Ministry of Environment of Denmark
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Survey and risk assessment of eyelash and eyebrow serums

Survey of chemical substances in consumer products No. 202

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Title:
Survey and risk assessment of eyelash and eyebrow serums

Purpose:
This project's purpose is to yield information about eyelash and eyebrow serums whose active ingredients are not prostaglandins or prostaglandin analogues. It accomplishes this through a sur-vey of eyelash and eyebrow serum products available on the market, primarily by reviewing ingre-dient lists on these products. Furthermore, the purpose of the project has been to conduct quantita-tive chemical analyses of selected ingredients and perform a risk assessment on them. In sum-mary, the purpose of the project is to investigate the content of specific selected active ingredients in eyelash and eyebrow serums, and to assess whether they are safe for consumers to use.

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Foreword

Survey and risk assessment of eyelash and eyebrow serums

This project investigated the active ingredients used in two specific types of cosmetic products: eyelash serums and eyebrow serums. This report presents the results of the survey of these two types of cosmetic products, as well as chemical analyses and hazard assessments for selected ingredients.

The project was carried out by FORCE Technology (survey and chemical analyses), with DHI as hazard assessment subcontractor.

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Summary

Eyelash and eyebrow serums are products that ostensibly promote the growth of and/or reduce the loss of eyelash and eyebrow hairs. Originally, the specific active ingredients used were prostaglandins and synthetic analogues of prostaglandins. In 2021, the EU's Scientific Committee on Consumer Safety (SCCS) investigated the safety of the use of these active ingredients in cosmetic products due to adverse effects caused by products of this type. In the assessment, the SCCS expressed a cause for concern about the use of prostaglandin analogues in cosmetic products.

Today, there are prostaglandin-free alternatives on the market. The active ingredients in these products are typically peptides. Peptides are organic substances composed of and linked together by different amino acids. Amino acids are found in all living organisms; these substances are the building blocks of proteins. There are typically 20 specific amino acids that are biologically used as building blocks for the formation of proteins and peptides.

Purpose
This project's purpose was to yield information about eyelash and eyebrow serums whose active ingredients are not prostaglandins or prostaglandin analogues. This is accomplished through a survey of eyelash and eyebrow serum products available on the market, primarily by reviewing ingredient lists on these products. In addition, it the project aimed to assess whether selected specific active ingredients in eyelash and eyebrow serums are safe for consumers to use.

Survey of serum products on the market
In the survey, a total of 58 unique serum products for eyelashes, eyebrows, or both were identified on online shops and platforms based in Denmark (DK), the EU, and non-EU countries. Only those serum products where a full ingredient list was available online were selected. For this reason, a sizeable majority of the selected products were Danish (more than half) or from the EU (more than one-third). Only five products with ingredient lists were identified on non-EU online platforms. For this reason, it was decided not to purchase serum products from non-EU countries for the chemical analyses. Of the 58 serum products, 14 products (24%) contained prostaglandins or prostaglandin analogues.

The information about the serum products presented on the online shops, as well as the product ingredient lists for the remaining 44 products without prostaglandins or prostaglandin analogues, were examined further. This review showed that the purpose of the vast majority of serum products (41 out of 44) is to increase the growth of hair strands, make them thicker/fuller, or both. Alternatively, some serum products presented their primary function as "protects against damage" or "strengthens lashes and brows".

Serum products are generally expensive. They typically contain between 5 and 10 ml of serum, but quantities as small as 2.4 ml and as large as 15 ml have been observed. Prices range from 30 DKK (for 10 ml) to 595 DKK (for 3 ml), equivalent to a range of approximately 30-2000 DKK per 10 ml. The target group for these types of products is not always specifically stated on either the products themselves or the websites selling them, but the products are typically found alongside products for women, or women are pictured using the products. Four products (of the 44) specifically state that they can be used by both men and women. One product recommends that it is only to be used by those ages of 16 years and up.
Most serums indicated that they were to be applied to the roots of the lashes and/or roots of the brows 1-2 times a day. Most serum products are designed to be applied with a small, fine,

round brush. Others are designed to be applied with a larger mascara-type brush or another application method. For most serum products, whether for brows or lashes, it was indicated that they should be used for a minimum of 4-8 weeks before an effect can be expected. In some cases, this period was up to 16 weeks. Most manufacturers state that use must be continued to maintain the desired effect; in other words, these products may potentially be used for long periods of time (years).
The amount of these serum products used per day is very small. In sales contexts, examples were found indicating (for example) that one serum product can last for six months of once-daily use on both brows and lashes. As a rough estimate, this corresponds to an amount down to about 0.06 ml per day, or about 60 mg per day (assuming a density of 1 g/ml). In the project, application experiments were conducted on artificial eyelashes and eyebrows where the amount applied was up to approximately 46 mg per application. However, the amount used varied greatly due to the wide variance in the consistency of the serum products purchased.

Ingredients in brow and lash serums
When reviewing the ingredient lists for the 44 serum products, the focus was on active ingredient content, plant extracts / essential oils, fragrances, and potentially problematic ingredients, such as allergens or suspected endocrine disruptors. The review showed that the majority of serum products contained peptides (82%) and/or amino acids (which peptides are made of) (18%) as active ingredients. A single peptide (Myristoyl Pentapeptide-17) was used in almost half of the surveyed products. Serum products without either peptides or amino acids (11%) contained vitamins (B3, B5, B7 or E) and/or various plant extracts. Vitamins were used in the majority of serum products (77%), with vitamins B5 and B7 being the most common vitamins. A total of 131 unique plant extracts were identified in the 44 serum products. The majority of serum products (82%) contained one or more plant extracts. It was clear that each manufacturer uses specific plant extracts, as each individual plant extract ingredient was found in no more than six of the 44 serum products. Hyaluronic acid, which exists naturally in all tissues where it is synthesised and binds water, was used in just over half of the serum products surveyed.

One serum product was identified to contain fragrance, but without listing any of the fragrances subject to declaration (cf. the Cosmetic Products Regulation). In addition, three products were identified to contain benzyl alcohol, which is one of the fragrances that must be declared, but which can also be used as a preservative. Four serum products contained some of the new fragrances that are mandatory to declare, such as various menthol, peppermint and citrus extracts. Other potentially problematic ingredients identified included methylparaben (a suspected endocrine disruptor) in one of the 44 serum products, and salicylic acid (a suspected endocrine disruptor) in two of the 44 serum products.

It was not considered relevant to measure the pH values of the serum products, as two Danish cosmetics companies stated that their serum products had a either a neutral pH relative to that of skin (i.e., between 5.0 and 5.5), or a pH of approximately 7. The same pH value was listed for a few of the serum products examined online (i.e., pH values between 5.5 and 8). In addition, a literature search showed that the optimal pH value for peptides is between 4 and 7. In comparison, the average skin pH is about 4.7, and the average tear pH is about 7.
Through the Danish trade associations, contact was made with cosmetics wholesalers and/or manufacturers in Denmark. The information obtained from the trade associations showed that two of the nine companies that responded to the enquiry were in the process of developing products of this type for their product assortment, and two companies already offered such products. The remaining five companies did not sell products of this type. Information from two companies showed that they use peptides as active ingredients in products of this type, and that the content in these products is low (typically less than 300 µg/g product).

Chemical analyses

Based on the identified ingredients in the 44 surveyed serum products, it was decided to analyse the content of the three most used peptides (Myristoyl Pentapeptide-17, Biotinoyl Tripeptide-1 and Acetyl Tetrapeptide-3) in the products. These peptides were used in 20, 16 and 7 of the 44 serum products analysed, respectively. The reason for this was that:

- These three peptides were found in several of the surveyed serum products.
- Plant extracts and essential oils are generally difficult to analyse, as they are mixtures of many different substances and their exact compositions depend on the manufacturing process and source. In addition, the use of plant extracts varied from manufacturer to manufacturer, and there were no particular plant extracts typically used in the products.
- Potentially problematic ingredients such as methylparaben and salicylic acid, both suspected endocrine disruptors, were identified in a few products (one and two of the 44 serum products, respectively). Furthermore, a limit value for the content of these two ingredients in cosmetic products has already been set via the Cosmetic Products Regulation.

A total of 19 serum products containing one or more of the three peptides were therefore purchased. Products from outside the EU were deliberately excluded, as very few of these products had ingredient information available and the content of these peptides was uncertain. The products purchased were from Denmark (DK, approx. 67%) and the EU (approx. 33%). The products were analysed by UPLC-MS^2 (a combination of liquid chromatography and mass spectrometry). The results of the chemical analyses showed that the three peptides are generally present at low concentrations. The highest concentration of any of the peptides in the 19 serums tested was approximately 60 µg/g (corresponding to 0.006%).

Hazard assessment

No toxicological data were identified for any of the three specific peptide ingredients. However, for related peptides, degradation of peptides due to the skin's enzyme content has been demonstrated, so it is relevant to conduct a hazard assessment of the peptides' individual components; that is, the amino acids. All of the amino acids included in the three peptide ingredients occur naturally in human foods, where they are part of the peptide chains and proteins in foods. All these amino acids are commonly found in the body, where they are part of the body's metabolic processes and structure, so any absorption of these amino acids in the body is not considered a concern. As for local effects (skin and eye irritation and skin sensitisation), available test data and data on the amino acids do not present any cause for concern in terms of skin irritant or sensitising effects. The three peptide ingredients are therefore not considered to result in toxic effects, and hence no critical effects can be identified for the ingredients for which a quantitative risk assessment can be performed.

Discussion

The three peptide ingredients and the building blocks (amino acids) they are made up of are generally not considered toxic. Taken together with the low daily use quantities of the serum products and the low concentrations of the peptide ingredients in the serum products, their presence in these serum products does not provide cause for health concerns. However, out of the many different ingredients comprising the products, only the peptides were analysed and evaluated in this project. Other frequently used ingredients in serum products (hyaluronic acid and vitamins) are also natural ingredients that are not considered to pose health concerns in such products.

However, a few of the serum products contain ingredients considered allergenic (benzyl alcohol and various plant extracts). The possibility of risks associated with these ingredients was not investigated further in this project. In addition, a few of the serum products contain suspected endocrine disruptors (methylparaben and salicylic acid). The Cosmetic Products Regulation establishes limit values for both of these ingredients.

Finally, it should be noted that the results of the survey and assessment in this report only cover serum products purchased in Denmark and the EU, as serum products outside the EU were not included in the project (as ingredient lists for them were not available at purchase time).

1. Introduction

The EU's Scientific Committee on Consumer Safety (SCCS) has assessed that there are concerns regarding the use of prostaglandin-based ingredients in eyelash serums. Peptides are now partly replacing prostaglandins.

Eyelash and eyebrow serums are products that ostensibly promote the growth of and/or reduce the loss of eyelash and eyebrow hairs. Originally, the active ingredients used were prostaglandins and synthetic analogues of prostaglandins. In 2021, the EU's Scientific Committee on Consumer Safety (SCCS) investigated the safety of the use of these active ingredients in cosmetic products due to discomfort caused by products of this type (SCCS/1635/21). The assessment of the SCCS was that there are concerns about the use of prostaglandin analogues in cosmetic products, especially if they are to be used around the eyes, as is the case for eyelash serums in particular.

Partly due to the SCCS's assessment, the Danish consumer organisation 'Forbrugerrådet Tænk Kemi'[1] has removed eyelash and eyebrow serums from their 'Kemiluppen' app,[2], such that it is no longer possible to search for these types of products in the app (Danish Consumer Council, 2023).

1.1 Background

In light of the SCCS's assessment of prostaglandins and prostaglandin analogues, "prostaglandin-free" alternatives are now on the market. The active ingredients in these products are typically peptides. In some cases, a single product may have multiple active ingredients.

However, there is insufficient information about the specific active ingredients used in these products and the safety of these products for consumers. The Danish Environmental Protection Agency (Danish EPA) has initiated this project out of a desire for more knowledge in this area.

1.2 Purpose

This project's purpose is to yield information about eyelash and eyebrow serums whose active ingredients are not prostaglandins or prostaglandin analogues. It accomplishes this through a survey of eyelash and eyebrow serum products available on the market, primarily by reviewing ingredient lists on these products. Furthermore, the purpose of the project has been to conduct quantitative chemical analyses of selected ingredients and perform a risk assessment on them. In summary, the purpose of the project is to investigate the content of specific selected active ingredients in eyelash and eyebrow serums, and to assess whether they are safe for consumers to use.

[1] Forbrugerrådet Tænk Kemi is a Danish consumer council organisation. Can in English be translated to 'Think Chemistry'.

[2] Kemiluppen ['The Chemistry Loupe'] is an app administered by the Danish Consumer Council's initiative ('Forbrugerrådet Tænk Kemi'), allowing consumers to scan cosmetic products and get environmental and health assessments of their ingredients on their smartphones.

1.3 Definitions

This section defines some of the terms used in this report, such as INCI names, prostaglandins, prostaglandin analogues, amino acids, peptides, cosmeticuticals, and essential oils.

1.3.1 INCI names and nomenclature in this report

INCI names are the international system of nomenclature used for ingredients in cosmetic products (International Nomenclature of Cosmetic Ingredients). Cosmetic products marketed in the EU must list their ingredients with the same common term (name) for a given ingredient. In that context, INCI names are normally used instead of other typical chemical names for the substances (such as trivial names or the official, unambiguous IUPAC names). As a general rule, the INCI names have been used in this report; however, some trivial names have been used, including:

- Ethyl tafluprostamide (INCI: dechloro dihydroxy difluoro ethylcloprostenolamide).

1.3.2 Prostaglandins and prostaglandin analogues

Prostaglandins are a group of important signalling substances that can be produced in almost all animal cells. Natural prostaglandins are fatty acids with 20 carbon atoms, consisting of a five-membered ring (a cyclopentane) with two side chains of seven and eight carbon atoms, respectively. One side chain contains an acid group (carboxylic acid, in position C1) and the other a double bond (C13 and C14), as well as an alcohol group at C15 (see FIGURE 1). In addition, alcohol groups (or oxo groups) are often found on the cyclopentyl ring (at C9 and C11), as well as a double bond between C5 and C6.

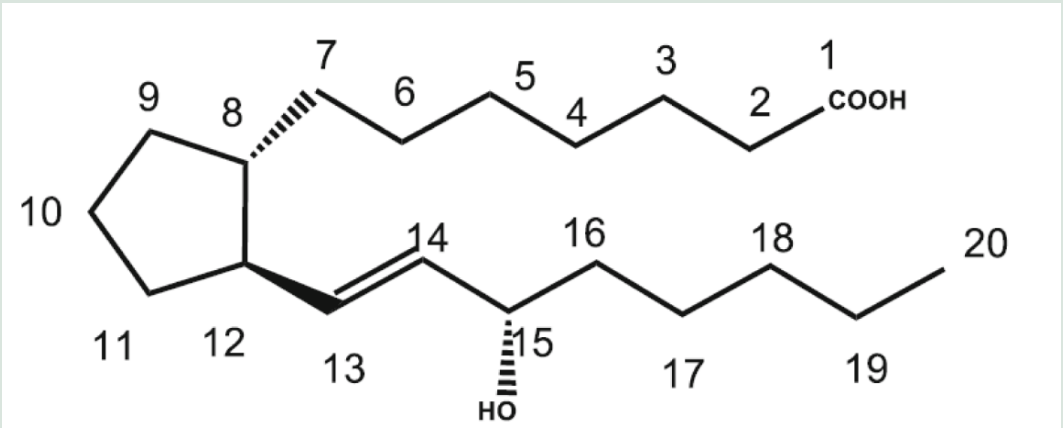

FIGURE 1. The general chemical structure of prostaglandins. This image is taken from the SCCS opinion on prostaglandins, SCCS/1635/21.

Certain prostaglandin analogues are used in cosmetic products and have been used in eyelash and eyebrow serums. These prostaglandin analogues are larger molecules with a benzene ring, possibly containing chlorine, on one of the two side chains.

In 2021, the SCCS conducted an assessment of two prostaglandin analogues used in eyelash and/or eyebrow serums: isopropyl cloprostenate and ethyl tafluprostamide (INCI name: dechloro dihydroxy difluoro ethylcloprostenolamide). In this SCCS opinion (SCCS/1635/21), 20 different prostaglandin analogues are described. In the CosIng database[3] (the EU's database of substances and ingredients in cosmetic products), a total of 16 prostaglandin analogues can

[3] https://single-market-economy.ec.europa.eu/sectors/cosmetics/cosmetic-ingredient-database_en

be found (search for "synthetic analogue of prostaglandin"), three of which are not listed in this SCCS opinion. This consolidated list of 23 prostaglandin analogues (see TABLE 1) serves as a starting point for this report. Given that the scope of this project excludes products containing prostaglandin analogues, this means that products containing these 23 ingredients are not investigated further in this project.

TABLE 1. Prostaglandin analogues. Products containing the 23 listed prostaglandin analogues were not investigated further in this project.

INCI name	CAS no.	Source
Benzothiophenyl dephenethyllatanoprost	-	SCCS/1635/21
Cyclopropylbimatoprost	1138395-12-6	SCCS/1635/21, CosIng
Cyclopropylmethylbimatoprost	1138395-10-4	SCCS/1635/21, CosIng
Dechloro cyclopropylcloprostenolamide	1138395-11-5	SCCS/1635/21, CosIng
Dechloro cyclopropylmethylcloprostenolamide	1138395-09-1	SCCS/1635/21, CosIng
Dechloro dihydroxy difluoro ethylcloprostenolamide (DDDE) (Ethyl tafluprostamide)	1185851-52-8	SCCS/1635/21
Dechloro ethylcloprostenolamide	1005193-64-5	SCCS/1635/21, CosIng
Dehydrolatanoprost	130209-76-6	SCCS/1635/21, CosIng
Dihydroxypropyl dehydrolatanoprostamide	-	SCCS/1635/21, CosIng
Dihydroxypropyl didehydrolatanoprostamide	1193782-16-9	SCCS/1635/21, CosIng
Dinitroglyceryl alprostadilate	-	CosIng
Dinitroglyceryl dinoprostonate	189940-83-8	CosIng
Dinoprostoyl isopropylserinate	2389059-87-2	CosIng
Ethyl travoprostamide	1005193-64-5	SCCS/1635/21, CosIng
Isopropyl cloprostenate	157283-66-4	SCCS/1635/21, CosIng
Keto travoprost	404830-45-1	SCCS/1635/21
Methyl bimatoprost acidate	38315-47-8	SCCS/1635/21, CosIng
Methyl travoprost	-	SCCS/1635/21
Norbimatoprost	155206-01-2	SCCS/1635/21
Nortafluprost	209860-89-9	SCCS/1635/21, CosIng
Tafluprost	209860-87-7	SCCS/1635/21
Trifluoromethyl dechloro ethylprostenolamide	1005193-64-5	SCCS/1635/21
Trifluoromethyl dehydrolatanoprost (travoprost)	157283-68-6	SCCS/1635/21, CosIng

1.3.3 Amino acids

Amino acids are found in all living organisms; these substances are the building blocks of proteins. Of the approximately 500 known amino acids, only 22 are proteinogenic, meaning that they are part of proteins in living organisms and can be genetically decoded. Of these 22, there are 20 which are primarily used as building blocks for the formation of proteins and peptides. Amino acids contain at least one primary amine group (i.e., the chemical group $-NH_2$) and at least one carboxylic acid group (i.e., -COOH), usually attached to the same carbon atom (so-called α-amino acids) (Lex.dk/aminosyrer, 2024).

Amino acids are often labelled with their one- or three-letter abbreviations. For example, lysine is abbreviated "Lys", histidine "His" and glycine "Gly".

FIGURE 2 shows the general structure of α-amino acids, as well as the 20 amino acids primarily used in the formation of proteins in cells.

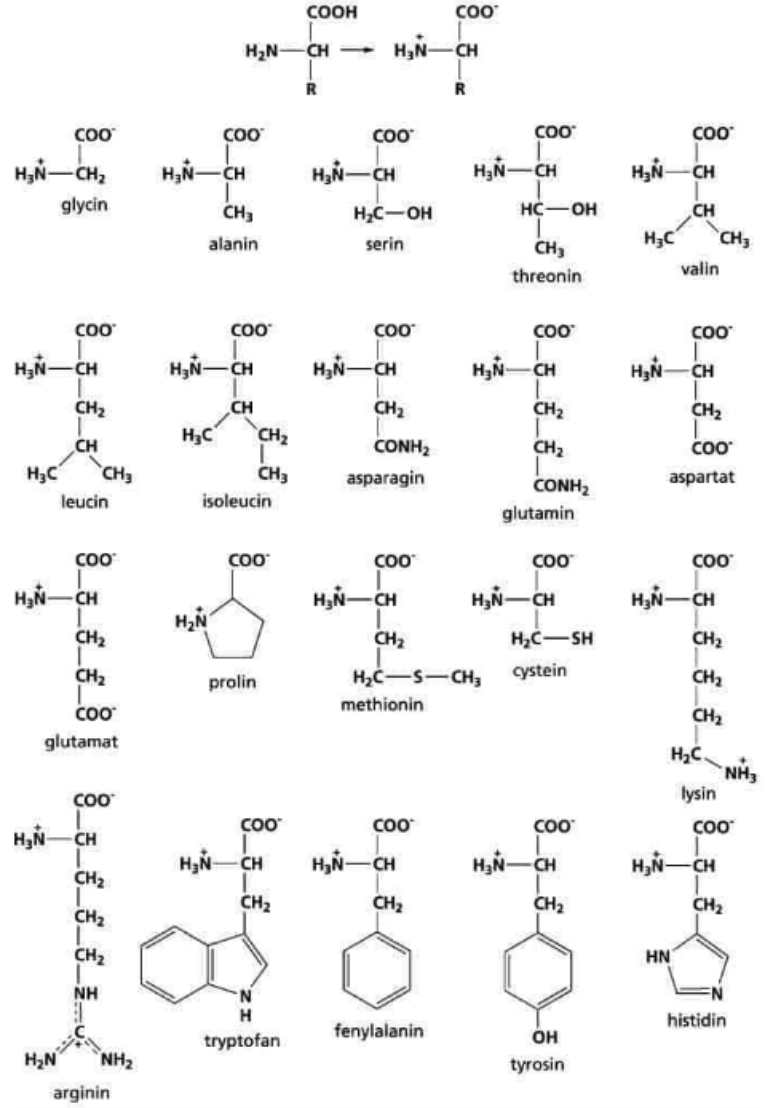

FIGURE 2. Amino acids. Top: The general structure of an α-amino acid, shown uncharged (left) and in zwitterion form - as amino acids appear at physiological pH values, where the amine group is positively charged and the acid group is negatively charged. Below the general structures, the 20 amino acids primarily used in the formation of proteins in cells are illustrated. The amino acids are shown as they appear at physiological pH values. This figure is sourced from denstoredanske.lex.dk/aminosyrer.

