reservoir. Hyaluronic acid is also involved in tissue repair. While it is abundant in extracellular matrices, it also contributes to tissue hydrodynamics, movement and proliferation of cells, and participates in a number of cell surface receptor interactions, notably those including its primary receptor, CD44.

When skin is excessively exposed to UVB rays, it becomes inflamed and the cells in the dermis stop producing hyaluronic acid and increase the rate of its degradation. With aging, the quantity of hyaluronic acid and its degree of polymerisation decreases leading to a decrease in the water content hold by the connective tissue and a loss of elasticity that manifests itself in wrinkles.

In normal human skin, hyaluronic acid exists as a polymer of medium molecular weight (600 to 1,000 kDa). The physiological degradation of hyaluronic acid in the skin proceeds via internalisation by the keratinocytes and fragmentation by hyaluronidases. High molecular weight hyaluronic acid has been used for many years as natural moisturizer in the cosmetic industry. In 2003 the FDA has approved hyaluronic acid injections for filling soft tissue defects such as facial wrinkles. Hyaluronic acid injections temporarily smooth wrinkles by adding volume under the skin, with effects typically lasting for six months.

This FDA approval and the significant trend for botox like cosmetics reinforced the demand for hyaluronic acid. Hyaluronic acid is now not only considered a moisturizer but as a relevant anti-aging ingredient. Our in vivo data on our medium molecular weight HyaCare® confirmed this statement.

In parallel, intensive developments have been made to check if molecular weight reduction would provide even more skin care benefits. This lead to the introduction of HyaCare® 50, a new and unique, very low molecular weight hyaluronic acid (50 kDa) produced by fermentation of Bacillus subtilisis using an environmentally friendly aqueous recovery process.

Properties

Ex vivo permeation study

Method: The penetration of different molecular weight fractions has been evaluated using side by side diffusion cells and tritiated hyaluronic acid on dermatomed skin from the porcine ear. The receptor was filled with PBS buffer and the donor with radio labelled HA solution. After incubation for 5 h and 22 h at room temperature, the receptor phase was removed and radioactivity was determined by liquid scintillation counting.

Results

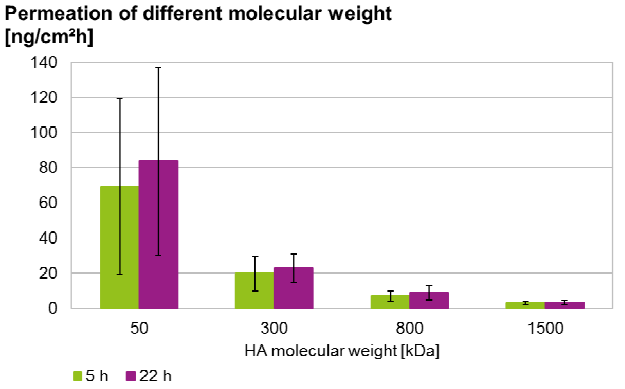

Figure 1: Influence of hyaluronic acid molecular weight on percutaneous transport

As seen in figure 1, HyaCare® 50 shows a much stronger permeation through the skin in comparison to higher molecular weight products.

In vitro gene expression analysis

Method: The study was performed at the Department of Clinical Chemistry at the University of Regensburg under supervision of Professor Gerd Schmitz.

SkinEthicTM reconstructed epidermis models were used. A thin, liquid formulation containing 0.5% HyaCare® 50, 0.5% HyaCare® or no active ingredient (vehicle) were topically applied for 48 hours. After that the skin tissue was harvested, lysed and RNA was isolated. The RNA from the skin models was transcribed into DNA and labelled with fluorescent dyes. Affymetrix® HGU133 Gene Chips were used to characterize genome wide expression.

Results: It was demonstrated that HyaCare® 50 was able to influence gene expression significantly in comparison to HyaCare®. More than 40 genes were regulated, including upregulation of junctional control genes.