1.3.4 Peptides

Peptides are a class of organic compounds consisting of chains of different amino acids linked together by amide/peptide bonds (-CONH-) between the carboxylic acid group (-COOH) of one amino acid and the amine group (-NH2) of the following amino acid. Peptides are classified as biological polymers and typically have a relatively high molar mass. They can be broadly divided into oligopeptides (≤ 20 amino acids) and polypeptides (20-50 amino acids). Dipeptides are the smallest peptides, consisting of two amino acids. Tripeptides consist of three amino acids, tetrapeptides consist of four amino acids, and so on. If there are more than

50 amino acid units in a chain, the compound is typically considered a protein (Lex.dk/peptid, 2024; Nhu Ngoc et al., 2023).

The composition (structure/sequence) of a peptide is traditionally stated starting from the end where the amine group is free (the so-called N-terminus), using the three-letter abbreviations of the amino acids. In abbreviated IUPAC (International Union of Pure and Applied Chemistry) form, a tripeptide consisting of the three amino acids lysine (Lys), histidine (His) and glycine (Gly) is specified as H-Lys-His-Gly-OH, where "H-" and "-OH" indicate that the amine group of lysine and the acid group of glycine, respectively, are free.

Peptides occur naturally in the body, partly as breakdown products of proteins, but several peptides also have a specific function in the body. For example, some peptides serve as hormones or growth factors (Lex.dk/peptid, 2024; Nhu Ngoc et al., 2023).

Bioactive peptides typically contain 3-30 amino acids (He et al., 2023). A possible 8,000 tripeptides (consisting of three amino acid units) can be formed from the 20 most commonly used amino acids alone, and the total number of potential unique peptides is incredibly large. This also means that many different peptides could potentially be used in the various serum products.

1.3.5 Cosmeceuticals

Biologically active peptides and proteins are used in both the medical and cosmeceutical fields. Cosmeceuticals are cosmetic products with bioactive ingredients claimed to have medicinal benefits, such as eyelash and eyebrow serums. The wide use of peptides in cosmetics can be noted in the CosIng database, where searches for the words "peptide" and "protein" yield around 1700 hits for peptides and 900 hits for proteins as ingredients in cosmetics.

1.3.6 Essential oils

Essential oils are extracts from plant materials obtained by distillation, pressing, or extraction (Lex.dk/æterisk olie, 2024). Essential oils typically consist of a mixture of highly volatile substances that have strong scents. Many of the ingredients in essential oils can be produced synthetically. A search for "essential oils" in the CosIng database returns 241 results, showing various extracts from the seeds, fruits, leaves, and peels of different plants. This report assumes that when a serum product contains one or more plant extracts, the product may contain essential oils.

1.4 Scoping and prioritisation

This project focuses exclusively on eyelash and eyebrow serums. Other types of serum are outside the scope of the project. Over the course of the project, products such as peptide-containing mascaras were identified, with claims that the peptides promote eyelash growth. This type of product is also outside the scope of the project.

The project focuses exclusively on eyelash and eyebrow serums with active ingredients other than prostaglandins and prostaglandin analogues. Consequently, serum products containing the ingredients listed in TABLE 1 were not included in this project. This means that in the search for eyelash and eyebrow serum products, products containing prostaglandins or prostaglandin analogues were excluded from the survey.

Much of the project's data on available products and their ingredients comes from scrutinising ingredient lists for these types of products in both physical and online stores, but primarily online stores. This type of information is primarily available from stores and online shops in Denmark and the EU, as the EU's Cosmetic Products Regulation requires a list of ingredients to be printed on the packaging. For this reason, products sold outside the EU ("non-EU

products") are only included in the project to a limited extent; namely, in the few cases where ingredient lists were provided for the products at online shops outside the EU.

It should be noted that the names and terms printed on the products have been stated in this report. There has been no check, whether the used ingredient names on the products actually are correct INCI-names.

2. Survey

This chapter describes the results of the survey conducted in this project. The survey was based on existing knowledge regarding eyelash and eyebrow serums.

The following activities were conducted as part of the survey:
- Contacting selected relevant industry organisations
- Contacting Videncenter for Allergi (the Danish Allergy Research Centre)
- Contacting Astma-Allergi Danmark (Asthma-Allergy Denmark)
- Searching for examples of specific products on the market in Denmark, in other EU countries, and outside the EU
- Reviewing ingredient lists for 44 eyebrow and/or eyelash serum products.
- Searching literature and the internet for relevant information about active ingredients
- Searching for relevant ingredients in Kemiluppen (the Danish Consumer Council's Tænk Kemi's initiative)

2.1 Regulating eyelash and eyebrow serums

In current practice, eyelash and eyebrow serums that promote the growth of eyelash and eyebrow hairs are marketed as cosmetic eye products in the EU. These products appear on websites alongside other cosmetic products where the products are promoted for their cosmetic effects.

The European Commission's CosIng database also includes cosmetic products for eyelash care; the database indicates that these products and their ingredients aim to increase the shine, diameter and length of eyelashes.

The fact that in 2019, the European Commission asked the Scientific Committee on Consumer Safety (SCCS) to assess the safety of the use of prostaglandins in cosmetics, with this assessment (SCCS, 2022) focusing on the use of prostaglandins as growth promoters in eyelash and eyebrow serums, also supports the idea that these are cosmetic products.

On this basis, eyelash and eyebrow serums are considered cosmetic products and thus subject to EU cosmetics legislation. Thus, the survey of these products is based on a search for data within the cosmetics industry.

2.2 Contacting the industry

Selected relevant organisations were contacted to gather information about the use of active ingredients in eyelash and eyebrow serums. The organisations contacted are as follows:
- Kosmetik- og hygiejnebranchen (Cosmetics and Hygiene Industry Association)
- Dansk Vask-, Kosmetik- og Husholdningsindustri (VKH) (Danish Cleaning, Cosmetics, and Household Products Industry Association (part of the Confederation of Danish Industry))
- Cosmetics Europe (the European industry association for cosmetic products)
- Videncenter for Allergi (the Danish Allergy Research Centre)
- Astma-Allergi Danmark (Asthma-Allergy Denmark)
- The Danish Consumer Council Tænk Kemi

2.2.1 Contacting Danish trade associations

Two Danish trade associations, Kosmetik- og hygiejnebranchen (the Cosmetics and Hygiene Industry Association) and VKH, were contacted at the start of the project. Both associations agreed to send out a document to their members describing the purpose of the project and

posing a series of questions regarding products on the market and their active ingredient content.

During the initial exchange, both industry associations stated that they were not of the impression that many of their members had this type of product in their portfolios, and that we should therefore not expect many responses.

The document containing the questionnaire was sent out to approximately 45-50 association members in total (including members of both associations). Some of these members are exclusively wholesalers without their own products and brands. Responses were received from a total of nine companies. The remaining 35-40 companies did not respond. This is assumed to be due to either a lack of time to respond or the absence of eyelash and eyebrow serums from their product portfolios.

The information received from the nine Danish companies is as follows:

- Five companies stated that their product portfolios include neither eyelash nor eyebrow serums.
- Two companies stated that they do not currently have eyelash or eyebrow serums in their product portfolios, but they are working on developing products in this category.
- Two companies provided detailed information about their eyelash and/or eyebrow serums. The responses from these two companies are presented in TABLE 2 below.

The responses received from the Danish manufacturers are presented combined. These responses include both eyelash and eyebrow serums, as well as multiple products from each manufacturer. Not all ingredients listed as active ingredients are used in the same product. The stated effects were provided by the manufacturers themselves. Thus, the data from the responding manufacturers is combined in TABLE 2.

TABLE 2. Information on eyelash and/or eyebrow serums received from Danish cosmetics manufacturers

Subject	Response
Active ingredients	Myristoyl Pentapeptide-17 at a concentration ≤ 0.01 %.
	Biotinoyl Tripeptide-1 at a concentration ≤ 0.001 %.
	Pisum sativum peptide at a concentration ≤ 1% (increases hair growth)
	Acetyl Tetrapeptide-3 at a concentration ≤ 0.3% (increases hair growth)
	Milk protein / lactis proteinum extract (CAS 91053-68-8) at a concentration ≤ 0.3 %.
	Seawater extract at a concentration ≤ 0.3%.
	Inositol (CAS 87-89-8) at a concentration ≤ 0.2 %.
	Hydrolysed rice protein at a concentration ≤ 0.01% (increases hair growth)
	Hydrolysed yeast protein at a concentration ≤ 0.01% (increases hair growth)
	Coffea arabica seed oil at a concentration ≤ 1% (stimulates hair follicles)
	Trifolium pratense flower extract, ≤ 0.1% (increases hair density)
Usage information	The products are intended to be used daily; and results can be seen after a few weeks to three months of daily use
	One package is enough for approximately one month of daily use
Content	Between 7 and 10 ml per product container
pH value	pH approx. 7 for both eyelash and eyebrow serums
	pH between 5.0 and 5.5 for both eyelash and eyebrow serums

Manufacturers generally stated that the active ingredients serve primarily to moisturise and nourish hair roots, which helps prevent hair breakage. Furthermore, they stated that peptides, in combination with proteins, increase hair growth and hair density/thickness.

The manufacturers stated that the same peptides are also used in various skincare products, such as face creams and make-up (lip gloss), as well as in other hair growth products, such as shampoo and conditioner.

2.2.2 Contacting the European trade association

The European trade association, Cosmetics Europe, was contacted at the beginning of the project. Cosmetics Europe was sent the same document (including a project description and questionnaire) that the Danish trade associations received. Cosmetics Europe initially discussed ways to contribute knowledge to the project with their expert group. Consequently, Cosmetics Europe has not circulated the questions to their members.

No information was received from Cosmetics Europe during the survey for this project.

2.2.3 Contacting Videncenter for Allergi (the Allergy Research Centre)

In March 2024, the Allergy Research Centre was contacted to find out if it had received reports of allergic reactions from eyelash or eyebrow serums. The centre responded that it has not received reports of patients experiencing problems with these products or allergies associated with them.

2.2.4 Contacting Astma-Allergi Danmark (Asthma-Allergy Denmark)

In March 2024, Asthma-Allergy Denmark was contacted to see if it had any experience related to ingredients in eyelash or eyebrow serums. The organisation responded to indicate that it has no specific knowledge of what this type of cosmetic product typically contains. In general, they are only slightly familiar with this product category.

2.2.5 Contacting the Danish Consumer Council's Tænk Kemi

The Danish Consumer Council's Tænk Kemi has developed the Kemiluppen ["the chemistry loupe"] app, which allows users to look up ratings (A, B, or C) by scanning the barcode of a cosmetic product, based on the Danish Consumer Council's assessment of the ingredients declared on the product. The database behind Kemiluppen contains information about all ingredients declared in the nearly 20,000 current/non-discontinued products on the Danish market (as of May 2024). The database also contains historical data on discontinued products. For this project, in April 2024, Tænk Kemi counted the eyelash and eyebrow serum products in its database and extracted a list of ingredients they contain.

In June 2020, Tænk Kemi decided that eyelash and eyebrow serums would no longer be included in the Kemiluppen database (Danish Consumer Council, 2023). According to the Tænk Kemi website, this decision is based on the use of insufficiently safe ingredients (prostaglandin and prostaglandin analogues) and uncertainty around the regulations that apply to this product group[4].
For this reason, it is no longer possible to search for eyelash and eyebrow serums in Kemiluppen. The available data is therefore outdated, as information about which of these products exist on the Danish market and what ingredients they contain is no longer updated. Tænk Kemi has stated that in-app scan counts for these products can still be used as an indication of their popularity, as scans continue to be recorded from the app even though the products are no longer searchable. However, the fact that these products are no longer shown in the app, as well as the fact that Tænk Kemi informs the app's users of this, could influence the number of product scans.

[4] The Danish Consumer Council Tænk Kemi is unsure whether eyelash and eyebrow serums that cause physiological changes (longer and stronger eyebrows and eyelashes) should be considered a cosmetic product or a medicinal product. https://taenk.dk/kemi/plejeprodukter-og-kosmetik/oejenvippeserum-undgaa-disse-stoffer

2.2.5.1 Old data (from 2020) on serum products from Kemiluppen

The data extracted from Kemiluppen shows that before information on these products was no longer updated, the database contained a total of 64 eyelash and/or eyebrow serum products. These were distributed across a total of at least 30 brands/manufacturers[5], and:

- 44 out of 64 products (69%) were eyelash serums
- 10 out of 64 products (16%) were eyebrow serums
- 6 out of 64 products (9%) were serums for both eyelashes and eyebrows
- The names of the remaining 4 out of 64 products (6%) made it impossible to tell whether they were serums for eyelashes alone, eyebrows alone, or both.

The ingredients in the 64 serum products were also listed in Kemiluppen. Because Tænk Kemi decided to stop including these products in the Kemiluppen database in 2020, the ingredient lists for these products have not been updated since that time. The data for these 64 serum products shows that 25 of them (39%) contain prostaglandin analogues as ingredients. This may not be representative of the same products today, as some manufacturers could have replaced prostaglandin analogues with other ingredients due to the 2021 SCCS assessment published in early 2022 (SCCS/1635/21).

Of the 64 serum products in the 2020 data, some of them do contain peptides as active ingredients:

- 42 contain some kind of peptide
- 25 contain one or more prostaglandin analogues
- Of these products, 13 contain both peptides and a prostaglandin analogue

The 13 products containing both a prostaglandin analogue and peptides all contain either one or two different peptides. The remaining 29 products containing peptide(s), but no prostaglandin analogue, all contain between one and eight peptides. However, most products (22) contain just a single peptide. This shows that different peptides were also used in conjunction with prostaglandin analogues.

The peptides used in these products, and which ones are most frequently used, are presented in TABLE 3 below. TABLE 3 shows that a total of 14 peptides are used in the 42 peptide-containing serum products. Four peptides occur in several of the 42 serum products, while the remaining 10 peptides appear in only one or two of the serum products. The comments column in TABLE 3 indicates the amino acids that comprise each peptide using their three-letter abbreviations (see section 1.3.3). For peptides appearing in the PubChem[6] database, the abbreviated IUPAC nomenclature (see section 1.3.4) is used as it appears in the database.

TABLE 3. Peptide compounds occurring in the 42 eyelash and eyebrow serum products identified in Kemiluppen. Note that product ingredient information dates back to 2020 or earlier and may not be accurate at the time of writing (spring 2024) as a result.

Peptide	CAS no.	No. of products (out of 42)	Comment / indication of amino acid composition
Biotinoyl Tripeptide-1	299157-54-3	29	A reaction product of biotin and tripeptide-1, per CosIng. Biotinyl-Gly-His-Lys-OH

[5] Not all products were listed with their brands in the database, and there may be manufacturers selling products under several distinct brands; this was not investigated further.

[6] https://pubchem.ncbi.nlm.nih.gov/

Peptide	CAS no.	No. of products (out of 42)	Comment / indication of amino acid composition
Myristoyl Pentapeptide-17	959610-30-1	11	A reaction product of myristic acid and pentapetide-17, per CosIng. Myristoyl-Lys-Leu-Ala-Lys-Lys-NH_2
Octapeptide-2	1054611-00-5	11	A synthetic peptide made from glutamic acid, glutamine, leucine, lysine, and threonine (i.e., amino acids), per CosIng. H-Lys-Leu-Lys-Lys-Thr-Glu-Thr-Gln-OH
Acetyl Tetrapeptide-3	827306-88-7	6	Produced by acetylation of tetrapeptide-3, per CosIng. Ac-Lys-Gly-His-Lys-NH_2
Pisum Sativum Peptide	90082-41-0	2	This peptide is the di/tri-peptide fraction isolated from peas (Pisum Sativum (Pea) Protein), per CosIng.
Myristoyl Hexapeptide-16	-	2	A reaction product of myristic acid and hexapeptide-16, per CosIng. Myristoyl-Leu-Lys-Lys-Ala-Leu-Lys-OH
Myristoyl Pentapeptide-4	1392416-25-9	1	A reaction product of myristic acid and the amino acids threonine, serine, and lysine, per CosIng. Myristoyl-Lys-Thr-Thr-Lys-Ser-OH
Palmitoyl Tripeptide-1	147732-56-7	1	A reaction product of palmitic acid and tripeptide-1, per CosIng. Palmitoyl-Gly-His-Lys-OH
Palmitoyl Tetrapeptide-7	221227-05-0	1	A reaction product of palmitic acid and tripeptide-7, per CosIng. Palmitoyl-Gly-Gln-Pro-Arg-OH
Sh-Polypeptide-9	-	1	A single-chain human peptide produced through fermentation by E. coli, per CosIng.
Sh-Polypeptide-11	-	1	A single-chain human peptide produced through fermentation by E. coli, per CosIng.
Sh-Polypeptide-1	-	1	A single-chain human peptide produced through fermentation by E. coli, per CosIng.
Sh-Oligopeptide-2	-	1	A single-chain human peptide produced through fermentation by E. coli, per CosIng.
Sh-Oligopeptide-1	-	1	A single-chain human peptide produced through fermentation by E. coli, per CosIng.

CAS numbers may not be listed for all peptides in the CosIng and PubChem databases.

The three-letter abbreviations denote the individual amino acids that comprise the peptides: Lys (lysine), Leu (leucine), Ala (alanine), Thr (threonine), Glu (glutamic acid), Gln (glutamine), Gly (glycine), His (histidine), Ser (serine), Pro (proline), and Arg (arginine).

2.2.5.2 New products in Kemiluppen (from 2024)

Although products in this category (eyelash and eyebrow serums) can no longer be searched for in the Kemiluppen app, it is still possible to scan barcodes for these products via the app. The products are added to the database, but the Danish Consumer Council Tænk Kemi does not maintain these products or add information about their ingredients.

While it is possible to extract name and brand information for 21 such new products from Kemiluppen, not all new products are updated with a name and brand. Evidently, eyelash serums continue to outnumber eyebrow serums, but several of the new products are for both brows and lashes. The data from Kemiluppen also shows that 10 new brands have been added to the minimum of 30 brands that could be found among the 64 serum products as of 2020. This could indicate that this product category has been growing over the past four years.

2.3 Surveying the market for eyelash and eyebrow serum products

This project began with a survey of the market for eyelash and eyebrow serum products. The survey was conducted primarily through internet searches, as products sold both inside and outside the EU needed to be included in the survey; the products for sale at physical stores in the Copenhagen area were also examined.

2.3.1 Market survey methodology

An overview was compiled, containing a total of 58 serum products. The products were segmented by type (for lashes or brows), sales channel (physical stores or online shops) and geographic area (online shops in Denmark (DK), online shops in EU countries (EU), and online platforms outside EU (non-EU)).

The survey included only those products for which ingredient lists were available. Ingredient lists can be found on all products in physical stores in Denmark, but they may not be listed online, as this is not a requirement under the Cosmetic Products Regulation. Therefore, only products whose ingredients were listed online appear in the overview.

The ingredient lists for the 58 products were then first reviewed to ensure that they did not contain prostaglandin analogues (ingredients listed in TABLE 1), as products containing prostaglandin analogues are outside the scope of the project. A total of 14 of the 58 products were found to contain prostaglandin analogues; these products were excluded from the survey and not investigated further.

For the remaining 44 products, the following information was noted in the overview:

- Product name
- Product type (eyelash serum, eyebrow serum, or both)
- Market (i.e., whether the product belongs to DK, EU, or non-EU)
- Target group (women, men, everyone)
- Expected effect and after how long
- Usage information
- Product size
- Price
- Ingredients
- Indication of active ingredients (based on product descriptions)
- Acid/base content (relevant for products' pH values)
- Store / online shop where products can be purchased

2.3.2 Market impression

This market research shows that serum products containing prostaglandin analogues are still available on the market. A total of 14 of the 58 serum products (24%) contained prostaglandin analogues, and 10 of these 14 products also contained peptides. Compared to the 2020 data on serum products in the Kemiluppen database (from the Danish Consumer Council Tænk Kemi), where 39% of the products contained prostaglandin analogues, there are indications that the use of prostaglandin analogues is decreasing. However, these figures are not directly comparable, as Kemiluppen only covers products on the Danish market, and the market

research in this project covers serum products marketed globally (provided that they can be shipped to Denmark).

A portion of this market research involved investigating the range of eyelash and eyebrow serum products available in physical stores. This took place exclusively in the Copenhagen area and covered typical stores where cosmetics are normally sold: supermarkets, health stores, chemists, pharmacies, and large department stores with a cosmetics department. In general, serum products appeared not to be particularly widely available, being sold only in a handful of shops and in large supermarkets and department stores.

On the other hand, eyelash and eyebrow serum products were easily found for sale from various online stores. Our market research identified serum products from a total of 25 different online shops (across the DK, EU and non-EU groups). Most serum products were identified in online shops based in Denmark and the European Union. This is because ingredient lists are rarely provided for cosmetic products on non-EU online platforms, such as Wish, TEMU, and Amazon. This project did not succeed in identifying serum products with ingredient information on these websites, which is why they were not included in our market research. However, there is a large number of eyelash and eyebrow serum products available on these websites that were nonetheless excluded from this project. Products from these sites are typically cheaper than those sold on Danish sites.

Out of the 44 serum products not containing prostaglandin analogues, the distribution across the DK, EU, and non-EU groups was as follows:
- 24 serum products from Danish physical/online shops
- 15 serum products from European online shops
- 5 serum products from online shops outside Europe

Non-EU products were primarily identified on Yesstyle.com, which provides ingredient lists for these products.

2.3.3 Information about the products' main purposes and claims

It is clear that the main purpose of the products studied is to promote longer hair growth, whether for eyelashes, eyebrows, or both. For the vast majority of these products (41 out of 44), the main purpose is to promote hair growth, make hair thicker/fuller, or both. Only three products do not directly mention increasing hair growth or hair fullness as their main purpose. Three products do, however, indicate that they protect hair from damage, moisturise hair, or strengthen lashes and brows.

These products make many subtly different claims. Some of the products make only a few of the claims listed below, while others make virtually all of them:
- Boost natural hair growth
- Visibly lengthen lashes and/or brows
- Make lashes/brows stronger, longer, and fuller
- Stimulate lash/brow volume and density
- Rebuild lashes/brows
- Prevent lash/brow breakage
- Protect lashes/brows from damage
- Increase the number of new hairs

2.3.4 Information about product types and prices

The 44 serum products included in our market research are distributed as shown in TABLE 4.

TABLE 4. Distribution of the 44 eyelash and eyebrow serum products by product type and seller's geographical location (DK, EU, non-EU).

Category	Eyelash serum	Eyebrow serum	Serum for both	Total
DK	11	3	10	24
EU	4	4	7	15
Non-EU	3		2	5
Total	**18**	**7**	**19**	**44**

As shown in TABLE 4, serums that can be used for both brows and lashes were the most commonly encountered products. The second most commonly encountered product type was eyelash serum. This picture is fairly consistent with the 2020 data from Kemiluppen, which showed that eyelash serums were most common, but combination products for both lashes and brows were on the rise.

The various serum products contained between 2.4 and 15 ml of serum, with half of them containing between 5 and 10 ml of serum. Product sizes were not listed online for a quarter of the products obtained from online shops.

Serum products generally command high prices. Prices per litre start at 3,000 DKK and run as high as 200,000 DKK. Prices per individual product range from 30 DKK (for 10 ml) and 595 DKK (for 3 ml). Without accounting for product sizes, average prices for the 44 serum products are listed in TABLE 5.

According to TABLE 5 below, the most expensive prices were generally found in Denmark, but prices did not differ appreciably between Denmark and EU. There is a clear price difference between serum products from Denmark and EU compared to those sold outside of EU, where products are typically available for around 100 DKK each. On TEMU and Wish, even cheaper products were seen (for around 35-50 DKK each), but these products were not included in our market research because their ingredients are not listed on these online platforms.

TABLE 5. Average price per product in DKK by region (DK, EU, and non-EU) across the different product types. The serum products studied contain between 2.4 and 15 ml of serum for the prices below.

Category	Eyelash serum	Eyebrow serum	Serum for both	Average
DK	385 DKK	179 DKK	259 DKK	306 DKK
EU	255 DKK	389 DKK	173 DKK	252 DKK
Non-EU	124 DKK	-	52 DKK	95 DKK
Total	**312 DKK**	**299 DKK**	**205 DKK**	**264 DKK**

- no brow serums were identified from outside the EU

2.3.5 Usage information

A variety of usage information was observed for the 44 eyelash and eyebrow serum products studied:

- Target group
- Application method
- Application frequency
- When can an effect be expected?
- Will the effect wear off if use is discontinued?

2.3.5.1 Target group

In general, there was little information provided about the target groups for these serum products. A few products (4 out of 44) indicated that eyelash and eyebrow serums can be used by everyone, regardless of gender. One product indicated that brow serums should only be used by ages of 16 years and older. A second product (an eyebrow serum) was labelled as not for use by pregnant women, while a third product (a combination lash/brow serum) indicated that it could be used even by pregnant women.

The vast majority of products (about 75%) offered no direct information about the target group, but images and advertisements for these products online depicted only women (i.e., no men), or were marketed alongside other products for women on the websites (e.g., listed in a women's section).

2.3.5.2 Application method

Many of the serum products, whether lash serum, brow serum, or both, indicated that they were to be applied along the roots of the lashes and brows. In other words, these serums are likely designed to act primarily on hair roots. Most of the products were sold with an applicator brush (a small, round, thin brush), suitable for applying a serum along the hair roots (see the top row of FIGURE 3). Less than a quarter of the products (7 out of 44) included only a mascara-type brush for serum application (whether for lashes or for brows; see the middle row of FIGURE 3).

Some products were sold a sort of hybrid applicator brush, consisting essentially of a mascara brush with a round ball at the tip (see the bottom row of FIGURE 3). In these cases, it was indicated that the serum should be applied along the hair roots using the ball, and along the length of the lashes and brows using the brush.

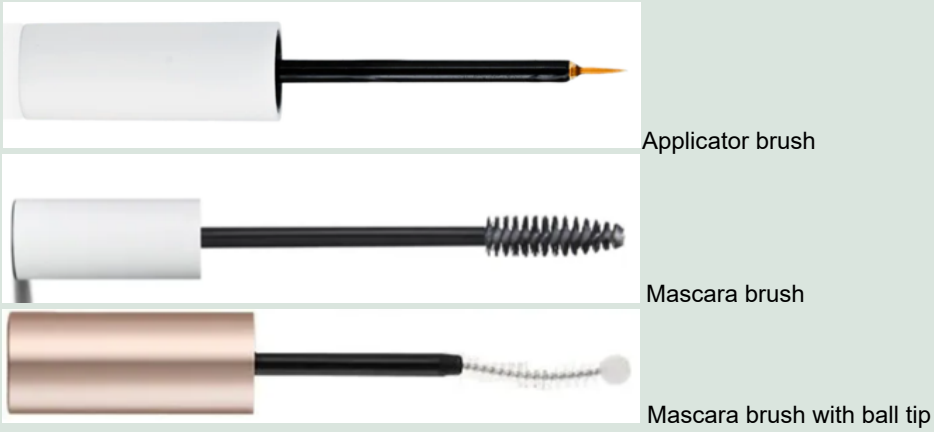

FIGURE 3. The three application methods observed for the 44 products surveyed

In a few cases, it was specifically stated that lash serum should only be applied to the upper lashes, not the lower lashes, as serum is automatically distributed across the upper and lower lashes by blinking.

2.3.5.3 Application frequency

In the vast majority of cases, the website, the product itself, or both indicated how often a serum should be used. About half of the products indicated that they were to be used once per day, such as before bedtime. About a quarter of the products indicated that they were to be used twice a day (in the morning and the evening). Some products indicated that they could be used one or two times a day, but two times would yield faster results.

There is no obvious difference in recommended usage frequencies depending on whether a given serum is for lashes, brows, or both.

2.3.5.4 Duration of use and quantity used

According to 66% of the products, an effect can be expected after a few weeks. Exactly when the effect is expected to be visible ranges from 2 to 16 weeks. However, the vast majority indicate that effects can be expected in 4 to 8 weeks. This applies to both lash and brow serums.

On the other hand, information on how long these products should be used was generally limited. The vast majority of products provided no information about this, although one product (a combination lash/brow serum) stated that users should take a one-month break after using the product for six consecutive months.

Apart from that, several products (16 out of 44, 36%) indicated that continued use is required to maintain results. To that end, users can apply the serum two to three times per week to maintain the results achieved. This applies to both lash and brow serums. Two products directly stated that lashes or brows will return to their normal state over time if not maintained with serum a few times per week after achieving the desired result.

Thus, eyelash and eyebrow serums constitute a class of products that can potentially be used daily for a very long period of time (e.g., years).

Only five products indicated how long they were expected to last, and only one manufacturer provided information on expected usage:

- 3 ml of lash serum is expected to last for three months of once-daily use
- 10 ml of combination brow/lash serum is expected to last for six months of once-daily use
- 8 ml of combination brow/lash serum is expected to last for three to four months of once-daily use
- 6 ml of combination brow/lash serum is expected to last for three months of once-daily use
- 3.5 ml of combination brow/lash serum is expected to last for two months of use one to two times daily
- 8 ml of combination brow/lash serum is expected to last for one month of once-daily use

This shows that the amount used per day is expected to be very small: somewhere between 0.06 and 0.27 ml per day for both lashes and brows, based on the figures above.

2.3.6 Information about pH value

There is limited information available from online shops regarding the pH value of serum products. The following information was provided on the websites:

- Product has a neutral pH value (7.0), corresponding to the average pH of tears (lash serum)
- Product has a neutral pH value (serum for both lashes and brows)
- Product has a slightly acidic pH value, similar to normal skin (approx. 5.5-6.5) (lash serum)
- Product has a pH value similar to tears (i.e., approx. 6-8) (lash serum)

Information on products' pH values was available primarily for non-EU products (three units) and for EU products (one unit).

The values given above correspond roughly to the responses received from industry contacts (manufacturers) regarding pH values for these substances (pH of 5.0-5.5 or pH of approx. 7).

One American cosmetics manufacturer states on its website that the optimal pH value for products containing peptides is between 4 and 7. If the pH value is either lower than 4 or

higher than 7, it could destroy the peptides, which are sensitive to pH changes. In practice, peptide bonds can be hydrolysed at an acidic or basic pH, breaking down the peptides into smaller peptides and amino acids. In addition to stability, pH also influences the solubility of peptides. Nugrahadi et al. (2023) write that hydrolysis is the main form of peptide degradation, and that hydrolysis is highly dependent on pH. They state that acid-catalysed hydrolysis occurs at a pH of 1-3, while base-catalysed degradation occurs at pH values above 7. Peptide hydrolysis can still occur at neutral pH, but the process is different. Consequently, a peptide mixture is typically optimised using a buffer solution to obtain the best pH value for the mixture in question and the product it is used in.

For comparison, the average normal skin pH is around 4.7 (Lambers et al., 2006), whereas the average pH of tears is normally around 7 (varies from 6.5 to 7.6) (Abelson et al., 1981).

2.3.7 Information about ingredients in lash and brow serums

This section reviews a selection of the listed ingredients in the 44 surveyed products. Only ingredients listed on websites and/or on product packaging seen in stores were considered for inclusion.

Information on active ingredients presented on websites selling these products was also considered. Thus, the active ingredients intended to promote eyelash and/or eyebrow hair growth that were highlighted or otherwise mentioned on each website are included in this review.

In addition, it is noted whether the surveyed serum products contain:

- Fragrances
- Plant extracts / essential oils
- Potential problematic ingredients (i.e., ingredients described as undesirable by the Danish Consumer Council Tænk Kemi)[7]; this category contains ingredients that are undesirable for health reasons, such as because they are allergens or suspected endocrine disruptors. This list is a result of a focus on allergens or suspected endocrine disruptors from amongst others the Danish Chemicals Initiative 2022-2025 from the Danish Ministry of Environment (Danish Ministry of Environment, 2022). Substances on this list originate from different knowledge source such as e.g. the Endocrine Disruptor Lists[8].

2.3.7.1 Active ingredients

Of the 44 serum products surveyed, the vast majority contain peptides as active ingredients. A total of 36 out of 44 serum products (82%) contain one or more peptides, and a further three products (7%) contain amino acids (which peptides are made of). Only five products (11%) contain neither peptides nor amino acids. These five products instead contain a variety of plant extracts, vitamins - such as niacinamide (vitamin B3), biotin (vitamin B7), panthenol (vitamin B5) and/or tocopherol (vitamin E) - or both.

Note that our literature review (section 2.4) has shown that some plant extracts themselves consist of peptides; hence, potentially all of the surveyed products may contain peptides or amino acids. However, this was not investigated further.

In general, for all 44 serum products, the substances identified as active ingredients belong to the following categories:

- Peptides
- Amino acids

[7] https://taenk.dk/kemi/plejeprodukter-og-kosmetik/personlig-pleje-se-efter-disse-stoffer

[8] Endocrine Disruptor List

- Vitamins
- Plant extracts

Many of the products contain various peptides, plant extracts, and vitamins. An overview of the peptides, plant extracts, and vitamins typically used in serum products is presented below.

Peptides

TABLE 6 below lists the peptides identified in the 44 serum products, as well as the number of products in which each peptide is used. As shown in TABLE 6, a total of 20 peptides was identified in the 44 serum products. As was previously mentioned, not all products contain peptides, and some products contain several peptides. Three peptides in particular were found in several of the surveyed serum products. More than half (24) of the products contain either Myristoyl Pentapeptide-17, Biotinoyl Tripeptide-1, or both.

According to the Danish Consumer Council Tænk Kemi, these three peptides (Myristoyl Pentapeptide-17, Biotinoyl Tripeptide-1, and Acetyl Tetrapeptide-3) are also used in other cosmetic hair care products; primarily mascara, but also beard serum, hair serum, shampoo, and conditioner.

A search for "peptide" in the European Medicines Agency's database[9] of active pharmaceutical ingredients did not return any medical uses for the short-chain peptides used in cosmetics. Long-chain peptides are typically used as part of antibody therapies with various immunoglobulins. Searching the Danish database Medicin.dk [“medicine.dk”] did not yield any additional data.

TABLE 6. The peptides used in the 44 serum products. The most commonly used peptides are listed first.

INCI name	CAS no.	No. of products (out of 44)	No. of lash – brow – combo serum products
Myristoyl Pentapeptide-17	959610-30-1	20	8 – 4 – 8
Biotinoyl Tripeptide-1	299157-54-3	16	5 – 3 – 8
Acetyl Tetrapeptide-3	827306-88-7	7	4 – 1 – 2
Copper Tripeptide-1	89030-95-5	4	3 – 0 – 1
Myristoyl Hexapeptide-16	959610-54-9	3	2 – 0 – 1
Myristoyl Pentapeptide-4	-	3	2 – 0 – 1
Oligopeptide-10	466691-40-7	3	0 – 2 – 1
Palmitoyl Tripeptide-5	623172-55-4 / 623172-56-5	2	1 – 0 – 1
Acetyl Hexapeptide-8	616204-22-9	2	1 – 0 – 0
Tripeptide-1	72957-37-0	2	1 – 0 – 1
Octapeptide-2		2	2 – 0 – 0
Pisum sativum peptide	90082-41-0	2	1 – 0 – 1
Oligopeptide-2	-	1	0 – 0 – 1
Myristoyl Tetrapeptide-12	959610-24-3	1	0 – 0 – 1
Caprylyl-triphopeptidglycol-1	-	1	0 – 1 – 0
Palmitoyl Pentapeptide-4	214047-00-4	1	1 – 0 – 0

[9] https://www.ema.europa.eu/en/medicines

INCI name	CAS no.	No. of products (out of 44)	No. of lash – brow – combo serum products
Hexapeptide-11	161258-30-6	1	0 – 0 – 1
Palmitoyl Tripeptide-1	147732-56-7	1	1 – 0 – 0
Myristoyl Pentapeptide-16	-	1	1 – 0 – 0
Glycine max polypeptide		1	1 – 0 – 0

Amino acids

In some serum products, amino acids (which peptides are made of) are used either together with or instead of peptides. Some products contain just a single amino acid, while others contain up to 17 amino acids. A total of 8 products contain amino acids, 3 of which contain only amino acids and no peptides. Overall, 39 out of 44 surveyed products (89%) contain peptides, amino acids, or both. Additionally, our literature review (section 2.4) has shown that some plant extracts themselves consist of peptides; hence, potentially all of the surveyed products may contain peptides or amino acids.

A search for "amino acid" in the European Medicines Agency's database[10] of active ingredients revealed that amino acids are used as active ingredients in preparations used in patients receiving parenteral nutrition or who have disorders related to amino acid metabolism. Searching the Danish database Medicin.dk [“medicine.dk”] did not yield any additional data.

Vitamins

A majority (34 out of 44, 77%) of the serum products contain one or more of the following vitamins: niacinamide (vitamin B3), biotin (vitamin B7), panthenol (vitamin B5) and tocopherol (vitamin E). Panthenol (vitamin B5) and biotin (vitamin B7) are the most commonly used vitamins, occurring in at least half of all products.

TABLE 7. The vitamins used in the 44 surveyed serum products. The most commonly used vitamins are listed first.

INCI name	CAS no.	No. of products (out of 44)	No. of lash – brow – combo serum products
Panthenol (Vitamin B5)	81-13-0 / 16485-10-2	29	14 – 4 – 11
Biotin (Vitamin B7)	58-85-5	22	7 – 4 – 11
Tocopherol (Vitamin E)	54-28-4 (gamma) / 16698-35-4 (beta) / 10191-41-0 (DL) / 119-13-1 / 1406-18-4 / 1406-66-2 / 2074-53-5 (DL) / 59-02-9 (D) / 7616-22-0	10	1 – 3 – 6
Niacinamide (Vitamin B3)	98-92-0	4	2 – 0 – 2

[10] https://www.ema.europa.eu/en/medicines

Plant extracts

In general, a majority of the serum products surveyed (36 out of 44, 82%) contain one or more plant extracts. Many different plant extracts are used in these products. In total, 131 plant extracts were identified in the 44 serum products. A complete list of the identified plant extracts is presented in Appendix 1.

Among those products which contained at least one plant extract, the average number of extracts per product was five. A large proportion of the identified plant extracts (107) were unique to a particular serum, appearing in just one of the 44 serum products. TABLE 8 below lists the 24 plant extracts that were observed in more than one serum product.

Some websites included descriptions of how the plant extracts in their products work and what effects they have on the growth of eyelashes or eyebrows. These claims come from the individual online shops marketing the products, and they may or may not be scientifically proven.

TABLE 8. The most frequently occurring plant extracts in the 44 serum products. The most common plant extracts are listed first.

INCI name	CAS number	No. of products (out of 44)	Online shops' comments about effects
Ricinus communis (castor) seed oil	8001-79-4	6	Prevents hair loss
Acorus calamus root extract	84775-39-3	5	Reduces hair loss and promotes hair growth
Trifolium pratense (red clover) flower extract	85085-25-2	5	Part of the CAPIXYL™ peptide complex
Camellia sinensis leaf extract	84650-60-2	4	
Aloe barbadensis leaf juice	85507-69-3 / 94349-62-9	3	
Centella asiatica extract	84696-21-9	3	
Curcuma longa callus conditioned media	-	3	Helps to increase hair strand diameter and prolong the hair growth phase, resulting in longer hair
Houttuynia cordata extract	164288-50-0	3	
Isochrysis galbana extract	1190414-92-6	3	Stimulates hair growth and increases hair density
Leuconostoc/radish root ferment filtrate	-	3	Probiotic preservative produced by fermenting radishes and Leuconostoc kimchii
Panax ginseng extract	-	3	Stimulates hair growth and promotes thicker, fuller, and stronger lashes
Panax ginseng root extract	84650-12-4	3	
Plankton extract	91079-57-1	3	Provides intense moisturisation for eyelashes; promotes stronger and thicker hair
Curcurbita pepo (pumpkin) seed extract	89998-03-8	3	Protects hair follicles from damage

INCI name	CAS number	No. of products (out of 44)	Online shops' comments about effects
Serenoa serrulata fruit extract	84604-15-9	3	
Swertia japonica extract	94167-11-0	3	Prevents hair loss
Amaranthus caudatus seed extract	223747-79-3	2	Increases hair strand diameter
Cocos nucifera oil	8001-31-8	2	Prevents damage
Helianthus annuus (sunflower) seed oil	84776-03-4 (generic) / 8001-21-6 / 164250-88-8 (generic)	2	
Larix europaea wood extract	91722-66-6	2	
Oryza sativa (rice) extract	90106-37-9	2	
Pisum sativum (pea) extract	90082-41-0	2	
Polygonum multiflorum root extract	-	2	
Vincetoxicum atratum extract	-	2	Provides intense moisturisation for eyelashes

Hyaluronic acid

Several of the serum products list hyaluronic acid as an active ingredient for its moisturising properties. Numerous serum products (24 out of 44, 55%) contain hyaluronic acid or a salt of this acid, such as sodium hyaluronate.

Hyaluronic acid is a carbohydrate with chains of varying lengths, comprising up to 50,000 monosaccharide units, alternating between glucuronic acid and N-acetyl-D-glucosamine. Hyaluronic acid is found in all tissues, where it is synthesised and binds significant amounts of water, forming a gel. Hyaluronic acid also plays a significant role in healing and tissue repair. (Lex.dk/hyaluronsyre, 2024). With age, the amount of hyaluronic acid in the body decreases; consequently, hyaluronic acid is often used in moisturising face creams[11], body lotions, and various facial care products (per the Danish Consumer Council Tænk Kemi).

2.3.7.2 Acids and bases

Acids and bases are used in cosmetic products to adjust their pH values. A majority of the serum products (33 out of 44, 75%) contain some form of acid, with citric acid being the most common (in 15 serum products). Five products contain both an acid and a base (sodium hydroxide), and two products contain only a base, also in the form of sodium hydroxide.

2.3.7.3 Fragrances and essential oils

Only one of the serum products tested contains fragrance. In this case, the term "fragrance (parfum)" appears in the list of ingredients, but none of the allergenic fragrances requiring individual declaration are listed.

Three other products contain benzyl alcohol, which is one of the 24 fragrances currently requiring individual declaration (Annex III of the Cosmetic Products Regulation), but none of these products contain "fragrance" according to their lists of ingredients. This may be because benzyl alcohol has been added to these products as a preservative. Benzyl alcohol is authorised as a preservative in cosmetic products (in Annex V of the Cosmetic Products Regulation).

[11] https://www.matas.dk/stories-hyaluronsyre

In July 2023, EU Regulation 2023/1545 was adopted, such that Annex III of the Cosmetic Products Regulation requiring individual declaration. However, the regulation provides for a transition period of three years from its entry into force (i.e., until 31 July 2026) for new products, and five years from its entry into force (i.e., until 31 July 2028) for products already on the market. This means that the newly listed fragrances do not yet have to be declared individually. However, some of the serum products tested contain some of the newly listed fragrances:

- One of the serum products studied contains menthol, which is one of the newly listed fragrances.
- Two of the surveyed serum products contain a so-called Mentha extract (peppermint), either Mentha piperita oil or Mentha piperita leaf extract. Mentha piperita oil is one of the newly listed fragrances, and Mentha piperita leaf extract has the same CAS number as Mentha piperita oil.
- One of the serum products contains citrus lemon peel extract, which is one of the new fragrance ingredients requiring individual declaration.

Apart from benzyl alcohol, menthol, and the Mentha extracts identified in six products, the serum products do not contain any of the fragrances listed in EU Regulation 2023/1545 which require individual declaration.

A majority of serum products (33 out of 44, 75%) contain some form of plant extract; such an extract may itself serve as a fragrance, in the case of essential oils.

Two products contain Rosmarinus officinalis (leaf oil and leaf extract), and one product contains a citrus extract (citrus orange fruit water); the Danish Consumer Council Tænk Kemi lists all of these as allergenic plant extracts[12]. This is due to the fact that these plant extracts contain known allergenic substances, e.g. citrus orange fruit water contains linalool, geraniol, citral and eugenol[13].