Tight junction and adherens junction proteins are important for the barrier function of the skin and the cellular elasticity.

In vitro protein expression analysis

In a previous gene expression study an upregulation of junctional genes by HyaCare® 50 was observed. Tight and adherens junction proteins connect the cells with each other and are very important for molecule transport, maintenance of ion homeostasis and protection of the viable layers of the epidermis. Therefore, the effect of HyaCare® 50 also on the expression of junctional proteins was analyzed.

Method: The study was performed at the University of Regensburg under supervision of Professor Gerd Schmitz.

For this study reconstructed human epidermis models (SkinEthic) were topically treated with 0.05% and 0.5% HyaCare® 50 for 72 h (n=4). After treatment the proteins were extracted and junctional proteins were analysed and quantified by Western Blotting (Nu-PAGE® Novex 4 12% Bis-Tris Gel (Invitrogen) using protein specific antibodies. The resulting intensities were standardized to GAPDH (Glyceraldehyde 3-phosphate dehydrogenase) by LumiAnalyst 3.0 in Biochemical Light Units [BLU]. Results:

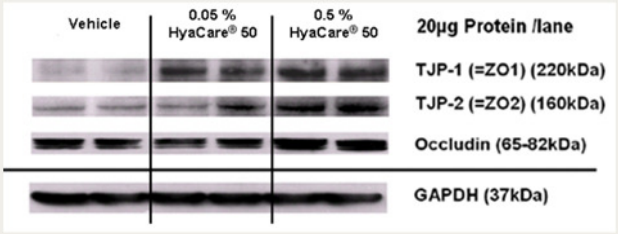

Figure 2: Example of Western Blot analysis of signify-cantly induced Tight Junction proteins (TJPs) in SkinEthic models treated with different concentrations of HyaCare® 50.

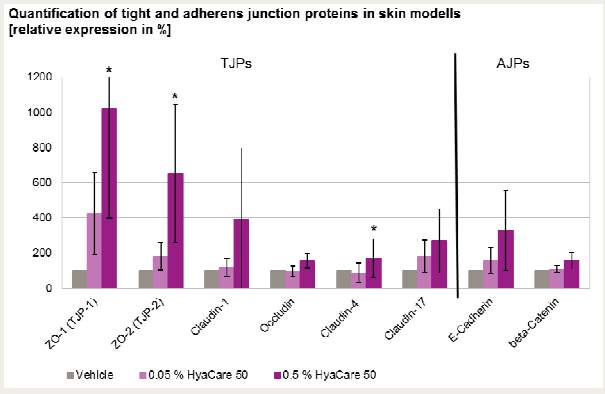

Figure 3: Quantification of TJPs and Adherens Junction proteins (AJPs) in SkinEthic skin models vs. GAPDH (*p<0.05).

The results of this study show that HyaCare®50 induces junctional proteins in human epidermis models. This confirms previous results obtained by gene expression analysis.

Properly performing Tight Junctions guarantee well balanced ion homeostasis, they protect the viable layers of the skin and finally lead to healthier, younger looking skin.

In vitro gene expression study

In this study the influence of HyaCare® 50 on Hyaluronan synthesis in human dermal fibroblasts was analyzed.

Method: The cells were treated with 0.001% and 0.01% HyaCare®50 for 24 h (n=3). After treatment the RNA was extracted and genes of the Hyaluronan synthesis/degradation pathways were analysed by quantitative real time PCR (qRT-PCR). The results were normalized to GAPDH (Glyceraldehyde 3-phosphate dehydrogenase) and compared to vehicle control. Results:

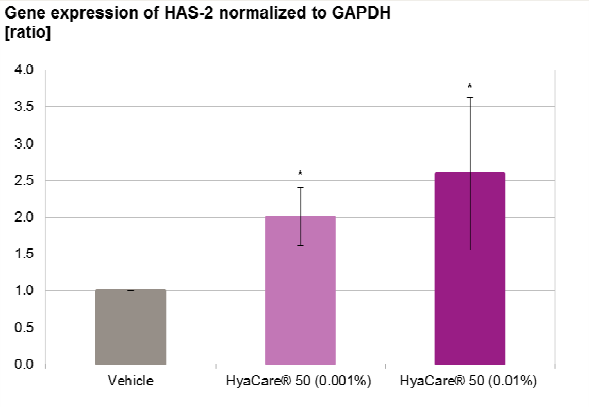

Figure 4: Expression of HAS-2 gene in human dermal fibroblasts treated with different concentrations of HyaCare® 50 compared to vehicle control and normalized to GAPDH (*p<0.05).

HAS-2 is the major Hyaluronan Synthase in fibroblasts and produces high molecular weight hyaluronic acid, which plays a very important role in the formation of the extracellular matrix. The up-regulation of this enzyme by HyaCare® 50 leads to a higher HAS-2 synthesis rate and therefore an induced production of hyaluronic acid in dermal fibroblasts. This leads to an increased content of Hyaluronic Acid in the skin. HyaCare® 50 fills the wrinkles from inside.

In vivo efficacy study

Long-term evaluation of skin elasticity and roughness

Method: The study was performed at ISPE s.r.l., Milan (Italy).

For the study 12 volunteers (female, mean age 51) were recruited. Each volunteer was required not to cleanse or moisture their faces for a minimum of at least 3 hours before the test procedure.

The study was carried out in a climatic room with defined conditions of 24 °C and 50% relative humidity. The assessment of the skin hydration was performed on the peri-ocular area of the face where an O/W cream without (vehicle) and with 0.1% HyaCare® 50 was tested and compared to each other (Half-side test). The application of the two products was randomized among the subject for each area. The volunteers applied the test formulation twice daily for 2 month.

After 4 weeks and at the end of the study skin elasticity and skin roughness was measured.

The skin elasticity was assessed with a Cutometer SEM 575 (Courage & Khazaka, Germany) Different parameters describing the skin elasticity were calculated. The parameter R2 describes the overall elasticity (R2 = UA/UF) while by R6 the viscoelastic ratio is calculated (R6 = UV/UE).

For the measurement of the wrinkle reduction skin replicas were prepared using a fast hardening synthetic polymer (Silflo, Flexico Ltd, UK) and an adhesive disc (3M, 24x40 mm). The replicas were analyzed by special image processing software (Quantilines, Monaderm). This software allows the calculation of Rz, the maximum roughness value. Rz describes especially the deep wrinkles.

Results: The area treated with the formulation containing HyaCare® 50 showed a significant increase of the overall elasticity value, equal to 14% after 4 and 8 weeks of application (figure 5).

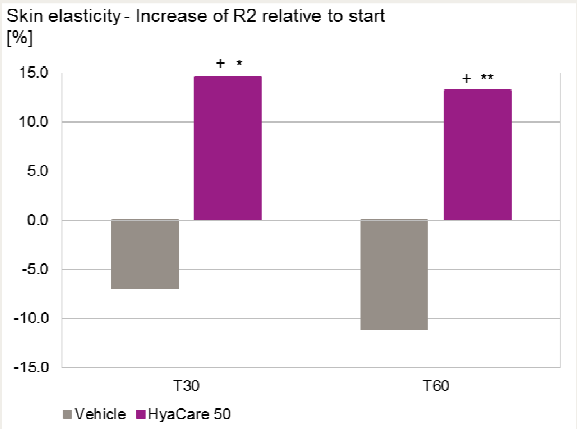

Figure 5: Overall skin elasticity after 4 and 8 weeks application of test formulations (+ significant compared to start, * significant compared to vehicle, ** highly significant compared to vehicle).