Of the 44 serum products, 6 (14%) contain no fragrance, benzyl alcohol, menthol, Mentha extracts, or any other kind of plant extracts (and thus any essential oils).

2.3.7.4 Other relevant ingredients

The 44 surveyed serum products were analysed for potentially problematic ingredients; namely, the substances on the list of undesirable ingredients published by the Danish Consumer Council Tænk Kemi[14]. That said, this list focuses on ingredients which are undesirable for health reasons, such as because they are allergens or suspected endocrine disruptors. TABLE 9 lists the potentially problematic ingredients observed.

TABLE 9. Potentially problematic ingredients (based on health concerns) in the 44 serum products.

INCI name	CAS no.	No. of products (out of 44)	Product type	Health effect
Benzyl alcohol	100-51-6	3	Eyebrow serum (1) and combination serum (2)	Allergenic

[12] https://taenk.dk/kemi/plejeprodukter-og-kosmetik/personlig-pleje-se-efter-disse-stoffer

[13] CITRUS AURANTIUM DULCIS FRUIT WATER – Ingredient - COSMILE Europe

[14] https://taenk.dk/kemi/plejeprodukter-og-kosmetik/personlig-pleje-se-efter-disse-stoffer

INCI name	CAS no.	No. of products (out of 44)	Product type	Health effect
Rosmarinus officinalis extracts	84604-14-8 8000-25-7	2	Combination serum	Allergenic
Citrus extracts	92346-89-9 / 84929-31-7 97766-30-8	2	Lash serum (1) and combination serum (1)	Allergenic
Salicylic acid	69-72-7	2	Combination serum	Suspected endocrine disruptor
Mentha piperita and other Mentha extracts	8006-90-4 / 84082-70-2	2	Combination serum	Allergenic
Methylparaben	99-76-3	1	Lash serum	Suspected endocrine disruptor
Menthol	89-78-1 / 1490-04-6 / 2216-51-5 / 15356-60-2	1	Eyebrow serum	Allergenic

As shown in TABLE 9, the potentially problematic ingredients observed consist primarily of various benzyl alcohol and various plant extracts, all of which are considered to be allergenic. In addition, methylparaben was identified as a preservative in one product and salicylic acid was observed in two of the products analysed. Both methylparaben and salicylic acid are suspected endocrine disruptors. Both of these substances have limit values set for their content in cosmetic products, as both are approved preservatives.

2.4 Literature review

The survey included a literature review encompassing tests of eyelash and eyebrow serums, as well as scientific literature on ingredients used in this type of product. Most of the review was carried out after reviewing the product samples, in order to gain prior knowledge of the ingredients typically found in the two types of serum.

2.4.1 Consumer testing of lash and brow serums

A literature search was conducted for consumer tests of eyelash and eyebrow serums. No tests were identified that examined the ingredients in these types of serums in official consumer publications. The following consumer testing platforms have discussed eyelash serums:

- The Danish Consumer Council Tænk Kemi (Denmark)
- Stiftung Warentest (Germany)

Tænk Kemi has not conducted testing on eyelash serums specifically, but in July 2023, it published a Danish article titled (translated to English) "Eyelash serums: Avoid these substances", previously mentioned in 2.2.5 (Danish Consumer Council, 2023). In this article, Tænk Kemi warns against the use of eyelash serums containing prostaglandin or prostaglandin analogues, noting that this product category is no longer included in its Kemiluppen app due to concerns about the effects of these ingredients. Tænk Kemi has not conducted any specific tests of eyelash or eyebrow serum products in its consumer magazine, known as Tænk ["Think"].

The same goes for an article by Stiftung Warentest (2019). It does not include any test results for lash serums in particular, but it does include similar warning about the presence of prostaglandin analogues in products of this type.

Again, serum containing prostaglandin or prostaglandin analogues was not investigated further in this project.

2.4.2 Other studies

In 2018, the Bundesinstitut für Risikobewertung (BfR), known in English as the German Federal Institute for Risk Assessment, conducted a review of 17 eyelash serum products purchased primarily online. It measured the concentrations of the prostaglandin analogues used and found them to range from 0.0006% to 0.022%. The focus of this study by BfR was on prostaglandins and the fact that some of the products contained prostaglandin analogues. Because it focused on prostaglandin analogues and their content, we chose not to review this study in detail.

No other studies were identified focusing on ingredients other than prostaglandin analogues in eyelash and eyebrow serums.

2.4.3 Information in the literature about active ingredients

We conducted a literature search for information about the active ingredients which, according to our survey of serum products for lashes and brows, appear to be the most important ingredients:

- Peptides
- Amino acids
- Vitamins
- Hyaluronic acid
- Certain plant extracts

We searched for information solely related to the mentioned active ingredients as they relate to cosmetic products. Our search encompassed published articles and the UL Prospector materials database[15]. Prospector is an international materials database where producers of raw materials and chemicals can upload information about their raw materials in the form of technical data sheets and descriptions. UL has offices in the US, China, Brazil, and Germany.

2.4.3.1 Peptides

Peptides have been noted to be important bioactive ingredients in today's cosmetic products (Nhu Ngoc et al., 2023), as they exhibit various kinds of biological activity, such as antioxidant, anti-inflammatory, anti-ageing and antimicrobial properties.

These bioactive peptides can generally be divided into four categories based, on their properties in cosmetic products (Nhu Ngoc et al., 2023; He et al., 2023):

- Signal peptides, whose functions include stimulating the synthesis of collagen (a protein in skin, tendons, connective tissue, etc.), stimulating the growth of cells in the outermost layer of the skin (the epidermis), and regulating melanin production (which causes skin pigmentation).
- Carrier peptides, which provide trace elements (such as copper and magnesium) for wound healing and enzyme processes, stimulate collagen synthesis, and improve skin elasticity.
- Neurotransmitter-inhibitor peptides, which moisturise, stimulate collagen synthesis, and inhibit melanin synthesis. The moisturising effect comes from effects on the synthesis of collagen (al-Atif, 2022).
- Enzyme inhibitor peptides, which moisturise and reduce the breakdown of collagen. According to al-Atif (2022), an increase in collagen helps to increase moisture.

[15] https://www.ulprospector.com/en/eu

According to a 2020 article by Salvador Ferreira et al., more than 80% of the peptides studied in around 100 peptide-containing cosmetic products sold in Portugal in 2018 were so-called signal peptides. However, this study covered face creams, not brow and lash serums.

Peptides can be produced synthetically, but they can also be extracted from natural sources, such as plants, animal sources (e.g., milk proteins) and marine sources (e.g., algae) (Nhu Ngoc et al., 2023).

Nhu Ngoc et al. (2023) and He et al. (2023) provide several examples of peptide-containing cosmetic products with anti-ageing and anti-wrinkle effects, but the scientific tests referenced are concerned solely with the effects of peptides in face creams. The peptides are combined with other active ingredients, such as vitamin E (tocopherol). Peptides also inhibit the breakdown of hyaluronic acid (a natural moisture binder in all tissues).

According to He et al. (2023), various modifications of these bioactive peptides, such as adding hydrophobic groups (fatty acids), can increase adsorption to the cell membrane and peptide efficacy, but at the expense of reduced solubility and peptide toxicity to cells.

Copper tripeptide is described as a carrier peptide with a wound healing effect. Copper tripeptide is also reported to have an effect in repairing damaged cells (Nhu Ngoc et al., 2023). Peptides extracted from soybeans are described as having a hair growth-promoting effect (Nhu Ngoc et al., 2023).

One American chemical manufacturer which produces ingredients used in cosmetic products sells a range of peptides used for hair growth. This manufacturer lists 22 peptides on its website[16] under "hair growth", including Acetyl Tetrapeptide-3 and myristoyl hexapeptide-16. On its website, the manufacturer states that these peptides have a positive impact on hair follicles by promoting blood circulation and increasing the availability of nutrients and oxygen to support hair growth. In addition, the peptides influence the body to produce more collagen and elastin, helping to strengthen the hair structure. Sadgrove and Simmonds (2021) write that Acetyl Tetrapeptide-3 and copper peptide are used in cosmetic products to promote hair growth. This effect is achieved by inhibiting proteins that have a negative impact on hair growth.

According to the UL Prospector materials database, the peptide with INCI name biotinyl-tetrapeptide-1 is formed from biotin (vitamin B7) and tetrapeptide-1. According to the manufacturer, biotinyl-tetrapeptide-1 has a proven stimulating effect on the metabolism of hair follicle cells and therefore stimulates hair growth. It also affects the ageing of hair follicle cells and prevents hair loss (ULProspector.com/Biotinyl-Tetrapeptide-1, 2024). Some of the surveyed serum products also claim that biotinyl-tetrapeptide-1 is a type of biotin that boosts hair growth by strengthening genes and increasing the proportion of anagen hair (active hair). In addition, the trade name Procapil™ is listed by some of the serum products in the survey. According to the UL Prospector database, Procapil™ contains seven different ingredients, including biotenyl-tetrapeptide-1 and PEG-40 hydrogenated castor oil. According to the manufacturer, Sederma, this mixture combats the ageing of hair follicles and thus prevents hair loss. The effect of Procapil™ is also described by a European cosmetics manufacturer, which states that Procapil™ has been shown in clinical studies to promote eyelash and eyebrow growth.

According to the UL Prospector materials database, the peptide with INCI name Myristoyl Pentapeptide-17 is claimed by its manufacturer to promote eyelash growth. This effect is

[16] This report deliberately does not reference specific manufacturers directly, so no source is specified.

achieved by stimulating the production of keratin and promoting the absorption of nutrients into hair follicle cells (ULProspector.com/Myristoyl Pentapeptide-17, 2024).

According to the UL Prospector database, a peptide mixture called SymPeptide® Xlash is also used, consisting of a mixture of glycerin, water and Myristoyl Pentapeptide-17. This mixture is claimed to enhance and emphasise the thickness of the eyelashes. Results can be obtained after two weeks (ULProspector.com/SymPeptide Xlash, 2024).

A few of the websites for the lash and brow serum products used mention the presence of Capixyl™ in these products. According to the UL Prospector material database, Capixyl™ consists of the peptide Acetyl Tetrapeptide-3 in combination with butylene glycol, aqua (water), dextran, and a plant extract identified as Trifolium pratense (clover) flower extract, one of the most frequently used plant extracts in serum products in this survey. According to UL Prospector, this mixture has anti-inflammatory properties, and the peptide stimulates tissue formation to better anchor the hairs and increase their vitality. Thus, this mixture ostensibly stimulates hair growth and limits hair loss, including for eyelashes (ULProspector.com/Capixyl, 2024)
One website states that studies have shown that Capixyl™ can increase hair length by up to 0.7 mm after 4 weeks and increase the number of new hairs by up to 27% after 8 weeks of use[17]. Sadgrove and Simmonds (2021) also write that Capixyl™, containing the peptide Acetyl Tetrapeptide-3, is used in cosmetic products that promote hair growth.

The concentrations of peptides used are stated in the literature to range from very small concentrations on the ppb scale to the ppm scale, typically around 1-30 ppm (Nhu Ngoc et al., 2023). However, this is at odds with industry-reported figures, which indicate levels of certain peptides up to 0.25% (equivalent to 2500 ppm).

A search of the CosIng database for tri-, tetra-, penta-, hexa-, hepta- and octapeptides reveals that these peptides are found in 258, 202, 212, 293, 111 and 84 substances in cosmetic products, respectively. A random sample of the search results suggests that these substances are typically used for skin and hair care purposes, as antioxidants and as skin protectants.
For eyelash care, which includes products that increase eyelash glossiness, diameter and length, the following three peptides are specifically listed:

- S-Water Bear Octapeptide-1
- S-Black Widow Spider Pentapeptide-1 SP
- S-Black Widow Spider Hexapeptide-1 SP

2.4.3.2 Amino acids

Khan et al. (2023) write that in cosmetic products, specifically in hair serums, amino acids are used to repair weak and damaged hair follicles. This is because the hair shaft is made up of keratin, and the production of keratin is dependent on amino acids. Khan et al. (2023) mention the following functions of specific amino acids:

- Arginine (Arg) repairs damaged hair strands and improves its hydrophobicity (i.e., its ability to shed water).
- Phenylalanine (Phe) increases the toughness of hair strands.
- Histidine (His) increases the toughness of hair strands.

2.4.3.3 Vitamins

Vitamins have been used in cosmetic products for many years (Lupo, 2001). We conducted a search on the use of vitamins in cosmetic hair care products and the effects mentioned. The search focuses solely on the four vitamins identified in the surveyed products in this project;

[17] https://comforth.dk/products/comforth-lash-brow-serum

namely, vitamins E, B3, B5 and B7. The descriptions of the effect of vitamins in hair care products in scientific literature are supplemented with descriptions of their claimed effects in relation to eyelash and eyebrow serum products on various online shops.

Tocopherol (Vitamin E)
Lupo (2001) states that vitamin E has potent antioxidant effects and acts as a UV shield. However, the positive properties of vitamin E are primarily described in terms of anti-ageing effects in skincare products. Similarly, Casas (2007) states that vitamin E is used in cosmetic products as an antioxidant for either the skin or the cosmetic product itself.

According to one of the online stores studied in this project (whose offerings include serum products), vitamin E helps support hair, as it has natural antioxidant effects that help maintain hair growth. The vitamin's antioxidant properties reduce oxidative stress and free radicals, which cause hair follicle cells to break down.

According to the CosIng database, tocopherol is used in cosmetic products as an antioxidant, fragrance, and skin conditioning agent.

Niacinamide (Vitamin B3)
Pappelbaum et al. (2024) state that treatment with niacinamide (vitamin B3) can prolong the hair growth phase. However, it is not specified whether this is by oral intake or by application of a cream to hair. According to Lupo (2001), niacinamide primarily has anti-inflammatory properties, but it also acts as a UV shield, guarding against photodegradation. Guryanov et al. (2022) simply state that cosmetic products containing niacinamide can stimulate hair growth. Casas (2007) writes that niacinamide is important for the release of energy in tissues and cells and is essential for cell growth.

According to the CosIng database, niacinamide is used in cosmetic products as a smoothing agent.

Panthenol (Vitamin B5)
According to Lupo (2001), panthenol has been used for years in various hair care products because this vitamin acts as a humectant, increasing the water content of the hair, which means that the elasticity of the hair is improved. Increasing the elasticity of individual hairs also means that fewer hairs break and become damaged. Guryanov et al. (2022) similarly describe the use of panthenol as a moisturiser and to stimulate hair growth. Casas (2007) states that panthenol is an important ingredient in skin and hair care products, its main properties being wound healing effects, anti-inflammatory effects, and moisturising effects; panthenol is also essential for the growth and normal maintenance of skin and hair. Casas also notes that panthenol can help protect and repair damaged hair, reduce split ends, and help thicken individual hairs (increase hair density).

According to one of the online shops studied in this project, panthenol helps strengthen hair, retain moisture, and improve the structure of damaged hair. The shop notes that panthenol is therefore the main ingredient in many hair masks and conditioners.

According to the CosIng database, panthenol is used in cosmetic products as an antistatic agent and as a hair and skin care agent.

Biotin (vitamin B7)
No descriptions of the effect of biotin in cosmetic products have been identified. According to one cosmetics vendor, biotin stimulates keratin production in the hair and actively increases follicle growth.

According to the CosIng database, biotin is used in cosmetic products as a hair and skin care agent.

2.4.3.4 Hyaluronic acid

According to Juncan et al. (2021), hyaluronic acid and its salts, such as sodium hyaluronate, are widely used as ingredients in somewhat more expensive cosmetic products which make claims regarding moisturisation, such as face creams. This is because hyaluronic acid is a moisturiser and plays a role in skin elasticity, as it can bind significant amounts of water to form a gel (Lex.dk/hyaluronsyre, 2024; Juncan et al., 2021).

As described previously, hyaluronic acid is a carbohydrate (polysaccharide) with chains of varying lengths made up of up to 50,000 monosaccharide units, alternating between glucuronic acid and N-acetyl-D-glucosamine (Lex.dk/hyaluronsyre, 2024). Hyaluronic acid is found in all tissues, including in humans. According to Juncan et al. (2021), there are typically 15 g of hyaluronic acid in a typical 70 kg human. One-third of the hyaluronic acid present (5 g) is replaced daily, as it degrades rapidly in the body. However, the rate of hyaluronic acid replacement in the body slows down with age.

Juncan et al. (2021), who have reviewed a large number of scientific articles on hyaluronic acid, note that there is scientific evidence that this substance has positive effects, especially in terms of moisturising and anti-ageing effects in relation to the skin, althought it also has biological effects in relation to the body's cell development, inflammation, and wound healing. Furthermore, Juncan et al. (2021) write that additional biological effects of hyaluronic acid have been demonstrated in conjunction with other active ingredients, including plant extracts, vitamins, amino acids, and peptides. According to the Cosmetic Ingredients Review (CIR) report on hyaluronic acid and its salts, hyaluronic acid is used in cosmetic products for both the skin and the hair (CIR, 2022). According to Danish Consumer Council Tænk Kemi, hyaluronic acid is an ingredient used in many products on the Danish market, and in several different product types.

The molecular size (and therefore molecular weight) of hyaluronic acid and the substances it binds to have an impact on its biological activity. Juncan et al. (2021) write that hyaluronic acid is important as an ingredient for the "delivery" of other active ingredients to the body. Examples include the use of hyaluronic acid with specific peptides; specific plant extracts; and specific vitamins, such as niacinamide (B3), panthenol (B5), and tocopherol (E).

According to the CosIng database, hyaluronic acid is used in cosmetic products as an antistatic agent, as a humectant and moisturising agent, and as a skin care agent.

2.4.3.5 Plant extracts

One of the websites for the lash and brow serum products used lists a mixture called Redensyl™ as an ingredient in these products. According to UL Prospector, Redensyl™ consists of the ingredients with the following INCI names: glycerin, aqua, sodium metabisulfite, Larix europaea wood extract, glycine, zinc chloride and Camellia sinensis leaf extract. The plant extracts Larix europaea wood extract and Camellia sinensis leaf extract are present in two and four of the analysed serum products, respectively. The UL Prospector database indicates that the Redensyl™ blend reactivates stem cells to promote hair growth.

In the search for specific examples of eyelash and eyebrow serums, the following descriptions of plant extract ingredients and their effects were identified.

Acorus calamus root extract
Acorus calamus root extract increases the shine and softness of lashes, reduces hair loss, and promotes hair growth, according to one of the online shops studied in this project.

According to the CosIng database, this substance is used in cosmetic products as a fragrance and skin care agent.

Amaranthus caudatus seed extract
Amaranthus caudatus seed extract appears to be the INCI name for a peptide with the trade name NaturePep® Amaranth[18]. According to the Prospector materials database (2024), this amaranth peptide (NaturePep® Amaranth) helps to increase the diameter of the hair shaft, resulting in stronger and thicker hair. Comforth Scandinavia (2024) also writes that an extract from the amaranth plant increases the diameter of the hair shaft.

According to the CosIng database, this substance is used in cosmetic products as a skin care agent.

Panax ginseng extract
Ginseng is an ancient Asian herb that naturally promotes hair growth by stimulating hair follicles, according to one of the investigated online shops. Ginseng helps prevent hair loss and enhances eyelash growth from root to tip, promoting thicker, fuller and stronger lashes.

According to the CosIng database, this substance is used in cosmetic products as an antioxidant, for skin care, and for oral care.

Ricinus communis (castor) seed oil
Ricinus communis (castor) seed oil has nourishing and repairing properties according to the UL Prospector database, and is therefore ideal as an ingredient in cosmetic hair products (ULProspector.com/Organic Castor Oil, 2024).
According to online pharmacy ApoPro (2024), castor oil is rich in minerals and fatty acids that increase blood circulation and provide improved nourishment to hair roots.

According to the CosIng database, this substance is used in cosmetic products as a fragrance and perfume ingredient, as well as for skin care.

Trifolium pratense flower extract
Trifolium pratense (red clover) flower extract is part of the Capixyl™ peptide mixture, according to a Danish online shop. The mixture also contains Acetyl Tetrapeptide-3 (also described above, under peptides). According to UL Prospector, this mixture has anti-inflammatory properties, and the peptide stimulates tissue formation to better anchor the hairs and increase their vitality. Thus, this mixture ostensibly stimulates hair growth and limits hair loss, including for eyelashes (ULProspector.com/Capixyl, 2024)

According to the CosIng database, this substance is used in cosmetic products as a fragrance and astringent (producing a tissue contraction effect).

[18] https://www.personalcaremagazine.com/story/38105/improving-hair-thickening-with-amaranth-peptides

3. Exposure scenarios

In order to conduct a risk assessment of the eyelash and eyebrow serum products, it is important to identify the target group and usage pattern for these products, as well as the concentrations of the ingredients they contain, to be able to calculate a user's exposure to these ingredients.

We can provide the following summary of these attributes based on the data obtained in the survey:

Target group: Largely younger women, including pregnant women, who are considered to be particularly sensitive to chemical exposure.

Application: The products are typically used daily for extended periods, and the products are applied 1-2 times daily. Approximately between 0.06 ml and 0.27 ml are used daily according to use ranges supplied on webshops.

Based on this, user exposure can be calculated in terms of milligrams of substance per square centimetre of surface area; this is an important exposure measure to assess how concentrated the exposure is on the skin, and thus the risk of local skin effects. User exposure can also be calculated in terms of milligrams per kilogram of body weight, which is a measure of exposure used to assess harmful effects of the portion of the ingredients that can be absorbed into the body and pose a risk of effects in the body's internal organs.

In light of this, exposure can be calculated as follows:

$$Exposure\ (mg/cm^2) = \frac{0.27\ ml\ product \times mg\ subs./ml}{exposed\ area\ (cm^2)}$$

$$Exposure\ (mg/kg\ lgv/dag) = \frac{0.27\ ml\ product\ \times mg\ subst./ml}{60\ kg}$$

The body weight of the user is fixed at 60 kg, as this is the recommended value to use for risk assessments; cf. SCCS Notes of Guidance for the Testing of Cosmetic Ingredients and Their Safety Evaluation (SCCS 2023).

When assessing a specific product, the amount of product used per day should be considered separately, as the amount sold will depend on the product's *instructions for use, viscosity, and application equipment.* For eyelash serum products containing prostaglandins, the SCCS (2022) used an exposure amount of 2.4 mg, corresponding to approx. 2.4 µl or 0.0024 ml.

No standard measurements for the skin area of eyebrows and eyelashes have been readily identified. On a website selling eyebrow stencils, different eyebrow shapes are depicted in relation to a centimetre measurement. The measurements for these eyebrows are 0.7 cm at the widest point and 5.7 cm in length. Similarly, measurements of fake eyebrows show that the length of the eyebrows are between 5.5 and 5.8 cm, and that the eyebrows are 0.7-0.8 cm wide, where they are the widest. This results in a maximum area per eyebrow of 4 cm^2, not accounting for the fact that the eyebrows are narrower at the sides. Measurements taken by the project team on different artificial eye lashes showed that the area of the eyelid edges were approximately 2 x (3.5 cm x 0.25 cm) = 1.8 cm^2.

4. Selection of substances

In collaboration with the Danish EPA, data from the survey was used to select which substances to focus on in quantitative chemical analyses and a subsequent risk assessment. A focus on the serum products' active ingredients was the premise for this project.

The market research in this project showed that peptides are widely used as active ingredients in eyelash and eyebrow serums. In addition to peptides, certain vitamins, hyaluronic acid, and various plant extracts are also used as active ingredients. Of these active ingredients, peptides were considered the most interesting to investigate further in the project. These ingredients consist of amino acids linked together. Amino acids are naturally found in the body. Developments in the design of synthetic peptides used as active ingredients in a new generation of cosmetic products known as "cosmeceuticals" (see, e.g., He et al (2023) and UK Research and Innovation (2023)) have been seen, in recent years, for this group of substances.

Hyaluronic acid and the vitamins (E, B3, B5, and B7) naturally found in the body are not considered to be of concern in cosmetic products. Several plant extracts can be allergenic, as they may contain various fragrances. However, it is only a few plant extracts that can be found in many of the serum products surveyed. The plant extracts used seem to vary from manufacturer to manufacturer and from product to product. The vast majority of the observed plant extracts occurred in only one of the 44 products analysed.
Many plant extracts (and fragrances newly requiring individual declaration) are difficult to analyse, as they consist of mixtures of many different substances. The exact composition of a plant extract depends on its manufacturing process, the source of the plant material, the batch of the extract, and so on. In addition, it is not known where each serum manufacturer sources its extracts. Precise quantification is therefore difficult and subject to significant uncertainty, as the composition of a plant extract purchased as a reference substance may differ substantially from the extract used by a given manufacturer.

The surveyed serum products were also analysed for possible problematic ingredients, such as substances suspected of being endocrine disruptors. In this regard, the ingredients methylparaben and salicylic acid were observed, but only in a few products. For both methylparaben and salicylic acid, the Cosmetic Products Regulation already sets limit values for the maximum allowed content in cosmetic products (for methylparaben, in Annex V, reference number 12; for salicylic acid, both in Annex III, reference number 98, and in Annex V, reference number 3).

For these reasons, it was decided to focus the quantitative analyses and risk assessment in this project on the three most commonly used peptides, occurring in 20, 16, and 7 of the 44 serum products surveyed in this project:

- Myristoyl Pentapeptide-17
- Biotinoyl Tripeptide-1
- Acetyl Tetrapeptide-3

Based on the survey, it was decided in collaboration with the Danish EPA that it would not make sense to measure the pH values of the serum products. Firstly, the survey of the investigated products showed that the serum products are likely to have a relatively neutral pH (between 5 and 7); secondly, the active ingredient peptides that the majority of the serum products contain will be sensitive to acidic or basic pH values (below 4 or above 7), increasing the risk of the peptides being broken down (hydrolysed) into smaller peptides and amino acids.

Thus, the stated pH range is not considered critical, despite the serum products being used in and around the eye area.

5. Selection and procurement of products

Based on the survey and the preliminary overview of 58 serum products, a total of 19 serum products were selected and purchased for the chemical analyses. The 19 products were selected according to the following criteria:

- Products were to contain one or more of the three selected peptides (Myristoyl Pentapeptide-17, Biotinoyl Tripeptide-1 or Acetyl Tetrapeptide-3)
- Products where the content of the peptide(s) was highest on the ingredient list were prioritised, as this may indicate that the concentration of peptides is highest in these products. According to the Cosmetic Products Regulation, ingredients with the highest concentrations must be listed first on the ingredient list, in decreasing order of concentration - however, ingredients with concentrations below 1% can be listed in any order.
- As the survey identified a predominance of products in Danish online shops, the majority of products were purchased in Denmark and from Danish online shops. Products outside the EU were deliberately excluded, as few products with ingredient information were identified on online platforms outside the EU. For this reason, DK and EU products were chosen with a distribution of approximately 67% and 33%, respectively.
- The survey showed that some products were sold as combination lash and brow serums, and that this group of products was similar in size to the lash serum group. The fewest serums identified in the survey were for brows only. For this reason, products purchased for the chemical analyses consisted primarily of lash and combination lash/brow serums.
- In addition, products were purchased to represent the most common application types among the 58 mapped products (fine brush, mascara-type brush, round foam pad).

A breakdown of the products purchased is presented in TABLE 10.

TABLE 10. Distribution of the 19 serum products purchased by source and type

Product type	Purchased in DK	Purchased in the EU	Total
Combination brow/lash serum	4	3	**7**
Eyebrow serum	1	3	**4**
Eyelash serum	8	0	**8**
Total	**13**	**6**	**19**

Between three and five units of each product were purchased, depending on the quantity (in ml) of serum in the product. One product was saved as a spare sample for the Danish EPA. The remaining samples were used for the chemical analyses.

The 19 purchased products are listed in TABLE 11 below, which contains information about product type, source (DK or EU), production country, application method, volume of product, and price. The products are labelled using the following information:

- Source (DK or EU)
- Number from 1 to 19
- Product type (B for brow serum, L for lash serum, or BL for both)

TABLE 11. Overview of the 19 products purchased and information about product type, application method, price, etc.

Product no.	Product type	Country, manufacturer	Application method	Product volume (ml)	Price
DK-1-BL	Both	Canada	Fine brush	5	123 DKK
DK-2-BL	Both	France	Mascara-type brush	6.5	154 DKK
DK-3-BL	Both	Denmark	Fine brush	8	295 DKK
DK-4-BL	Both	Sweden	Fine brush	12	370 DKK
DK-5-L	Lash serum	Denmark	Fine brush	8	225 DKK
DK-6-L	Lash serum	China	Fine brush	5	465 DKK
DK-7-L	Lash serum	Italy	Fine brush	6	379 DKK
DK-8-B	Eyebrow serum	Denmark	Mascara-type brush	6	225 DKK
DK-9-L	Lash serum	Denmark	Fine brush	6	359 DKK
DK-10-L	Lash serum	Denmark	Fine brush	6	250 DKK
DK-11-L	Lash serum	Switzerland	Mascara-type brush	10	245 DKK
DK-12-L	Lash serum	Denmark	Fine brush	4	159 DKK
DK-13-L	Lash serum	Denmark	Fine brush	5	370 DKK
EU-14-BL	Both	Türkiye	Mascara-type brush	3.5	324 DKK
EU-15-BL	Both	Italy	Mascara-type brush	5	119 DKK
EU-16-BL	Both	Germany	Fine brush	6	189 DKK
EU-17-B	Eyebrow serum	China	Fine brush	3	287 DKK
EU-18-B	Eyebrow serum	China	Round foam pad	5	375 DKK
EU-19-B	Eyebrow serum	USA	Fine brush	3.5	535 DKK

The content of the three selected peptides according to the products' ingredient lists is listed in TABLE 12 below.

TABLE 12. Overview of the 19 purchased products and their content of the three peptides selected for the chemical analyses

Product no.	Peptide	Product no.	Peptide
DK-1-BL	Myristoyl Pentapeptide-17, Biotinoyl Tripeptide-1, Acetyl Tetrapeptide-3	DK-11-L	Biotinoyl Tripeptide-1
DK-2-BL	Biotinoyl Tripeptide-1	DK-12-L	Biotinoyl Tripeptide-1
DK-3-BL	Acetyl Tetrapeptide-3	DK-13-L	Myristoyl Pentapeptide-17
DK-4-BL	Myristoyl Pentapeptide-17	EU-14-BL	Biotinoyl Tripeptide-1
DK-5-L	Acetyl Tetrapeptide-3	EU-15-BL	Biotinoyl Tripeptide-1
DK-6-L	Myristoyl Pentapeptide-17, Biotinoyl Tripeptide-1	EU-16-BL	Myristoyl Pentapeptide-17
DK-7-L	Acetyl Tetrapeptide-3	EU-17-B	Myristoyl Pentapeptide-17, Biotinoyl Tripeptide-1
DK-8-B	Myristoyl Pentapeptide-17	EU-18-B	Myristoyl Pentapeptide-17, Biotinoyl Tripeptide-1
DK-9-L	Myristoyl Pentapeptide-17, Biotinoyl Tripeptide-1	EU-19-B	Myristoyl Pentapeptide-17, Acetyl Tetrapeptide-3
DK-10-L	Myristoyl Pentapeptide-17		

6. Chemical analyses

As described in chapter 5, in collaboration with the Danish EPA, it was decided to focus on the three most commonly used peptides in the surveyed serum products, and 19 serum products were purchased for the chemical analyses:

- Myristoyl Pentapeptide-17 (ingredient in 11 of the 19 serum products)
- Biotinoyl Tripeptide-1 (ingredient in ten of the 19 serum products)
- Acetyl Tetrapeptide-3 (ingredient in five of the 19 serum products)

The 19 purchased products were analysed quantitatively with regard to the amount of the selected peptide(s) present that were listed on the products' ingredient lists. The analysis method and results are described below.

6.1 Method of analysis

The three peptides were quantified using UPLC-MS^2 (Ultra-Performance Liquid Chromatography-Tandem Mass Spectrometry). True duplicate determinations were performed for all analyses. The analysis method used is based on an analysis method described in the literature for the quantification of palmitoyl-functionalised peptides in anti-wrinkle creams (Chirita et al., 2009). The method from Chirita et al. (2009) measures short peptides in anti-wrinkle creams, including palmitoyl tripeptide-1 (pal-GHK), with dilution as the only sample preparation with the addition of salt (NaCl) and acetic acid in methanol. As this method does not encompass measurement of the three peptides to be measured in this project, but only of the internal standard used - namely, palmitoyl tripeptide-1 (pal-GHK) - the method was modified so that the three peptides and the internal standard could all be measured.

Initially, experiments were carried out with the addition of salt to the samples, as used in the method from the literature. Doing so can salt out any proteins in the sample, thereby providing cleaner samples for analysis. However, the addition of salt was found to prevent the measurement of individual peptides.

In this project, samples were therefore only diluted in acidified methanol. The internal standard (palmitoyl tripeptide-1 (pal-GHK)) was added, and samples were analysed directly via UPLC-MS^2 after filtration. The eluent (the liquid used in the UPLC) was a mixture of water and acetonitrile (MeCN) trifluoroacetic acid, and the method was isocratic (meaning that the composition of the eluent does not change during analysis). UPLC-MS^2 parameters and methods were customised for each peptide and measured using multiple reaction monitoring (MRM) methods. MRM is a special measurement technique that can be performed on certain types of mass spectrometers (tandem mass spectrometers). This technique is widely used for peptides and protein characterisation, ensuring high selectivity and the lowest possible limits of detection. For each of the three peptides and the internal standard, a minimum of 4 ions specific to the peptide were identified and measured.

Calibration rows were prepared in acidified methanol. Both controls (acidified methanol) and standard addition were used on selected products to investigate the effect of the matrix.

6.1.1 Sample preparation

The sample was weighed into a vial, and a known amount of internal standard (palmitoyl tripeptide-1) and methanol with 1% glacial acetic acid was added. The solution was then diluted with methanol, mixed well, filtered through a syringe filter and transferred to LC-MS vials before analysis.

6.1.2 Procedure

As part of the UPLC-MS^2 analysis, at least one sample of pure solvent was run before and after each of the 19 samples to check for carry-over between samples and reduce any effects of contamination introduced by the serum samples. Standard rows were run first, last, and in the middle of the analysis sequence. Due to contamination introduced by the serum samples, the column was rinsed with trifluoroacetic acid in acetonitrile for every fifth sample. Controls were distributed throughout the sequence, and the sensitivity of the instrument was regularly checked with a sample with known content of all peptides, as well as after every rinse.

6.1.3 Limits of detection, recovery and uncertainties

In general, the limit of detection is relatively high for the analysed samples compared to what should be achievable with UPLC-MS^2. This is partly due to the high degree of dilution required, as the sample preparation did not involve a purification step; and the fact that the samples contain much more than the peptides, including salts that can affect the analysis. It was also necessary to use trifluoroacetic acid in the eluent. Trifluoroacetic acid is known to negatively affect sensitivity in LC-MS analyses, as it can affect how efficiently a compound, such as one of the measured peptides, is converted to ions in the mass spectrometer during analysis (ion suppression), and thus the magnitude of the signal measured.
The limits of detection were 0.6 µg/g, 3 µg/g and 6 µg/g for Biotinoyl Tripeptide-1, Acetyl Tetrapeptide-3 and Myristoyl Pentapeptide-17, respectively. However, in several instances, the actual limits were higher due to impurities introduced by the samples. Multiple peaks next to or on top of each other disturb the measurement (interference) and make it harder to separate and identify the three peptides. This means that it is only possible to identify the three peptides at higher concentrations, corresponding to a higher limit of detection. Using MRM methods minimises this effect, but several of the samples contained substances that particularly interfered with the measurement of Acetyl Tetrapeptide-3. In addition, substances other than the measured peptides in the samples may mean that one or more of the peptides are not converted to ions as efficiently (ion suppression), resulting in a weaker signal and thus lower sensitivity / higher limits of detection, or substances in the samples may affect other parts of the analysis. Furthermore, it was observed that the sensitivity decreased as more samples were analysed due to general contamination of the instrument. This meant that sensitivity to Acetyl Tetrapeptide-3 and Myristoyl Pentapeptide-17 in particular decreased, thought to be significant for samples 14-19, in which very low concentrations could not be measured.

The average recovery rates of the analysis was 100%, 92%, and 86% for Biotinoyl Tripeptide-1, Acetyl Tetrapeptide-3 and Myristoyl Pentapeptide-17, respectively, at concentrations corresponding to 20 µg/g in the samples; and to 74% and 109% for Biotinoyl Tripeptide-1 and Acetyl Tetrapeptide-3, respectively, at concentrations equivalent to 4 µg/g in the samples. The content of Myristoyl Pentapeptide-17 at this low level of concentration could not be measured. For Acetyl Tetrapeptide-3, the low concentration level of 4 µg/g in the samples is below the limit of quantification, and close to the limit of quantification for Biotinoyl Tripeptide-1. The recovery rate is the percentage of a known amount of added substance measured in the analysis; it serves as an indication of the accuracy of the analysis. Recovery rates can exceed 100% (i.e., greater content measured than was actually added).
Given the low recovery at the limit of quantification, it is likely that the measured content of Biotinoyl Tripeptide-1 and Myristoyl Pentapeptide-17 is underestimated when close to the limit of quantification.

Standard addition to four selected samples also showed that recovery is highly dependent on the matrix. For example, recovery rates of the three peptides added to sample DK-1-BL was 54% for Biotinoyl Tripeptide-1, while Acetyl Tetrapeptide-3 and Myristoyl Pentapeptide-17 were below the detection limit; meanwhile, 105% of the added Myristoyl Pentapeptide-17 was

recovered by standard addition to sample DK-10-L. The content of each sample therefore has a significant impact on how much of a given peptide is measured in the sample.

The expanded uncertainty of the analysis was measured at 20%, 46%, and 43% for Biotinoyl Tripeptide-1, Acetyl Tetrapeptide-3 and Myristoyl Pentapeptide-17, respectively, at concentrations corresponding to 20 µg/g in the samples; and at 28% and 47% for Biotinoyl Tripeptide-1 and Acetyl Tetrapeptide-3, respectively, at concentrations equivalent to 4 µg/g in the samples. For Acetyl Tetrapeptide-3, the low concentration level of 4 µg/g in the samples is below the limit of quantification.

6.2 Results of the quantitative analyses

The results of the quantitative analyses are presented in TABLE 13 below. Although not all three peptides were used in all 19 serum products (not all three peptides were necessarily listed in the ingredient lists), the analysis covered all three peptides for all 19 samples, as the analytical method was developed to measure all peptides together. Deviations from the ingredient list are marked with a light green background in the table. This means that if a peptide is on an ingredient list but not identified by the quantitative analysis (marked with <), the corresponding cell will have a light green background.

TABLE 13. Results of the quantitative analyses of the three peptides in 19 purchased products. Deviations from the ingredient list on the product are marked with a light green background.

Product no.	Myristoyl Pentapeptide-17 (µg/g)	Biotinoyl Tripeptide-1 (µg/g)	Acetyl Tetrapeptide-3 (µg/g)
DK-1-BL	< 6**	0.6-2**	< 3**
DK-2-BL	< 6	0.6-2	< 6
DK-3-BL	< 6	< 0.6	11
DK-4-BL	< 6	< 0.6	3-8*
DK-5-L	< 6	< 0.6	< 6
DK-6-L	< 6	< 0.6	3-8*
DK-7-L	< 6	< 0.6	3-8
DK-8-B	61	< 0.6	< 3
DK-9-L	7	49	< 6
DK-10-L	41	< 0.6**	< 6**
DK-11-L	< 6	0.6-2	< 6
DK-12-L	< 6	0.6-2	< 6
DK-13-L	6	< 0.6	< 6
EU-14-BL	< 6	0.6-2	< 6
EU-15-BL	< 6	0.6-2	3-8*
EU-16-BL	31	< 0.6	< 6
EU-17-B	< 6	0.6-2	< 6
EU-18-B	< 6**	< 0.6	< 6**
EU-19-B	52***	< 0.6	9***

* Possible false positive

** Standard addition to the sample indicates that any content in the sample is underestimated.

*** Standard addition to the sample indicates that the content in the sample is overestimated.

6.3 Discussion of analysis results

All 19 serum products contain a wide range of ingredients - between 12 and 78 ingredients - some of which are some form of plant extract. Such extracts themselves contain many different substances. This has contributed to high limits of detection and low recovery with standard addition. In addition, many of the 19 serum products contain some form of salt or pH-regulating ingredient (e.g., sodium hydroxide), which can have a major impact on the result and recovery rates of the peptides.

As shown in TABLE 13, the three peptides generally occur at low concentrations. The highest concentration of any of the peptides in the 19 serums tested is approximately 60 µg/g (corresponding to 0.006%). Myristoyl Pentapeptide-17 was identified at the highest concentration in six products, at concentrations between 6 and 61 µg/g. Biotinoyl Tripeptide-1 was identified in seven products at concentrations between 0.6-2 and 49 µg/g. Acetyl Tetrapeptide-3 was identified in six products at concentrations between 3-8 and 11 µg/g.

According to the two companies that answered questions about the concentration of peptides in eyelash and eyebrow serums in the survey in this project, the concentration of the three investigated peptides in their products is ≤ 0.01%, ≤ 0.001% and ≤ 0.3%, corresponding to 100 µg/g, 10 µg/g and 3000 µg/g for Myristoyl Pentapeptide-17, Biotinoyl Tripeptide-1 and Acetyl Tetrapeptide-3, respectively. It should be noted, however, that all concentration levels provided by the companies were given as "less than" values, meaning that the actual concentrations may well be significantly lower than the stated maxima. No information on the content of the three investigated peptides in this type of serum that could be used for comparison has been identified in the literature. However, in the article containing the method from the literature (Chirita et al., 2009), the peptide palmitoyl-lysyl-threonyl-threonyl-lysyl-serine (pal-KKTKS) was identified in six anti-wrinkle creams at concentrations between 1 and 6.5 µg/g.

As shown by the highlighted cells in TABLE 13, in several cases there is a discrepancy between the peptides we identified in the 19 serums investigated and what is stated in the products' ingredient lists. This is mainly due to the fact that we were unable to identify these peptides at sufficiently low concentrations (i.e., the limit of detection was high). Several of the peptides also contain salts that can influence the analysis results. This could cause us to either be unable to identify a peptide or measure a lower concentration than the actual one (i.e., to underestimate the concentration). For instance, this is the case with sample DK-1-BL, which according to the ingredient list should contain all three peptides, but where only Biotinoyl Tripeptide-1 was measured below the limit of quantification, and where standard addition of a known amount of each peptide to the sample showed that any content in the sample was significantly underestimated. The added amounts of Myristoyl Pentapeptide-17 and Acetyl Tetrapeptide-3 could not be measured at all.

7. Exposure assessment

7.1 Application experiments

To get an idea of the amount of serum used per application, single application experiments were performed using different types of serum with different application methods. Application experiments were carried out for a total of three products. We selected products with enough serum remaining after the chemical analyses to complete these trials, including different types of products with different application methods (fine brush, foam pad, and mascara-type brush).

Serum products generally come in small quantities, between 2.4 and 15 ml of serum per product. In the sale context, some products indicate that one unit will last for one or more months of use. For this reason, it is clear that the amount to be used per application is very small. The application experiments were therefore carried out by weighing on a microbalance that can measure down to a single µg. Serum was applied as indicated on the products, in the following ways for the three application experiments performed:

1. With a fine brush, along the roots of the artificial eyelashes
2. Along the roots and on the lashes themselves with a mascara-type brush
3. On the artificial eyebrows with a pad, both on the hair and as close to the roots as possible

The artificial eyelashes and eyebrows were weighed before and directly after serum application. Application experiments with the artificial eyelashes were performed on a new pair of artificial eyelashes each time. However, it should be noted that the artificial eyelashes were not identical - they had different sizes and shapes, numbers and lengths of hairs, etc. The different weighed amounts can therefore illustrate not only variations in the amount applied, but also differences in the size of the eyelashes. For the artificial eyebrows, the brows were dried in a heat cabinet before a second application of the same serum was repeated on the same eyebrows. This was because we only had two sets of artificial eyebrows for use in the trials.

The difference in weight before and after serum application is assumed to be the weight of the serum applied. 10 trials were performed with each serum due to expected large variations in measurements.