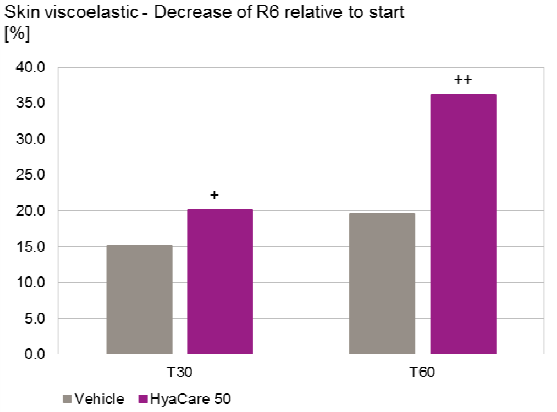

Figure 6: Decrease in the skin viscoelastic ratio after 4 and 8 weeks application of test formulations (+ signify-cant compared to start, ++ highly significant compared to start).

The area treated with HyaCare® 50 showed as well a highly significant decrease in the viscoelastic ratio (R6) equal to 36% after 8 weeks. This reflects the rejuvenation of the skin through the increase of its elastic component (figure 6).

Skin image analysis showed a significant decrease of maximum skin roughness of 10% of the area treated with HyaCare® 50 (figure 7).

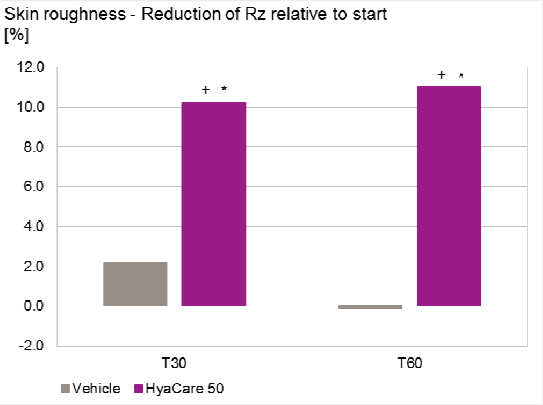

Figure 7: Maximum Roughness ratio after 4 and 8 weeks application of test formulations (+ significant compared to start, * significant compared to vehicle).

These data confirmed the anti-wrinkle activity of HyaCare® 50 mainly in terms of decrease of deep wrinkles as illustrated in figures 8 and visible reduction of crow feet as shown in figure 9.

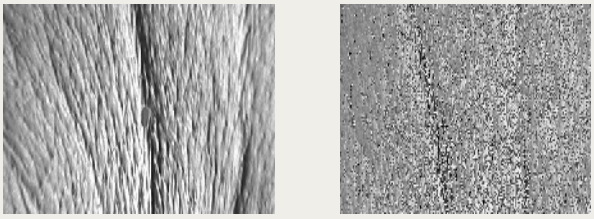

Figure 8: Skin image analysis before and after 4 weeks of treatment with HyaCare® 50.

Figure 9: Skin image analysis before, after 4 and 8 weeks of treatment with HyaCare® 50.

Preparation

HyaCare® 50 is completely soluble in water and can be processed cold. For the preparation of both O/W or W/O emulsions it should be added to the water phase. In O/W emulsions HyaCare® 50 might reduce the viscosity of the emulsion. The desired viscosity of O/W emulsions can be ad-justed by increasing the amount of polyacrylates (e. g. TEGO® Carbomer) or by increasing the con-centration of consistency enhancers, e. g. TEGO® Alkanol 18 (Stearyl Alcohol), TEGO® Alkanol 1618 (Cetearyl Alcohol), TEGIN® M or TEGIN® 4100 (Glyceryl Stearate). HyaCare® 50 shows good compatibility with other ingredients.

Recommended usage concentration

0.01 – 0.2% of HyaCare® 50

Applications

HyaCare® 50 is suitable for O/W and W/O formulations of:

- Anti-wrinkle eye creams
- Anti-wrinkle face cream
- Anti-aging foundations

Hazardous goods classification

Information concerning

- classification and labelling according to regulations for transport of chemicals
- protective measures for storage and handling
- measures in case of accidents and fire
- toxicological and ecotoxicological effects

is given in our safety data sheets.

Guideline formulations

Fresh and Mild Eye-Roll-On (CHN BR084-1-3)	
Phase A	
TEGO® Care PBS 6 (Polyglyceryl-6 Stearate (and) Polyglyceryl-6-Behenate)	3.00%
TEGO® Alkanol 1618 (Cetearyl Alcohol)	0.20%
TEGOSOFT® DC (Decyl Cocoate)	4.00%
TEGOSOFT® OER (Oleyl Erucate)	3.00%
TEGOSOFT® SH (Stearyl Heptanoate)	3.0%
TEGO® Xymenynic (Caprylic/Capric Triglyceride; Xymenynic Acid)	0.40%
Helianthus Annuus (Sunflower) Seed Oil	0.50%
Phase B	
HyaCare® (Sodium Hyaluronate)	0.05%
HyaCare® 50	0.05%
TEGO® Cosmo C 100 (Creatine)	0.50%
Glycerin	3.00%
Panthenol	0.00%
Allantoin	0.20%
Keltrol CG-SFT (Xanthan Gum)	02.0%
Water	46.68%
Phase C	
Water	30.00%
KELCOGEL CG-HA (Gellan Gum)	0.02%
Phase D	
TEGO® Arjuna S (Terminalia arjuna extract; Pentylene Glycol)	2.00%
Phase E	
Microcare SBB (Benzyl Alchol; Benzoic Acid; Sorbic Acid)	0.70%
Phase Z	
Sodium Hydroxid (10% in water)	q. s.

Preparation

1. Heat phase A and B separately to approx. 70 – 75 °C.
2. Add phase A to phase B with stirring.[1)]
3. Homogenize.
4. Gellen Gum should be dispersed in pure water and add phase C to about 85 °C for 25 minutes.
5. Add phase C, homogenize for a short while.
6. Cool down with gentle stirring add phases D, E, Z below 40 °C.

[1)] Important:

If phase A has to be charged into the vessel first, phase B must be added **without stirring**.

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Power Serum for aged Skin (MM 216/2)	
Phase A	
TEGO® Care 450 (Polyglyceryl-3 Methyl-glucose Distearate)	2.0%
TEGOSOFT® DEC (Diethylhexyl Carbonate)	5.0%
TEGOSOFT® OP (Ethylhexyl Palmitate)	5.0%
TEGOSOFT® OER (Oleyl Erucate)TEGOSOFT* OP (Ethylhexyl Palmitate)	1.5%
Persea Gratissima (Avocado) Oil	1.5%
Phase B	
TEGO® Pep 4-17 (Tetrapeptide-2; Glycerin; Butylene Glycol; Aqua)	2.0%
HyaCare® 50	0.1%
Glycerin	3.0%
Water	75.2%
Phase C	
TEGO® Carbomer 141 (Carbomer)	0.2%
TEGOSOFT® OP (Ethylhexyl Palmitate)	0.8%
Phase D	
TEGO® Stemlastin (Cyanidium Caldarium Extract)	3.0%
Phase E	
Sodium Hydroxid (10% in water)	q. s.
Phase F	
Euxyl PE 9010 (Phenoxyethanol, Ethylhexylglycerin)	0,7%
Phase Z	
Perfume	q. s.

Preparation

1. Heat phase A and B separately to approx. 70 to 75 °C.
2. Add phase A to phase B with stirring.[1)]
3. Homogenize.
4. Cool with gentle stirring to approx. 60 °C and add phase C
5. Homogenize for a short time
6. Cool with gentle stirring and add phases D, E and F below 40°C

[1)] Important:

If phase A has to be charged into the vessel first, phase B must be added **without stirring**.

Remarks

Viscosity: 15 Pas (Brookfield RV DV-1, sp 5,5 rpm).