A microbalance consists of a small cylindrical chamber that can be closed. The weighing instrument is extremely sensitive to interference from sources like drafts and vibrations in the room. It takes time for the microbalance to stabilise and indicate an exact weight. This is also because solvents in the serum slowly evaporate. The weight of a pair of artificial eyelashes/eyebrows with serum applied was noted at the time when the weight shown on the display was stable and did not change (was lowered). The applied amounts of serum are given in milligrams in TABLE 14 on the next page. The amounts indicated are the amount applied to one pair of eyelashes or one pair of eyebrows; that is, the total amount applied to both eyes.

The results of the application experiments (TABLE 14) show that there are large variations between the 10 application experiments with the same serum. This is due to the solvent(s) in the serum evaporating, the artificial eyebrows not being the same for the 10 application experiments performed, some serum "clumping" (due to its viscosity), and eyebrows and eyelashes being held with tweezers while the serum was applied. It is therefore not possible to apply the same amount every time. For example, serum DK-2-BL is so viscous that it was not possible to extract the product with a pipette for the chemical analyses, while products DK-5-L

and EU-18-B could be pipetted. The fact that DK-2-BL is so viscous is probably the main reason why approximately 10 times more of serum DK-2-BL is used compared to serum DK-5-L. However, in reality, different subjects will also apply different amounts of serum to their eyes.

In addition, the quantities used are very small, so there will naturally be greater variations. Finally, the artificial eyelashes are loose eyelashes where the serum is applied. These artificial eyelashes consist of a thin, self-adhesive foam rubber strip to which artificial hairs are attached. It cannot be ruled out that the foam rubber strip will absorb more serum than would be absorbed when applicating to natural lashes/eyes. Due to the small amounts applied, it was not possible to otherwise estimate the amount of serum applied.

The results do, however, give an indication of the order of magnitude of serum used for eyebrows and eyelashes, respectively.

TABLE 14. Application experiments with a total of three purchased serums. The application method of the serum is indicated in parentheses. The amount is the amount applied to both eyes.

Trial no.	**DK-5-L** **Amount on eyelashes** **(fine brush)** **mg**	**DK-2-BL** **Amount on eyelashes** **(mascara-type brush)** **mg**	**EU-18-B** **Amount on eyebrows** **(foam pad)** **mg**
Application method	Along the roots of the lashes	Along the roots of the lashes and on the lashes themselves	On the hairs themselves, as well as at the hair roots
1	1.544	15.946	38.639
2	0.842	7.276	42.263
3	3.661	27.708	31.898
4	0.924	13.644	37.471
5	3.215	29.502	29.822
6	1.470	45.526	30.998
7	4.954	42.943	37.328
8	4.908	34.984	41.637
9	3.511	22.696	30.902
10	1.420	29.070	31.009
Average of the 10 trials	**2.645**	**26.930**	**35.197**
Maximum value for the 10 trials	**4.954**	**45.526**	**42.263**

7.2 Exposure calculations

7.2.1 Local exposure on skin/mucous membranes (mg/cm²)

As stated in chapter 3, to assess the risk of local effects (skin sensitisation and irritation), it is important to know the extent of local exposure on the skin/mucosal surface indicated as *mg of substance / cm² of exposed area:*

$$Exposure\ (mg/cm^2) = \frac{ml\ product \times mg\ subst./ml}{exposed\ area\ (cm^2)}$$

Based on the maximum application amount of the product on the eyelashes (45.5 mg) and eyebrow (42.3 mg), the maximum exposure on the eyelid borders (1.8 cm^2) and eyebrow skin (8 cm^2) (areas given in chapter 3) can be calculated, using the highest concentrations measured for three ingredients (TABLE 13) serving as a worst-case exposure value. (Note that the concentrations from TABLE 15 in connection with the calculations are given in ng substance/mg instead of µg/g, as given in TABLE 13):

Eyelid border exposure (1.8 cm^2)

$$Expsoure\ (myristoyl\ pentapep.\ 17)(ng/cm^2) = \frac{45.5\ mg\ product\ \times\ 61\ ng\ subst./mg}{1.8\ cm^2} = 1542 ng/cm^2$$

$$Exposure\ (biotinoyl\ tripep.\ 1)(ng/cm^2) = \frac{45.5\ mg\ product\ \times\ 49\ ng\ subst./mg}{1.8\ cm^2} = 1239 ng/cm^2$$

$$Exposure\ (acetyl\ tetrapeptide\ 3)(ng/cm^2) = \frac{45.5\ mg\ product\ \times\ 11\ ng\ subst./mg}{1.8\ cm^2} = 278 ng/cm^2$$

Eyebrow skin exposure (8 cm^2)

$$Exposure\ (myristoyl\ pentapep.\ 17)(ng/cm^2) = \frac{42.3\ mg\ product\ \times\ 61\ ng\ subst./mg}{8\ cm^2} = 323\ ng/cm^2$$

$$Exposure\ (biotinoyl\ tripep.\ 1)(ng/cm^2) = \frac{42.3\ mg\ product\ \times\ 49\ ng\ subst./mg}{8\ cm^2} = 259 ng/cm^2$$

$$Exposure\ (acetyl\ tetrapeptide\ 3)(ng/cm^2) = \frac{42.3\ mg\ product\ \times\ 11\ ng\ subst./mg}{8\ cm^2} = 58 ng/cm^2$$

As can be seen from the calculations, approximately five times higher exposure is achieved on the eyelid borders compared to the eyebrow skin.

7.2.2 Total exposure (mg/kg bw/day)

As stated in chapter 3, the following formula is used for calculating the total exposure of a substance:

$$Exposure\ (mg/kg\ bw/dag)\ = \frac{ml\ product/day\ \times\ mg\ subst./ml}{60\ kg}$$

Based on the maximum application rate of the product on the eyelashes (45.5 mg) and eyebrows (42.3 mg); i.e. 87.8 mg in total, the maximum exposure can be calculated using the highest concentrations measured for the three ingredients (TABLE 13 as a worst-case exposure value. (Note that the concentrations from TABLE 15 in connection with the calculations are given in ng substances/mg instead of µg/g as given in TABLE 13):

$$Exposure\ (myristoyl\ pentapep.\ 17)(ng/kg\ bw/day) = \frac{87.8\ mg/day\ \times\ 61\ ng\ subst./mg}{60\ kg} = 89\ ng/kg\ bw/day$$

$$Exposure\ (biotinoyl\ tripep.\ 1)(ng/kg\ bw/day) = \frac{87.8\ mg/day\ \times\ 49\ ng\ subst./mg}{60\ kg} = 72\ ng/kg\ bw/day$$

$$Exposure\ (acetyl\ tetrapeptide\ 3)(ng/kg\ bw/day) = \frac{87.8\ mg/day\ \times\ 11\ ng\ subst./mg}{60\ kg} = 16\ ng/kg\ bw/day$$

7.2.3 Exposure overview

TABLE 15 below are the calculated worst-case exposures of the three peptide substances based on the highest application amount measured on lashes and brows, and for the highest concentrations measured for the three peptide ingredients.

TABLE 15. Worst-case user exposure to the selected peptide ingredients

	Myristoyl Pentapetide-17	**Biotinoyl Tripeptide-1**	**Acetyl Tetrapeptide-3**
Highest concentrations measured	61 ng/mg	49 ng/mg	11 ng/mg
Maximum amount of product applied (lashes + brows)	87.8 mg	87.8 mg	87.8 mg
Maximum exposure (ng/kg bw/day)	89	72	16
Maximum exposure ng/cm^2	1542	1239	278

8. Hazard assessment

8.1 Description of approach

The following three peptide cosmetic ingredients have been selected for a hazard and risk assessment.

- Myristoyl Pentapeptide-17
- Biotinoyl Tripeptide-1
- Acetyl Tetrapeptide-3

As previously mentioned, peptides and amino acids are essential for building the body's organs and tissues, and take part in the metabolic processes. The bioactive peptides in cosmetics are typically used to maintain and support skin and hair growth.

The table below shows the composition and function of the three selected substances according to the CosIng database:

Peptide compound	Composition	Function
Myristoyl Pentapeptide-17 CAS 959610-30-1	Myristoyl-Lys-Leu-Ala-Lys-Lys-NH_2	Skin care
Biotinoyl Tripeptide-1 CAS 299157-54-3	Biotinyl-Gly-His-Lys-OH	Hair care
Acetyl Tetrapeptide-3 CAS 827306-88-7	Acetyl-Lys-Gly-His-Lys-NH_2	Skin care

On a cosmetics company's website, Myristoyl Pentapeptide-17 is claimed to stimulate the expression of the gene for keratin, which is an important structural protein in hair. This is consistent with Ngoc et al. (2023) stating that bioactive peptides can act as signalling substances.

The three peptide ingredients above represent only a very limited number of peptide ingredients used, as the CosIng database lists 251 tripeptides, 199 tetrapeptides and 199 pentapeptides as active cosmetic ingredients.

Although these peptides are often referred to as biologically active ingredients, the safety of their use in cosmetics has not been assessed by the SCCS, except for a single tetrapeptide coupled to platinum in nano form (*Acetyl Tetrapeptide-17 Colloidal Platinum (nano*)).

For the description and hazard assessment of the three peptide ingredients in this project, data was obtained from:

- Pubchem
- CIR (Cosmetic Ingredient Review) database
- Relevant REACH registration dossiers
- EFSA opinions
- The Danish Veterinary and Food Administration's database of dietary supplements on the market
- Other literature

An important source is the American CIR (Cosmetic Ingredient Review) database, which contains expert toxicological assessments of thousands of cosmetic ingredients. The database was created by the cosmetics and personal care industry with support from the US FDA.

In connection with the description and hazard assessment of the substances, data was sought for each cosmetic ingredient itself (the three peptide substances) and the individual components that comprise the peptide substance, as hydrolysis/degradation of the peptide substances into the single amino acids could potentially occur when applied to moist skin and when mixed with tears.

Based on the toxicological data, the purpose of the hazard assessment is to identify critical effects and the highest no-observed-adverse-effect levels (NOAELs) or lowest-observed-adverse-effect levels (LOAELs) related to local skin and eye exposure (i.e., skin and eye irritation and skin sensitisation effects), and to identify critical organ (systemic) effects related to absorption of the substances through skin/mucous membranes.

A detailed hazard assessment of the peptide ingredients and their individual components / degradation products is provided in Appendix 2.

The results of the hazard assessments are summarised below.

8.2 Hazard assessment results

The tables below summarise the data availability for each cosmetic ingredient and its individual components, and which critical effects can be identified. Then, in column three, an overall conclusion for the hazard assessment of the cosmetic ingredient is given.

TABLE 16. Hazard assessment of Myristoyl Pentapeptide-17

Chemical substance	Toxicological data	Critical effects	Conclusion
Ingredient			
Myristoyl Pentapeptide-17	No data	-	No toxicological data was found for the ingredient Myristoyl Pentapeptide-17.
Components			Data for the individual components into which the substance can be broken down by hydrolysis (myristic acid and the amino acids lysine, leucine and alanine) do not present any identifiable critical systemic effects.
Myristic acid	Yes	No critical effects identified	
Pentapeptide-17	No data	-	
Amino acids			The substance Myristoyl Pentapeptide-17 is expected to be of very low toxicity, as no critical effects or N(L)OAEL can be identified for risk assessment.
Lysine	Yes	No critical effects identified	
Leucine	Yes	No critical effects identified	
Alanine	Yes	No critical effects identified	

TABLE 17. Hazard assessment of Biotinoyl Tripeptide-1

Chemical substance	Toxicological data	Critical effects	Conclusion
Ingredient			No toxicological data was found for the ingredient Biotinoyl Tripeptide-1. Data for the individual components into which the substance can be broken down by hydrolysis (viz., biotin and the amino acids glycine, histidine and lysine) do not present any identifiable critical local or systemic effects. Tripeptide-1 bound to palmitic acid caused eye irritation in rabbits at a concentration of 0.001%*. The substance Myristoyl Biotinoyl Tripeptide-1 is expected to have very low systemic toxicity, as no critical effects or N(L)OAEL can be identified for risk assessment. For local effects, data regarding eye irritation is too uncertain for use in risk assessment, as the exposure was associated with a finished product that likely contains other substances as well.
Biotinoyl Tripeptide-1	No data	-	
Components			
Biotin, Vitamin B7	Yes	No critical effects identified	
Tripeptide-1	Yes; minimal data	No critical effects identified (eye irritation at 0.001%)*	
Amino acids			
Glycine	Yes	No critical effects identified	
Histidine	Yes	No critical effects identified	
Lysine	Yes	No critical effects identified	

*Data very uncertain, as exposure was conducted with a cosmetic product with other possible ingredients

TABLE 18**.** Hazard assessment of Acetyl Tetrapeptide-3

Chemical substance	Toxicological data	Critical effects	Conclusion
Ingredient			No toxicological data was found for the ingredient Acetyl Tetrapeptide-3. Data for the individual components into which the substance can be broken down by hydrolysis (viz., acetic acid, Tetrapeptide-3 and the amino acids lysine, glycine and histidine) do not present any identifiable critical systemic effects.
Acetyl Tetrapeptide-3	No data	-	
Components			
Acetic acid	Yes	No critical effects identified	
Tetrapeptide-3	No data	-	
Amino acids			
Lysine	Yes	No critical effects identified	
Glycine	Yes	No critical effects identified	
Histidine	Yes	No critical effects identified	

As can be seen from these tables, no toxicological data was found on any of the three cosmetic ingredients. For a related ingredient, *Palmitoyl Pentapeptide-4*, degradation of this ingredient has been demonstrated when the substance is added to skin extraction fluid,

caused by the enzyme content of the fluid. This emphasises the importance of hazard assessments of the individual components of ingredients.

The amino acids listed are naturally present in the human diet, where they are part of peptide chains and proteins. All of these amino acids are thus commonly found in the body and involved in the body's metabolic processes and structure.

Based on this and the above review of the peptides and their building blocks, *systemic exposure* to these peptides and the amino acids they contain is not considered to constitute a health concern.
The same applies to the vitamin biotin (B7), which is marketed as a dietary supplement. Finally, myristic acid is also considered a non-toxic fatty acid, as it is a naturally occurring fatty acid in plant oils and thus commonly found in foods.

Regarding *local effects* (skin and eye irritation and skin sensitisation), the available test data does not indicate these as critical effects. Nevertheless, it should be added that relevant *in vitro* and *in vivo* skin sensitisation tests are generally lacking for the peptide substances *per se*; however, data for the individual components, which are linked together to form the peptides, do not give cause for concern for sensitising effects.

Overall, and due to the non-toxic nature of the substances, it is not possible to identify critical effects for the peptide ingredients and their components and relevant N(L)OAEL values that could provide a basis for a quantitative risk assessment.

9. Discussion and conclusion

As described in the hazard assessment in chapter 8, no toxicological data was found on any of the three selected peptides (Myristoyl Pentapeptide-17, Biotinoyl Tripeptide-1 or Acetyl Tetrapeptide-3); therefore, the components of these peptides - amino acids and other acids (myristic acid, biotin (vitamin B7) and acetic acid) - were assessed for hazards instead. All of these amino acids are commonly found in the body and involved in the body's metabolic processes and structure. The same is true for vitamin B7, which is marketed as a dietary supplement. Similarly, no critical effects were identified for acetic acid, and myristic acid is a fatty acid commonly found in plant oils and thereby in foods. The three selected peptides and their use in cosmetic products are consequently of no health concern.

9.1 Uncertainties/limitations

In spite of the above conclusion, there are a number of uncertainties and limitations to this study, which are discussed in this section.

Survey

The survey covers 44 identified serum products for lashes, brows or both sold primarily on the Danish and European markets. The methodology used in the project focused solely on reviewing the ingredient lists for these products. As, generally speaking, only serum products on the market in Denmark and the EU have ingredients listed online at the time of purchasing, this means that the survey covers only these markets. Products sold from outside the EU, including those sold on such online platforms as Temu and Wish, were deliberately excluded from the project, as these products were presented online in a manner that did not show their ingredients. Chemical analyses of all the ingredients in these products would be a completely different and somewhat larger analytical task that could not be accommodated within the scope of this project. It is therefore possible that serum products purchased outside the EU may contain completely different ingredients, potentially including problematic ingredients that have not been investigated in this project. This possibility is relevant for further investigation, as the survey in this project shows that serum products from outside the EU may sell for less than half the price of serum products purchased within Denmark or the EU.

Finally, it should be noted that serum products are typically composed of many different ingredients (often over 20 ingredients per product); meanwhile, for the purposes of this project, it was decided to focus exclusively on the peptide ingredients, which are often emphasised as active ingredients in the presentation of these products.

Chemical analyses

All 19 serum products purchased and analysed for the content of the three selected peptides contained a wide range of ingredients: between 12 and 78 unique ingredients per product. Some of these ingredients were some form of plant extract, and these extracts themselves contain many different substances. These other ingredients contributed to relatively high limits of detection (between 0.6 and 6 µg/g) and low recovery rates in the analytical method used for the three peptides. However, this simply means that it was not possible to identify any low levels of peptides (below 6 µg/g). Even so, this does not change the results of the chemical analyses, which show that the three peptides are present at low concentrations in the 19 purchased serum products. The highest concentration of any of the peptides in the 19 serums tested was approximately 60 µg/g (corresponding to 0.006%).

Exposure assessment
There is relatively limited uncertainty in the exposure assessment (i.e., in the amounts of the selected peptides that consumers are exposed to daily). This is because the uncertainty around the maximum daily application rate and the estimated area of exposed skin/mucosa is estimated to be relatively low (± a factor of 1-3).

Hazard/risk assessment
There is also considered to be limited uncertainty regarding the hazard assessment. Although there is no concrete testing data for the three peptide ingredients, there is sufficient data on their sub-components to produce a reliable hazard assessment.

However, it should be noted that the assessment in the project only includes the content of the three peptide ingredients, and that all other ingredients in the products are not included in the assessments. Of the ingredients that are widely used in this type of product (i.e., included in at least a quarter of the 44 products analysed), the majority are vitamins and hyaluronic acid, in addition to the three peptides assessed.

9.2 Conclusion

The three peptides and their components (amino acids and myristic acid, biotin (vitamin B7) and acetic acid) are generally not considered toxic. In addition to the low daily use quantities of the serum products themselves (maximum 46 mg/day) and the low concentrations of the peptides in the serum products (maximum 61 µg/g), the presence of these peptide ingredients in the serum products is not a cause for health concerns. However, out of the many different ingredients comprising the products, only the three selected peptides were analysed and evaluated in this project. Several other peptides did occur in the surveyed serum products, but they were not investigated in this project. Other frequently used ingredients in serum products (hyaluronic acid and vitamins) are also natural ingredients that present no immediate cause for health concerns in these products.

However, a few of the serum products contain ingredients considered allergenic (benzyl alcohol and various plant extracts). As mentioned, any risk associated with these were not analysed further in this project. In addition, a few of the serum products contain suspected endocrine disruptors (methylparaben and salicylic acid). However, the Cosmetic Products Regulation establishes limit values for both of these ingredients.

Finally, it should be noted that the results of the survey and assessment in this report only cover serum products purchased in Denmark and the EU, as serum products outside the EU were not included in the project (as ingredient lists for them were not available at the time of purchase).

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Appendix 1. Plant extracts

This appendix contains an overview of the plant extracts used and identified in the 44 serum products included in this project's survey. It also indicates in how many of the 44 products the individual plant extracts were identified in.

Some online shops presented descriptions of how certain plant extracts work and how they affect the growth of eyelash or eyebrow hairs. These claims come from the individual online shops marketing the products, and they may or may not be scientifically proven.

TABLE 19. Plant extracts listed as ingredients in the 44 serum products analysed in this project, and the number of serum products they were identified in. The plant extracts are listed in alphabetical order.