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EVONIK
POWER TO CREATE

Product specification

Material HYACARE 50
Spec.Code K00 STANDARD

Evonik Nutrition & Care GmbH
Personal Care Business Line
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45127 Essen
Phone: + 49 (201) 173-2524
Fax: + 49 (201) 173-1828
http://www.evonik.com/personal-care
personal-care@evonik.com

Inspection Characteristics	Method	Limits	Units	Z
Identification	GM_1559_01	conform		C
Salts of Hyaluronate	GM_1576_01	> = 90	%	X
Glucuronic Acid	GM_1575_01	> = 43.5	%	X
Molecular Weight	GM_1577_01	20-70	KdA	C
pH-Value	GM_1565_04	4.5-8.5		X
Ash content	GM_1574_01	< = 20	%	X
Loss on drying	GM_1552_02	< = 10	%	X
Protein	GM_1573_01	< = 0.1	%	X
Iron	GM_1551_03	< = 80	ppm	X
Heavy Metals	GM_1551_03	< = 20	ppm	X
Arsenic	GM_1551_03	< = 2	ppm	X
Total Plate Count	GM_3000_04	< 100	CFU/g	X

Identification	conform

Report on inspection certificate: X = specific/actual value, C = unspecific value/conformity, T = not reported

Appearance @ 25°C: white to beige powder

pH determined as a 0.5% solution in water

This document is computer printed and therefore valid without signature.
All warranty claims in respect of the conformity of our product are subject to our General Terms and Conditions of Sale and Delivery. The data listed above reflects the criteria for our internal quality tests. We do not hereby make any express or implied warranty, whether for specific properties or for fitness for any particular application or purpose. All values are valid for the product when despatched from the works.

The Standard Test Methods can be obtained from specialized publishers. Evonik's test methods are available on request.

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Mat. Number G305694

HyaCare® 50

Product data record (PDR)

1. General information

1.1 Supplier

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Personal Care Business Line
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D-45127 Essen / Germany
Phone: +49 (201) 173-2524
Fax: +49 (201) 173-1828
personal-care@evonik.com
http://www.evonik.com/personal-care

1.2 Product Description

1.2.1 Raw material category Cosmetic Active Ingredient based on Hyaluronic Acid

1.2.2 Ingredients according to INCI

Hydrolyzed Hyaluronic acid

1.2.3 Composition

Components	Source	Ratio
Hydrolyzed Hyaluronic acid	vegetable / microbial	100 %

This composition information serves for information of our customers only.
It is neither relevant for the composition listing according to Regulation (EC) No 1223/2009, nor does it reflect the chemical composition according to the different chemical regulations in the world which is disclosed in the table "information on ingredients/hazardous components" in the relevant parts of the respective (Material) Safety Data Sheets.

1.2.4 Solvents, preservatives and other additives

	CAS No.	EINECS / EC No.	content	Function
no additives				

Unless mentioned in our PDR under section 2.1 (By products) or 2.2 (CMR), no components which are listed in Annex II of the Regulation (EC) No 1223/2009 and its modifications and updates are added to and are not to be expected in the above mentioned product due to the raw materials used and the production process.

2. Information on production process

General description of production process:
HyaCare® 50 is produced using Biotechnology (microbial synthesis, water based recovery process, concentration, purification, controlled hydrolysis to a MW range of 20 – 70 kDa, spray drying to a fine powder).

Hyaluronic acid (HA) is produced by fermentation. The production host is a well known strain of Bacillus subtilis with a long history of safe use in industry. The production strain was constructed by insertion of the HA synthetic genes from a strain of Streptococcus equisimilis. The production organism is genetically modified. Fermentation and recovery, including the use of the production strain, takes place under the EU directive for contained use.

HyaCare® 50 is produced in the strictest absence of any animal derived material of any type.

HyaCare® 50 final product is free of genetically modified organism (GMO).

2.1 By products

		method
Residual solvents	not applicable	
Free amines	not applicable	Chromatography
Nitrosamines	not applicable	
Monochloroacetic acid	not applicable	Chromatography
Dichloroacetic acid	not applicable	Chromatography
1,4-Dioxane	not applicable	
Pesticides	meets the valid regulatory requirements for limits on agricultural pesticides	
Heavy metals (Cu; Pb; Pt; Pd; Hg; As; Cd; Ni)	max. 20 ppm	AAS-ICP
As	max. 2 ppm	AAS-ICP
Latex	not to be expected in the product due to the raw materials used and the production process	
VOC	< 3 % according to SR (Swiss Right) 814.018	

2.2 CMR (Carcinogenic, Mutagenic or Reprotoxic)

The use in cosmetic products of substances classified as CMR substances, of category 1A or 1B or 2 under Part 3 of Annex VI to Regulation (EC) No 1272/2008 shall be prohibited.

Further Information:
http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2009:342:0059:0209:en:PDF

Some of the CMR substances mentioned below and listed in Annex VI to Regulation (EC) No 1272/2008 are used as starting materials or solvents for the production of our cosmetic raw materials and may require reporting under California Proposition 65 or the Safe Cosmetics Act, SB 484.

The presence of these prohibited substances has to be seen as non-intended. It is stemming from impurities of the starting materials or the manufacturing process which is technically unavoidable in good manufacturing practice.

CMR substance	Starting material	max. concentration	method
Ethylene Oxide	no		
Propylene Oxide	no		
Octamethylcyclotetrasiloxane (D4)	no		
2-Ethylhexanoic Acid	no		
n-Hexane	no		
Methyl Chloride	no		
Dimethyl Sulphate	no		

2.3 "Allergens" according to the Regulation (EC) No 1223/2009

The presence of substances, the mentioning of which is required under the column 'Other' in Annex III, shall be indicated in the list of ingredients in addition to the terms perfume or aroma.

The cosmetic raw materials and the cosmetic actives supplied by Evonik Personal Care are manufactured without the use of perfumes and fragrances. An analytical proof for the absence in traces of the substances to be mentioned in addition to the terms perfume or aroma is not performed in cosmetic raw materials, which are chemically produced.

None of these substances have been intentionally added to our cosmetic raw materials or are formed during the manufacturing process according to our knowledge of the chemistry.

2.4 Food Ingredients listed in Annex IIIa of Commission Directive 2007/68/EC.

None of these substances have been intentionally added to our cosmetic raw materials or are formed during the manufacturing process according to our knowledge of the chemistry.

3. Microbiological status

Total Viable Count	max. 100 cfu/g
Pathogens*	absent/g

*Pathogens are: Enterobacteria, Pseudomonas, Enterococci, Candida albicans, Staphylococci

4. Shelf life / storage conditions

1080 days after production at 4 – 10 °C (in unopened original packaging under clean and dry conditions). Product is hydroscopic. Transport and short-term storage (up to 1 week) at RT (22 +/-2 °C) possible.

5. Regulatory Status

5.1 HS-Code 391390
EU-CN-Code 39139000

5.2 Regulatory status (chemical regulations)

Europe

Components	REACH status	CAS No.	EINECS / EC No.
Hydrolyzed Hyaluronic acid	Polymer	9004-61-9	232-678-0

Other countries

Country		yes / no	Remark
Australia	AICS:	yes	
China	IECSC:	yes	
Canada	DSL: NDSL:	yes	
Taiwan	TCSI:	yes	

In the following countries the relevant authorities currently do not require pre-market approval for cosmetic raw materials:

Brazil, Japan, South Korea, Philippines, USA

5.2.1 Regulatory status (cosmetic regulation)

Country		yes / no	Remark
China	CFDA:	yes	
Japan	JSQI:	no	

6. Toxicology and Ecotoxicology

Refer to summary of ecotoxicological and toxicological data

7. Packaging

0.5 kg