INCI name	No. of products (out of 44)	Online shops' comments about effects
Acorus Calamus Root Extract	5	Reduces hair loss and promotes hair growth
Adansonia Digitata Seed Oil	1	
Aesculus Hippocastanum Leaf Extract	1	
Alchemilla Vulgaris Leaf Extract	1	
Allium Sativum (Garlic) Bulb Extract	1	
Allium Sativum Onion Oil	1	
Aloe Barbadensis Leaf Extract	1	
Aloe Barbadensis Leaf Juice	3	
Aloe Barbadensis Leaf Water	1	
Alteromonas Ferment Extract	1	
Althea Officinalis (Marshmallow) Root Extract	1	
Amaranthus Caudatus Seed Extract	2	Increases hair strand diameter
Anemarrhena Asphodeloides Extract	1	
Angelica Sinensis Extract	1	
Argania Spinosa Kernel Oil	1	Anti-inflammatory
Argania Spinosa Seed Oil	1	
Aronia Melanocarpa Fruit Extract	1	
Avena Sativa (Oat) Kernel Extract	1	
Bambusa Arundinacea Stem Extract	1	
Bergamot Peel Water	1	
Beta Vulgaris Root Extract	1	
Brassica Oleracea Capitata Sprout Extract	1	
Brassica Oleracea Italica (Broccoli) Sprout Extract	1	
Caesalpinia Spinosa Gum	1	
Camellia Japonica Flower Extract	1	
Camellia Sinensis Leaf Extract	4	
Cannabis Sativa Seed Oil	1	

INCI name	No. of products (out of 44)	Online shops' comments about effects
Caulerpa Lentilllifera Extract	1	
Centella Asiatica Extract	3	
Centella Asiatica Leaf Extract	1	
Chamomilla Recutita (Matricaria) Extract	1	
Chamomilla Recutita (Matricaria) Flower Extract	1	
Chantharellus Cibarius (Chanterelle) Extract	1	
Chondrus Crispus Extract	1	
Cinnamomum Cassia Bark Extract	1	
Citrus Lemon Peel Extract	1	
Citrus Orange Fruit Water	1	
Coconut Fruit Juice	1	
Cocos Nucifera (Coconut) Fruit Extract	1	
Cocos Nucifera Oil	2	Prevents damage
Coffea Arabica Seed Oil	1	Growth-enhancing ingredient
Corthellus Shiitake (Mushroom) Extract	1	
Crataegus Pinnatifida Fruit Extract	1	
Cucumis Sativus (Cucumber) Fruit Extract	1	
Curcurbita Pepo (Pumpkin) Seed Extract	3	Protects hair follicles from damage
Cucurbita Pepo (Pumpkin) Seed Oil	1	
Curcuma Longa Callus Conditioned Media	3	Helps to increase hair strand diameter and prolong the hair growth phase, resulting in longer hair
Dianthus Caryophyllus Flower Extract	1	
Empetrum Nigrum (Black Crowberry) Fruit Juice	1	
Equisetum Arvense Extract	1	
Equisetum Arvense Leaf Extract	1	
Euphorbia Cerifera Cera (Candelilla) Wax	1	
Europaea Wood Extract	1	
Euterpe Oleracea Fruit Extract	1	
Ganoderma Lucidum (Mushroom) Extract	1	
Glycine Max (Soybean) Seed Extract	1	
Glycine Soja (Soybean) Seed Extract	1	
Glycyrrhiza Glabra Root Extract	1	
Glycyrrhiza Uralensis (Licorice) Root Extract	1	
Helianthus Annuus (Sunflower) Seed Oil	2	
Helianthus Annuus Seed Oil	1	
Hibiscus Sabdariffa Flower Extract	1	
Houttuynia Cordata Extract	3	
Inonotus Obliquus (Mushroom) Extract	1	
Isochrysis Galbana Extract	3	Stimulates hair growth and increases hair density
Juglans Regia Peel Extract	1	

INCI name	No. of products (out of 44)	Online shops' comments about effects
Juniper Wood Oil	1	
Larix Europaea Wood Extract	2	
Lavandula Angustifolia Flower Oil	1	
Lavender Flower Water	1	
Lepidium Meyenii Root Extract	1	
Leuconostoc/Radish Root Ferment Filtrate	3	Probiotic preservative produced by fermenting radishes and Leuconostoc kimchii
Melaleuca Alternifolia Leaf Oil	1	
Mentha Piperita (Peppermint) Leaf Extract	1	
Mentha Piperita Oil	1	
Morus Alba Fruit Extract	1	
Morus Alba Root Extract	1	
Nigella Sativa Seed Oil	1	
Oryza Sativa (Rice) Extract	2	
Paeonia Albiflora Root Extract	1	
Panax Ginseng Extract	3	Stimulates hair growth and promotes thicker, fuller, and stronger lashes
Panax Ginseng Root Extract	3	
Paullinia Cupana Fruit Extract	1	
Phaseolus Radiatus Sprout Extract	1	
Pinus Pinea Seed Oil	1	
Piper Longum Fruit Extract	1	
Pisum Sativum (Pea) Extract	2	
Plankton Extract	3	Provides intense moisturisation for eyelashes; promotes stronger and thicker hair
Polygonum Fagopyrum (Buckwheat) Seed Extract	1	
Polygonum Multiflorum Extract	1	
Polygonum Multiflorum Root Extract	2	
Portulaca Oleracea Extract	1	
Propolis Extract	1	
Prunus Mume Fruit Extract	1	
Pseudoalteromonas Ferment Extract	1	
Puntia Streptacantha Stem Extract	1	
Quinoa ferment extract filtrate	1	
Raphanus Sativus (Radish) Root Extract	1	
Ribes Nigrum (Black Currant) Fruit Extract	1	
Ricinus Communis (Castor) Seed Oil	6	Prevents hair loss
Rosmarinus Officinalis (Rosemary) Leaf Extract	1	
Rosmarinus Officinalis Leaf Oil	1	
Rubus Chamaemorus (Cloudberry) Fruit Extract	1	
Rubus Coreanus Fruit Extract	1	

INCI name	No. of products (out of 44)	Online shops' comments about effects
Saccharomyces Cerevisiae Extract	1	
Saccharomyces Ferment Filtrate	1	
Sambucus Nigra Fruit Extract	1	
Serenoa Serrulata Fruit Extract	3	
Simmondsia Chinensis Seed Oil (jojoba oil)	1	
Sinensis Leaf Extract	1	
Solanum Melongena (Eggplant) Fruit Extract	1	
Sophora Flavescens Extract	1	
Sophora Flavescens Root Extract	1	
Sophora Japonica Root Extract	1	
Swertia Japonica Extract	3	Prevents hair loss
Theobroma Cacao (Cocoa) Extract	1	
Thymus Vulgaris Leaf Extract	1	
Thymus Vulgaris Leaf Oil	1	
Trifolium Pratense Extract	1	
Trifolium Pratense (red clover) Flower Extract	5	Part of the CAPIXYL™ peptide complex
Tropaeolum Majus Extract	1	
Urtica Dioica (Nettle) Leaf Extract	1	
Vaccinium Myrtellus (Bilberry) Fruit Juice	1	
Vaccinium Myrtellus (Bilberry) Seed Oil	1	
Vincetoxicum Atratum Extract	2	Provides intense moisturisation for eyelashes
Viola Mandshurica Flower Extract	1	
Viscum Album (Mistletoe) Extract	1	
Vitis Vinifera (Grape) Fruit Extract	1	
Vitis Vinifera (Grape) Seed Extract	1	
Zingiber Officinale Root Extract	1	
Zingiber Officinalis Root Oil	1	

Appendix 2.Hazard assessment

As previously mentioned, peptides and amino acids are essential for building the body's organs and tissues, as well as for metabolism. The bioactive peptides in cosmetics are typically used to maintain and support skin and hair growth.

The following three peptide ingredients were selected for hazard and risk assessments:

- Myristoyl Pentapeptide-17
- Biotinoyl Tripeptide-1
- Acetyl Tetrapeptide-3

The table below shows the composition and function of the three selected ingredients according to the CosIng database:

Peptide compound	Composition	Function
Myristoyl Pentapeptide-17 CAS 959610-30-1	Myristoyl-Lys-Leu-Ala-Lys-Lys-NH_2	Skin care
Biotinoyl Tripeptide-1 CAS 299157-54-3	Biotinyl-Gly-His-Lys-OH	Hair care
Acetyl Tetrapeptide-3 CAS 827306-88-7	Acetyl-Lys-Gly-His-Lys-NH_2	Skin care

On a cosmetics company's website, Myristoyl Pentapeptide-17 is claimed to stimulate the expression of the gene for keratin, which is an important structural protein in hair. This is consistent with Ngoc et al. (2023) stating that bioactive peptides can act as signalling substances.

The three peptide ingredients above represent only a very limited number of tri-, tetra- and pentapeptides used in cosmetics. The CosIng database for cosmetic ingredients lists 251, 199 and 199 active ingredients containing tri-, tetra- and pentapeptides, respectively.

Although these peptides are often referred to as biologically active ingredients, the safety of their use in cosmetics has not been assessed by the SCCS, except for a single tetrapeptide coupled to platinum in nano form (*Acetyl Tetrapeptide-17 Colloidal Platinum (nano*)).

For this project, web-based literature searches were conducted for toxicological data on low-molecular-weight peptides and the building blocks of the three peptides (acid moieties and amino acids).

Conditions of note regarding the hazard assessment
As seen from the exposure assessment in section 7.2, exposure to the three peptide ingredients is extremely low (the highest values are 1541 ng/cm^2 and 89 ng/kg bw/day for Myristoyl Pentapeptide-17). This is a result of the very low levels of peptide ingredients found in the cosmetic products, and the very small daily usage amounts of the products. The importance of this low exposure is put into perspective below.

In assessing the risk of **systemic effects**, a margin of safety (MoS) value is calculated as stated in the SCCS Notes of Guidance (2023) based on the following formula:

MoS = PoD / SED

where PoD (Point of Departure) is typically based on an oral NOAEL from a 90-day animal study, and SED is the systemic exposure of the cosmetic ingredient; that is, the dose that penetrates the skin and is absorbed into the body.
An MoS value of 100 is generally considered safe with respect to harmful effects, as the exposure is 100 times lower than the PoD (NOAEL) value in the animal study.

If we conservatively assume that absorption through the skin is as high as that which occurs with oral exposure in an animal study, the calculated exposure to Myristoyl-Pentaptide-17 can be used to calculate the size of the NOAEL value that results in an MoS value of 100:

100 = NOAEL / 89 ng/kg bw/day

NOAEL = 8900 ng/kg bw/day, corresponding to 0.0089 mg/kg bw/day

This is an extremely low NOAEL value, which is only seen for very toxicologically potent substances.
This means that toxicological data from the majority of other cosmetic ingredients, where NOAELs are typically in the range of 10-100 mg/kg bw/day, cannot be considered critical in this project, as NOAEL values of this magnitude will result in MoS values exceeding 1000 - 10,000.

This has implications for the subsequent hazard assessment of the peptide ingredients, as data indicating NOAEL values for the most critical effects in the range of 10-100 mg/kg bw/day cannot be considered relevant for further risk assessment.

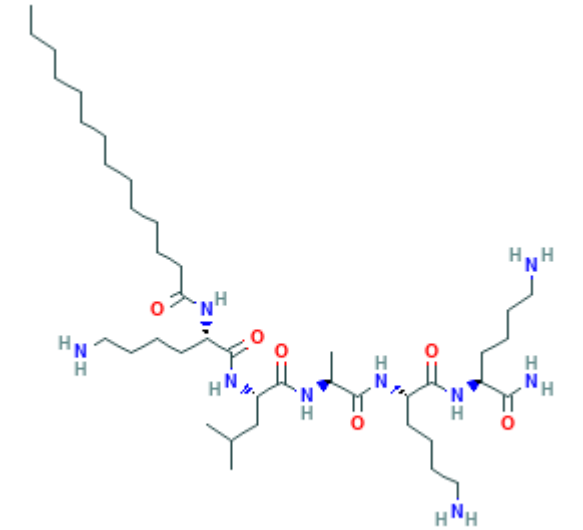
The same applies to risk assessment for local effects, where skin sensitising properties are particularly critical. Based on the exposure assessment for Myristoyl Pentapeptide-17, the greatest exposure is achieved on the eyelid border, as the entire application volume on the eyelashes is estimated to provide the eyelid border with a maximum mucosal exposure of 1.5 µg/cm^2.

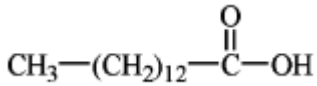
This means that only the most potent skin sensitisers could pose a risk. Chilton et al. (2022), based on an assessment of data for over 500 skin sensitising substances, have divided the substances into non-reactive, reactive and highly reactive skin sensitising substances. By statistically processing these data, exposure thresholds that would protect against sensitising effects with 95% confidence were then calculated at 710 µg/cm^2, 73 µg/cm^2 and 1 µg/cm^2 for the three substance groups, respectively.
This means that data for Myristoyl Pentapeptide-17 must show very clear skin sensitising effects to cause concern for skin sensitisation at an exposure of 1.5 µg/cm^2.

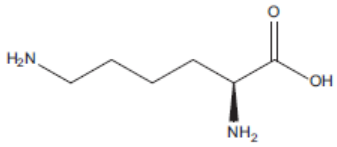
Below in the tables, hazard assessments of the three peptide ingredients are listed based on the data collected and taking into account the above considerations in connection with the identification of possible critical effects.

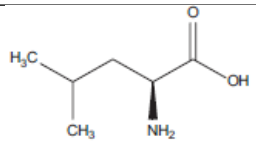
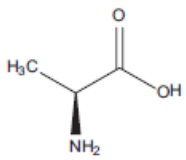
Appendix 2.1 Myristoyl Pentapeptide-17

The table below lists data obtained from searches for use in hazard assessment of the substance.

Chemical substance	Chemical identity and toxicological data	Reference
Myristoyl Pentapeptide-17	 CAS: 959610-30-1 Formula: $C_{41}H_{81}N_9O_6$ Molecular weight: 796.1 g/mol No toxicological data was found for Myristoyl Pentapeptide-17. CIR (2024) has assessed the safety of the substances *Myristoyl Pentapeptide-4, Palmitoyl Pentapeptide-4, and Pentapeptide-4* for cosmetic use, where the pentapeptide chain Pentapeptide-4 contains the amino acids *lysine, serine,* and *threonine*, whereas Myristoyl Pentapeptide-17 contains the amino acids *lysine, leucine* and *alanine*. *Palmitoyl Pentapeptide-4* is reported to be used at concentrations up to 0.0012% in eye lotions. In skin extraction fluid tests, degradation of *Palmitoyl Pentapeptide-4* was detected due to the proteolytic enzyme content of the extraction fluid. *In vitro* and *in vivo* skin irritation tests with *Palmitoyl Pentapeptide-4* at concentrations up to 0.12% showed no irritation. Similarly, human testing with a concentration up to 0.018% showed no irritation. In *in vitro* eye irritation tests, a concentration of 0.01% caused moderate irritation in a HET-CAM test with eggs, while a concentration of 0.036% in an OECD 492 test with human corneal cells produced no irritation response. Similarly, no eye irritation was found in a 0.01% test in rabbits. *In vitro* skin sensitisation tests (OECD 442C and OECD 442D) showed no sensitising potential. In a GPMT test with induction concentrations of 0.0075% and 0.01% and a provocation concentration of 0.0025%, no sensitisation was found.	Pubchem CIR (2024)

Chemical substance	Chemical identity and toxicological data	Reference
	In human subjects, skin sensitisation tests (HRIPT) with concentrations of 0.01% and 0.018% resulted in neither skin irritation nor skin sensitisation. Pentapeptide-4 showed no mutagenic effects in *in vitro* mutagenic tests with bacteria or human lymphocytes. **Assessment** Degradation of the pentapeptide in skin extraction fluid indicates that some degradation of the substance may occur in moisture from skin and mucous membranes. Based on read-across, negative skin and eye irritation tests and skin sensitisation tests with Palmitoyl Pentapeptide-4 do not suggest similar effects for Myristoyl Pentapeptide-17.	
Component	**Chemical identity and toxicological data**	**Reference**
Myristic acid	 CAS: 544-63-8 This substance is a known food additive and occurs as a saturated fatty acid in vegetable oils such as palm kernel oil, from which it is extracted. The substance is reported to promote skin permeability of other cosmetic ingredients. The substance is used in cosmetics at concentrations up to 28.7% in rinse-off products, and up to 1% in products used in the eye area. The substance has been assessed by CIR (2019) in a group with other vegetable fatty acids (lauric, oleic, palmitic and stearic), as these substances are considered to have similar biological effects. Myristic acid has low acute toxicity, with an oral LD50 in rats above 5 g/kg bw. In tests with rabbits, 5% myristic acid in ethanol caused mild skin irritation. In clinical studies, skin exposure to 50% myristic acid in mineral oil caused no irritation in subjects. In eye irritation tests with rabbits, myristic acid has caused mild irritation at a concentration of 1.5%. Myristic acid has been tested but has not caused mutagenic effects in bacteria. No data were found for sub-chronic or chronic exposure to the substance, nor in studies for reproductive toxicity or sensitising effects. Based on the overall set of data for the fatty acids, CIR concluded that myristic acid and the other fatty acids in cosmetics do not pose a health risk. The Danish QSAR database indicates myristic acid as negative regarding skin sensitisation base on CAS Ultra, Leadscope and SciQSAR modelling. **Assessment**	CIR (2019)

Chemical substance	Chemical identity and toxicological data	Reference
	Based on the available data, myristic acid is not considered to have critical effects that are relevant for further risk assessment in the project.	DTU QSAR
Pentapeptide-17	Lys-Leu-Ala-Lys-Lys-NH_2 CAS: Not specified No toxicological data was found for Pentapeptide-17. CIR (2024) has assessed the safety of the substances *Myristoyl Pentapeptide-4, Palmitoyl Pentapeptide-4, and Pentapeptide-4* for cosmetic use, where the pentapeptide chain contains the amino acids *lysine, serine, and threonine*, whereas Myristoyl Pentapeptide-17 contains the amino acids *lysine, leucine and alanine*. Data from these pentapeptides is listed above in connection with the hazard description of Myristoyl Pentapeptide-17. **Assessment** Based on read-across from other pentapeptides, negative skin and eye irritation tests and skin sensitisation tests with *Palmitoyl Pentapeptide-4* do not suggest similar effects for Myristoyl Pentapeptide-17 or Pentapeptide-17 alone.	CIR (2024)
Amino acids	**Chemical identity and toxicological data**	**Reference**
Lysine	 CAS: 56-87-1 This substance is listed in Annex VI of the REACH Regulation (no. 1907/2006) as a substance that does not have critical effects and is therefore not subject to registration under REACH. Lysine is marketed in Denmark as a dietary supplement with an indicated daily dose of approximately 1 gram. CIR (2013) has conducted a safety assessment of amino acids used in cosmetics and states that amino acids are primarily used in hair and skin care products. Lysine is reported to be used at a concentration of up to 0.7% in skincare products and up to 0.04% in products used in the eye area. No *in vitro* or animal tests have been reported regarding skin and eye irritation and skin sensitisation. In skin sensitisation tests with patients, neither irritation nor sensitisation was observed with semi-occlusive facial exposure to cosmetic products containing 0.65% lysine. CIR (2013) concluded that due to the low concentrations used in cosmetics, and in relation to use as a food additive, its use in cosmetics is safe. Based on *in vivo* tests (OECD 404 and OECD 405), lysine hydrochloride is not considered to be a skin or eye irritant. A GPMT test performed with lysine hydrochloride did not cause a sensitising response in any of the animals.	CIR (2013) REACH-reg Ly

Chemical substance	Chemical identity and toxicological data	Reference
	In a 90-day feeding study in rats, no adverse effects were observed at the highest lysine hydrochloride dose of 914 mg/kg/day. Lysine hydrochloride showed no mutagenic effects *in vitro* in bacteria and mammalian cells. In oral teratogenicity studies in rats, the NOAEL was set at a maximum dose of 1000 mg/kg/day. **Assessment** Based on the available data, no critical effects relevant for further risk assessment in the project can be identified.	
Leucine	 CAS: 61-90-5 L-Leucine is an approved food additive (E641). CIR (2013) states that leucine is used in leave-on products in concentrations up to 0.001%. CIR (2013) does not provide toxicological data for leucine. In the REACH registration for L-leucine, the oral LD0 is stated as > 2000 mg/kg in rats. Based on read-across on data for L-valine, the substance is considered neither irritant nor sensitising*. The NOAEL in rats in a 90-day study is given as 3300 mg/kg bw/day. L-leucine has not shown genotoxic effects in either bacteria or mammalian cells. Oral exposure to L-leucine caused no adverse effects in teratogenic studies at the highest dose of 1000 mg/kg bw/day. The dermal DNEL for consumers is given as 416 mg/kg bw/day, while the oral DNEL is given as 41.6 mg/kg bw/day. The Danish QSAR database lists L-leucine as negative regarding skin sensitisation basen on Leadscope and SciQSAR modelling. * In the REACH registration dossier for L-alanine, L-isoleucine is stated not to cause eye irritation in rabbits in an OECD 405 test. Similarly, this dossier states that a GPMT test with L-tert-leucine did not lead to sensitisation of the animals (REACH reg. A). **Assessment** Based on the available data, leucine is not considered to have critical effects relevant for further risk assessment in the project.	CIR 2013 REACH-reg Le DTU QSAR
Alanine	 CAS: 56-41-7	

Chemical substance	Chemical identity and toxicological data	Reference
	CIR (2013) states that alanine is used in leave-on products in concentrations up to 0.1% and in concentrations up to 0.05% in products used in the eye area. In skin sensitisation studies with patients, facial application of a cosmetic product containing 0.04% alanine caused neither skin irritation nor sensitisation. In the REACH dossier for L-alanine, the oral LD50 for rats is given as > 5110 mg/kg. By read-across from skin and eye irritation data for L-valine and L-isoleucine and from data on di- and tripeptides containing alanine, alanine is not considered to be a skin or eye irritant. Based on read-across from a GPMT with L-iso-leucine, alanine is not considered to be sensitising. In a 26-week oral feeding trial with 26% DL-alanine content in rats, no adverse effects were observed. L-alanine did not show mutagenic effects in bacterial tests. No fertility and foetal developmental data are provided in the REACH dossier, but the substance is assessed as not suspected for these effects, as alanine plasma levels resulting from exposure through protein ingestion are not considered to pose a risk. The dermal DNEL for consumers is indicated to 160 mg/kg bw/day, while the oral DNEL is indicated to 16 mg/kg bw/day. The Danish QSAR database lists L-alanine as negative in terms of skin sensitisation in connection with Leadscope and SciQSAR modelling. **Assessment** Based on the available data, alanine is not considered to have critical effects relevant for further risk assessment in the project.	CIR (2013) REACH-reg A DTU QSAR

Sources:

Chilton et al (2022). Updating the Dermal Sensitisation Thresholds using an expanded dataset and an *in silico* expert system. Regulatory Toxicology and Pharmacology 133 (2022) 105200.

CIR (2013). Safety Assessment of α-Amino Acids as Used in Cosmetics. International Journal of Toxicology 32 (Supplement 4) 41S-64S.

CIR (2019). Safety Assessment of Fatty Acids & Fatty Acid Salts as Used in Cosmetics. Final Report. cir-reports.cir-safety.org

CIR (2024). Safety Assessment of Myristoyl Pentapeptide-4, Palmitoyl Pentapeptide-4, and Pentapeptide-4 as Used in Cosmetics. Tentative Report for Public Comment

DTU QSAR. Danish (Q)SAR Database (dtu.dk)

PubChem. PubChem (nih.gov)

Pure&Care (2024). Home: Peptides & Argireline serum | What are peptides? " Pure & Care (pureandcare.dk)

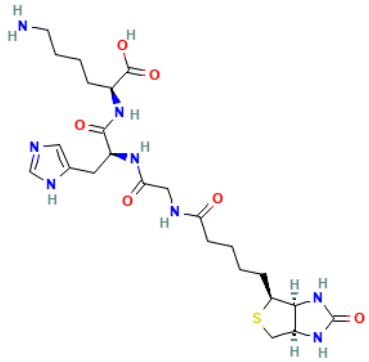
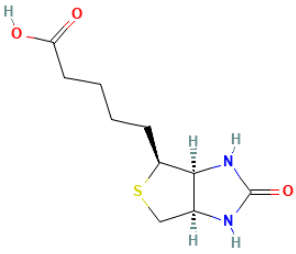
REACH-reg A. REACH registration dossier for L-alanine. Registration Dossier - ECHA (europa.eu) (accessed September 2024)

REACH-reg Le. REACH registration dossier for lysine hydrochloride. Registration Dossier - ECHA (europa.eu) (accessed September 2024)

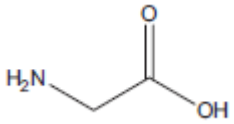
REACH-reg Ly. REACH registration dossier for lysine hydrochloride. Registration Dossier - ECHA (europa.eu) (accessed September 2024)

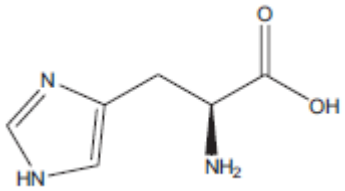
Appendix 2.2 Biotinoyl Tripeptide-1

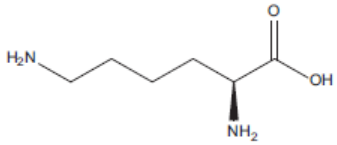
The table below lists data obtained from searches for use in hazard assessment of the substance.

Chemical substance	Chemical identity and toxicological data	Reference
Biotinoyl Tripeptide-1	 CAS: 299157-54-3 Formula: $C_{24}H_{38}N_8O_6S$ Molecular weight: 566.7 g/mol No toxicological data was found for Biotinoyl Tripeptide-1	Pubchem
Component	**Chemical identity and toxicological data**	**Reference**
Biotin, Vitamin B7	 CAS: 58-85-5 EFSA states that there are no toxicological data available for biotin to establish maximum values for the beneficial intake dose of this vitamin. Based on current dietary intake in the general population, an adequate intake for adults is estimated to be 40 µg/day or 6-7 µg/kg bw/day.	EFSA (2014)

Chemical substance	Chemical identity and toxicological data	Reference
	In Denmark, biotin has been registered in the Danish Veterinary and Food Administration's Dietary Supplement Register (foedevarestyrelsen.dk) with a recommended dose of 5 mg/day. CIR (2001) states that biotin as a water-soluble vitamin is used in hair and skin care products in concentrations up to 0.6%. The oral LD50 in rats is reported to be > 10 g/kg. In mice, rats and hamsters, serious adverse effects on foetal development have been observed in animals fed diets without biotin content. Subcutaneous and intramuscular injection of 0.1% biotin solution did not cause any signs of irritation at the puncture site in guinea pigs or rabbits. When 0.1% biotin solution was injected into the eyes of rabbits, transient and mild irritation was observed. No data are provided for skin sensitisation studies. It is stated that extensive clinical use of biotin has not resulted in reports of skin irritation and sensitisation. CIR (2001) cites a number of older studies where subcutaneous injection of 50-150 mg biotin/kg bw/day in pregnant mice and rats resulted in increased resorption of the foetuses and anti-oestrogenic effects in the mothers. However, a more recent study from 1996 found no effects on foetal development in rats dosed with 0.1% biotin in the diet or further dosing by subcutaneous injection with 150 mg biotin/kg bw on days 0, 6 and 12 of gestation. Based on an overall assessment, CIR (2001) concluded that the use of biotin in cosmetics does not pose a health risk. The Danish QSAR database indicates that the chemical structure of biotin cannot be predicted for skin sensitisation in the CAS Ultra, Leadscope and SciQSAR models. **Assessment** Based on the available data, no relevant data to establish a critical N(L)OAEL for biotin have been identified for use in a risk assessment in this project.	CIR 2001 DTU QSAR
Tripeptide-1	Gly-His-Lys-OH CAS: 49557-75-7 CIR (2018) has assessed the use of Palmitoyl Tripeptide-1 in cosmetics and states that the substance is used at a concentration up to 0.001% in leave-on products and up to 0.0004% in products for use in the eye area. Tripeptide-1 has been shown to be rapidly metabolised in the blood. Palmitoyl Tripeptide-1 in a finished formulation has been tested for skin irritation in rabbits at a concentration of 100 ppm (0.001%), resulting in mild redness. In	CIR (2018)

Chemical substance	Chemical identity and toxicological data	Reference
	GMPT tests, where the same formulation was used, resulted in an induction concentration of 1% (of the formulation of 0.001%) and provocation test of 75% (of the formulation of 0.001%), no allergic skin reaction was observed in the animals. In skin irritation tests with human volunteers, occlusive exposure for 48 hours at a concentration of 0.1% Palmitoyl Tripeptide-1 triggered no skin reactions. In a human skin sensitisation test with the same test solution, a mild to moderate degree of transient skin reaction was observed, but not sufficient to be considered a positive response. In eye irritation tests with rabbits, exposure to 0.001% Palmitoyl Tripeptide-1 in a finished product caused mild eye irritation. In bacterial tests, Palmitoyl Tripeptide-1 showed no mutagenic effect. Based on the above, CIR (2018) assessed that Palmitoyl tripeptide-1 is safe to use in cosmetics at the known concentrations. **Assessment** Palmitoyl Tripeptide-1 as a component of a finished product at concentrations of 0.001% is reported to cause eye irritation. However, the data is too uncertain to be used for read-across to Biotinoyl Tripeptide-1, partly because other components of the tested formulation are not known and partly because cleaved palmitic acid may have caused the irritant response.	
Amino acids	**Chemical identity and toxicological data**	**Reference**
Glycine	 CAS: 56-40-6 Glycine is sold in Denmark as a dietary supplement in tablets with a content of 1000 mg. CIR (2013) has conducted a safety assessment of amino acids used in cosmetics and states that amino acids are primarily used in hair and skin care products. Glycine is reported to be used at concentrations up to 4% in skincare products and up to 0.3% in products used in the eye area. In an *in vitro* EPISKIN test with 2% glycine, no signs of irritation were found, while an *in vitro* test with the cornea of a calf (BCOP test) showed a slight degree of irritation at the same concentration. 7.5% glycine in an eye liner did not cause any signs of eye irritation during a 14-day test period with human subjects. A number of skin sensitisation tests have been performed with glycine in cosmetic products. Repeated use of moisturiser with 2% glycine and roll-on antiperspirant with 2.8% glycine did not cause irritation/sensitisation in test panels of over 100 test subjects.	CIR (2013)

Chemical substance	Chemical identity and toxicological data	Reference
	EFSA (2014) has assessed the safety of the use of glutamyl-valyl-glycine tripeptide as a flavouring agent. It states that after ingestion, the tripeptide is rapidly hydrolysed into individual amino acids. Based on *in vitro* and *in vivo* mutagenicity tests, the tripeptide was found to pose no risk in terms of mutagenic/genotoxic effects. In a 28-day oral study in rats, the NOAEL was set at the highest dose of 1000 mg/kg bw/day of the tripeptide. EFSA did not consider it relevant to derive a tolerable dose for the tripeptide. Glycine tested in an LLNA test showed no skin sensitising response. In a 28-day oral study in rats, a NOAEL of 2000 mg/kg/day was found. In an older (1994) chronic long-term study in rats, an oral LOAEL of 1561 mg/kg/kgv (the lowest dose in the study) was found for histopathological effects in the kidneys. In an older (1977) teratogenic rat study, no effects on the offspring were found at the highest dose of 855 mg/kg/day. **Assessment** Based on the above information, glycine is not considered to have critical systemic effects that are relevant for further risk assessment in this project.	EFSA (2014) REACH-reg G
Histidine	 CAS: 71-00-1 The Norwegian Scientific Committee on Food Safety (VKM) has assessed that a daily L-histidine dietary supplement for adults and children (over 10 years of age) in the range of 550-600 mg does not pose any health concerns. CIR (2013) has conducted a safety assessment of amino acids used in cosmetics and states that amino acids are primarily used in hair and skin care products. Histidine is reported to be used at concentrations up to 0.05% in skincare products and up to 0.001% in products used on mucous membranes. Histidine and histidine hydrochloride have been tested for skin sensitisation with volunteers where repeated skin exposure to concentrations of 0.05% and 0.07% caused no signs of irritation or sensitisation in a test panel of 104 human subjects. Histidine has been tested in OECD 435 and OECD 439 *in vitro* tests and showed no potential for skin irritation. In an *in vitro* test (OECD 437), the substance showed no potential for eye irritation. In a long-term feeding study in rats, the NOAEL at the highest dose was 558 mg/kg/day.	VKM (2016) CIR (2013) REACH-reg H

Chemical substance	Chemical identity and toxicological data	Reference
	There is no data on skin sensitisation for histidine, but the Danish QSAR database lists histidine as negative in terms of skin sensitisation in connection with Leadscope modelling for the substance. **Assessment** Based on the above information, histidine is not considered to have critical systemic or local effects relevant for further risk assessment in this project.	Danish QSAR
Lysine	 CAS: 56-87-1 This substance is listed in Annex VI of the REACH Regulation as a substance that does not have critical effects and is therefore not subject to registration under REACH. Lysine is marketed in Denmark as a dietary supplement with an indicated daily dose of approximately 1 gram. CIR (2013) has conducted a safety assessment of amino acids used in cosmetics and states that amino acids are primarily used in hair and skin care products. Lysine is reported to be used at a concentration of up to 0.7% in skincare products and up to 0.04% in products used in the eye area. No *in vitro* or animal tests have been reported regarding skin and eye irritation and skin sensitisation. In skin sensitisation tests with patients, neither irritation nor sensitisation has been observed with semi-occlusive facial exposure to cosmetic products containing 0.65% lysine. CIR (2013) concluded that due to the low concentrations used in cosmetics and compared to its use as an additive in foods, its use in cosmetics is safe. Based on *in vivo* tests (OECD 404 and OECD 405), lysine hydrochloride is not considered to be a skin or eye irritant. A GPMT test performed with lysine hydrochloride did not cause a sensitising response in any of the animals. In a 90-day feeding study in rats, no adverse effects were observed at the highest lysine hydrochloride dose of 914 mg/kg/day. Lysine hydrochloride showed no mutagenic effects *in vitro* in bacteria and mammalian cells. In oral teratogenicity studies in rats, the NOAEL was set at a maximum dose of 1000 mg/kg/day. **Assessment** Based on the available data, no critical effects relevant for further risk assessment in the project can be identified.	CIR (2013) REACH-reg Ly

Sources:

CIR (2001). Final Report on the Safety Assessment of Biotin. Final Report on the Safety Assessment of Biotin (cir-safety.org)

CIR (2013). Safety Assessment of α-Amino Acids as Used in Cosmetics. International Journal of Toxicology 32 (Supplement 4) 41S-64S.

CIR (2018). Safety Assessment of Tripeptide-1, Hexapeptide-12, Their Metal Salts and Fatty Acyl Derivatives, and Palmitoyl Tetrapeptide-7 as Used in Cosmetics. International Journal of Toxicology 2018, Vol. 37 (Supplement 3) 90S-102S

DTU QSAR. Danish (Q)SAR Database (dtu.dk)

EFSA (2014). Scientific Opinion on Flavouring Group Evaluation 401 (FGE.401): γ-Glutamyl-valyl-glycine from chemical group 34. EFSA Journal 2014;12(4):3625

PubChem. PubChem (nih.gov)

REACH-reg G. REACH registration dossier for glycine. Registration Dossier - ECHA (europa.eu) (accessed September 2024)

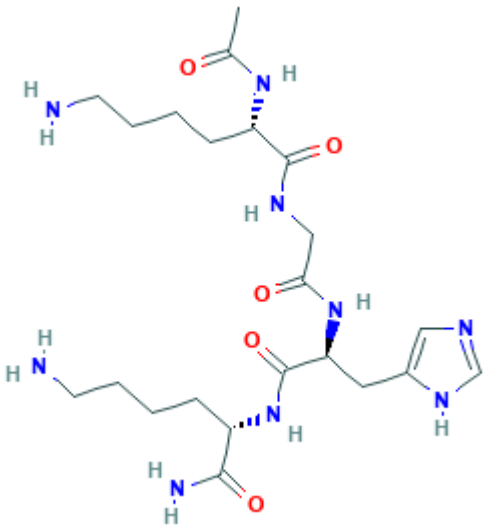
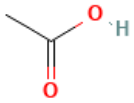
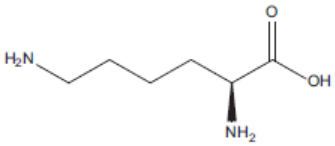
REACH-reg H. REACH registration dossier for Histidine. Registration Dossier - ECHA (europa.eu) (accessed September 2024)

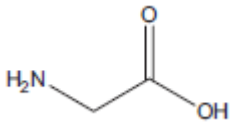
REACH-reg Ly. REACH registration dossier for lysine hydrochloride. Registration Dossier - ECHA (europa.eu) (accessed September 2024)

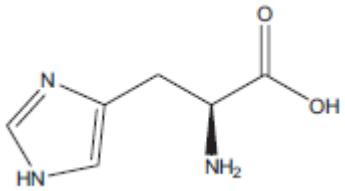
VKM (2016). Risk assessment of "other substances" - L-histidine. Opinion of the Panel on Nutrition, Dietetic Products, Novel Food and Allergy of the Norwegian Scientific Committee for Food Safety. VKM Report 2016: 24

Appendix 2.3 Acetyl Tetrapeptide-3

The table below lists data obtained from searches for use in hazard assessment of the substance.

Chemical substance	Chemical identity and toxicological data	Reference
Acetyl Tetrapeptide-3	 Formula: $C_{22}H_{39}N_9O_5$ Molecular weight: 509.6 g/mol CAS: 827306-88-7 No toxicological data was found for Acetyl Tetrapeptide-3.	Pubchem
Component	**Chemical identity and toxicological data**	**Reference**
Acetic acid	 CAS: 64-19-7 This substance is widely used in food and is a common component of the human diet. **Assessment** The substance is assessed to have no critical effects that are relevant for further risk assessment in the project.	
Tetrapeptide-3	Lys-Gly-His-Lys-NH_2 CAS: 827306-97-8 No toxicological data was found for Tetrapeptide-3.	Pubchem
Amino acids	**Chemical identity and toxicological data**	**Reference**
Lysine	 CAS: 56-87-1	CIR (2013)

Chemical substance	Chemical identity and toxicological data	Reference
	This substance is listed in Annex VI of the REACH Regulation as a substance that does not have critical effects and is therefore not subject to registration under REACH. Lysine is marketed in Denmark as a dietary supplement with an indicated daily dose of approximately 1 gram. CIR (2013) has conducted a safety assessment of amino acids used in cosmetics and states that amino acids are primarily used in hair and skin care products. Lysine is reported to be used at a concentration of up to 0.7% in skincare products and up to 0.04% in products used in the eye area. No *in vitro* or animal tests for skin and eye irritation and skin sensitisation have been reported. In skin sensitisation tests with patients, neither irritation nor sensitisation has been observed with semi-occlusive facial exposure to cosmetic products containing 0.65% lysine. CIR (2013) concluded that due to the low concentrations used in cosmetics and compared to its use as an additive in foods, its use in cosmetics is safe. Based on *in vivo* tests (OECD 404 and OECD 405), lysine hydrochloride is not considered to be a skin or eye irritant. A GPMT test performed with lysine hydrochloride did not cause a sensitising response in any of the animals. In a 90-day feeding study in rats, no adverse effects were observed at the highest lysine hydrochloride dose of 914 mg/kg/day. Lysine hydrochloride showed no mutagenic effects *in vitro* in bacteria and mammalian cells. In oral teratogenicity studies in rats, the NOAEL was set at a maximum dose of 1000 mg/kg/day. **Assessment** Based on the available data, no critical effects relevant for further risk assessment in the project can be identified.	REACH-reg Ly
Glycine	 CAS: 56-40-6 Glycine is sold in Denmark as a dietary supplement in tablets with a content of 1000 mg. CIR (2013) has conducted a safety assessment of amino acids used in cosmetics and states that amino acids are primarily used in hair and skin care products. Glycine is reported to be used at concentrations up to 4% in skincare products and up to 0.3% in products used in the eye area.	CIR (2013)

Chemical substance	Chemical identity and toxicological data	Reference
	In an *in vitro* EPISKIN test with 2% glycine, no signs of irritation were found, while an *in vitro* test with the cornea of a calf (BCOP test) showed a slight degree of irritation at the same concentration. 7.5% glycine in an eye liner did not cause any signs of eye irritation eye liner 14-day test period with human subjects. A number of skin sensitisation tests have been performed with glycine in cosmic products. Repeated use of moisturiser with 2% glycine and roll-on antiperspirant with 2.8% glycine did not cause irritation/sensitisation in test panels of over 100 test subjects. EFSA (2014) has assessed the safety of the use of glutamyl-valyl-glycine tripeptide as a flavouring agent. It states that after ingestion, the tripeptide is rapidly hydrolysed into individual amino acids. Based on *in vitro* and *in vivo* mutagenicity tests, the tripeptide was found to pose no risk in terms of mutagenic/genotoxic effects. In a 28-day oral study in rats, the NOAEL was set at the highest dose of 1000 mg/kg bw/day of the tripeptide. EFSA did not consider it relevant to derive a tolerable dose for the tripeptide. Glycine tested in an LLNA test showed no skin sensitising response. In a 28-day oral study in rats, a NOAEL of 2000 mg/kg/day was found. In an older (1994) chronic long-term study in rats, an oral LOAEL of 1561 mg/kg/kgv (the lowest dose in the study) was found for histopathological effects in the kidneys. In an older (1977) teratogenic rat study, no effects on the offspring were found at the highest dose of 855 mg/kg/day. **Assessment** Based on the above information, glycine is not considered to have critical systemic effects that are relevant for further risk assessment in this project.	EFSA (2014) REACH-reg G
Histidine	 CAS: 71-00-1 The Norwegian Scientific Committee on Food Safety (VKM) has assessed that a daily L-histidine dietary supplement for adults and children (over 10 years of age) in the range of 550-600 mg does not pose any health concerns. CIR (2013) has conducted a safety assessment of amino acids used in cosmetics and states that amino acids are primarily used in hair and skin care products. Histidine is reported to be used at concentrations up to 0.05% in skincare products and up to 0.001% in products used on mucous membranes.	REACH-reg H VKM (2016) CIR (2013)

Chemical substance	Chemical identity and toxicological data	Reference
	Histidine and histidine hydrochloride have been tested for skin sensitisation with volunteers where repeated skin exposure to concentrations of 0.05% and 0.07% caused no signs of irritation or sensitisation in a test panel of 104 human subjects. Histidine has been tested in OECD 435 and OECD 439 *in vitro* tests and showed no potential for skin irritation. In an *in vitro* test (OECD 437), the substance showed no potential for eye irritation. In a long-term feeding study in rats, the NOAEL at the highest dose was 558 mg/kg/day. There is no data on skin sensitisation for histidine, but the Danish QSAR database lists histidine as negative in terms of skin sensitisation in connection with Leadscope modelling for the substance. **Assessment** Based on the above information, histidine is not considered to have critical systemic or local effects relevant for further risk assessment in this project.	REACH-reg H DTU QSAR

Sources:

CIR (2013). Safety Assessment of α-Amino Acids as Used in Cosmetics. International Journal of Toxicology 32 (Supplement 4) 41S-64S

DTU QSAR. Danish (Q)SAR Database (dtu.dk)

EFSA (2014). Scientific Opinion on Flavouring Group Evaluation 401 (FGE.401): γ-Glutamyl-valyl-glycine from chemical group 34. EFSA Journal 2014;12(4):3625

PubChem. PubChem (nih.gov)

REACH-reg G. REACH registration dossier for glycine. Registration Dossier - ECHA (europa.eu) (accessed September 2024)

REACH-reg H. REACH registration dossier for Histidine. Registration Dossier - ECHA (europa.eu) (accessed September 2024)

REACH-reg Ly. REACH registration dossier for lysine hydrochloride. Registration Dossier - ECHA (europa.eu) (accessed September 2024)

VKM (2016). Risk assessment of "other substances" - L-histidine. Opinion of the Panel on Nutrition, Dietetic Products, Novel Food and Allergy of the Norwegian Scientific Committee for Food Safety. VKM Report 2016: 24

Appendix 2.4 Overall assessment

As can be seen from these tables, no toxicological data was found on any of the three cosmetic ingredients. For a related ingredient, *Palmitoyl Pentapeptide-4*, degradation has been demonstrated when the substance is added to skin extraction fluid caused by the fluid's enzyme content. This emphasises the importance of hazard assessments of the individual components of the peptide ingredients.

The amino acids listed are naturally present in the human diet, where they are part of peptide chains and proteins. All of these amino acids are thus commonly found in the body and involved in the body's metabolic processes and structure.

Based on this and the above review of the peptides and their building blocks, *systemic exposure* to these peptides and the amino acids that may be cleaved from them is not considered to constitute a health concern.
The same applies to the vitamin biotin, which is marketed as a dietary supplement.
Finally, myristic acid is also considered a non-toxic fatty acid, as it is a naturally occurring fatty acid in plant oils and thus commonly found in foods.

Regarding *local effects* (skin and eye irritation and skin sensitisation), the available test data does not indicate these as critical effects. Nevertheless, it should be noted that relevant *in vitro* and *in vivo* skin sensitisation tests are generally lacking for the peptide substances *per se*; however, data for the individual components comprising the peptides do not give cause for concern for sensitising effects.

Due to the non-toxic nature of these substances, it is not possible to identify critical effects for the substances or relevant N(L)OAEL and PoD values that could provide a basis for a quantitative risk assessment.

Survey and risk assessment of eyelash and eyebrow serums

Eyelash and eyebrow serums are products that ostensibly promote the growth of and/or reduce the loss of eyelash and eyebrow hairs. Originally, the active ingredients used were substances known as prostaglandins and synthetic analogues of these, but consumers have experienced adverse effects when using products containing these substances. Today, prostaglandin-free alternatives are available. The aim of this report was to gain knowledge on the active ingredients used in prostaglandin-free serums. The project investigated the safety of peptides used as active ingredients in serum products.

Almost 90% of the surveyed serum products contained peptides and/or amino acids as active ingredients. Other active ingredients used in serums include vitamins, hyaluronic acid and various plant extracts. Peptides are organic substances made up of amino acids found in all living organisms, and are building blocks for the formation of proteins used to build the body's cells, hormones, tissues etc.. The use of 20 unique peptides was identified in the 44 serum products surveyed in the project. Three of these peptides were used in several of the surveyed serum products, and a detailed hazard assessment was performed for these.

Although no specific toxicological data was found on the peptide substances, data on the components of these substances (fatty acids, vitamin B7 and various amino acids) did not identify any critical effects, and the three peptide substances were assessed to be unproblematic for health. It was therefore concluded that the use of the three peptide substances in the serum products does not pose an immediate concern for health risks.

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