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Mapping and risk assessment of food proteins in cosmetic products

Survey of chemical substances in consumer products No. 196

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Preface

This report describes the results of a study of cosmetic products containing food-based ingredients, as well as the risk of consumers developing food allergy when using these products. The database behind the Kemiluppen app from the Danish Consumer Council THINK Chemicals (Forbrugerrådet TÆNK Kemi) was used for mapping cosmetic products with food-based ingredients in the Danish market. In addition, websites in and outside the EU were screened for products. Products containing foods that most commonly cause food allergy were identified. With focus on products for children, products were purchased for analysis of their food protein contents. Based on the scientific literature and a survey of the diet in young children, an assessment was made of the risk of developing food allergy after exposure to food proteins in cosmetic products intended for children.

The assignment was performed in the period from May 2022 to April 2023 by DTU Food, Technical University of Denmark. The project group would like to thank the Danish Consumer Council THINK Chemicals for making the Kemiluppen database available to the project. A thank-you is also extended to the company Eurofins for acting as a sounding board in the preparation of the validation and analysis plan. Eurofins provided ELISA assays for use in the project at a reduced price, but was not involved in conducting analyses, performing data analysis, or interpreting data.

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Summary

Introduction

Food-based ingredients are widely used in the production of cosmetic products. Several studies conducted over the past few decades have indicated that food allergy may be developed through skin exposure to foods. It is thus relevant to assess whether the use of cosmetic products containing food-based ingredients poses a risk to consumers of developing food allergy. People normally develop tolerance to foods when they eat them. This occurs in young children when they are gradually introduced to new foods. The tolerance protects against sensitization via the skin. If you are exposed to a food via the skin before oral tolerance has been established, you risk developing food allergy. The risk is particularly high when the skin barrier is not intact, as seen in atopic dermatitis (infantile eczema). This report therefore focuses on products aimed at children and products aimed at specific skin types.

Mapping of cosmetic products

The database behind the Kemiluppen app from the Danish Consumer Council THINK Chemicals was used to map cosmetic products in the Danish market. The database was reviewed for identification of products with food-based ingredients that typically cause food allergy. A total of 3,741 cosmetic products containing food-based ingredients were identified, corresponding to 27 per cent of the 13,845 products in the database. Of these, 28 per cent contained two or more food-based ingredients. The ingredients could be divided into four categories: 1. Protein extracted from the food, 2. The whole food (e.g. milk) or part of the food (e.g. seeds, shells, or the like), 3. Extracts extracted from the food and 4. Oil or fat extracted from the food.
Protein ingredients typically come from milk, cereals, and soy. Extract ingredients are typically produced from soy and cereals. Oil ingredients are typically based on tree nuts, soy, sesame, and cereals. In total, 76 products aimed at children were identified as containing food-based ingredients that typically cause food allergy.
Cosmetic products containing food allergens marketed for use on sensitive, inflamed, damaged, itchy, and dry skin pose a specific risk to children with atopic dermatitis. 154 products containing food-based ingredients and aimed at specific skin types were identified.
In addition, products available from non-Danish webshops in and outside the EU were examined. The results were comparable to the Danish data from Kemiluppen.

It can be concluded that food-based ingredients are widely used in cosmetic products in both unmodified form and modified form. Cosmetic products aimed at children and specific skin types contained oils from almond or soy, in particular. In addition, there were oats in many products as a whole ingredient or an extract. Finally, it was found that milk in certain products is added as a protein ingredient or whole ingredient.

Consumption of allergenic foods in children

Based on a dietary survey from 2014-15, DTU Food has examined when Danish children were introduced to the various foods that typically cause food allergy. The survey comprised two parts: a questionnaire on the time of introduction of different foods in children aged 0-24 months and diet registration among children aged 6-36 months. In the diet registration, the parents noted the foods and beverages their child ate and drank each day over a period of one week.
The diet registration showed that virtually all children aged 6-7 months (91 per cent) had consumed a milk-based product. The proportion consuming foods with wheat was high (>70 per cent users) in the group aged 6-7 months, increasing to nearly 100 per cent at 8-9 months of age. The proportion consuming oat-containing products was >80 per cent in the group aged

6-7 months, with a slightly declining proportion in older age groups. The proportion who ate eggs, fish, or shellfish was <50 per cent in the group aged 6-7 months. The proportion increased in the older age groups, where the proportion who ate eggs reached more than 90 per cent in the group aged 10-11 months. The proportion who ate peanuts, tree nuts, and sesame was low (<10 per cent) in the group aged 6-7 months. The proportion increases, but only reaches >50 per cent around the age of 12 months.
The dietary survey shows variation in the introduction and consumption of foods that typically cause food allergy. The results indicate early introduction of milk and cereals (around 4-6 months of age), followed by eggs, fish, and shellfish (around 6-9 months of age), and then peanuts, tree nuts, and sesame (1-2 years of age).

Analysis of food protein in cosmetic products

It was found in connection with the mapping that cosmetic products aimed at children and specific skin types typically contain ingredients based on almond, soy, oats, cow's milk, wheat, and/or macadamia nuts (among the foods that typically cause food allergy). Based on the mapping, it was decided to focus on analysis of proteins from the following foods: 1. Cow's milk, which most frequently causes food allergies in children and is typically used as a protein or whole ingredient in cosmetic products. 2. Almond, as almond oil finds frequent use in cosmetic products and as individual products contain almond extract, and 3. Soy, which is typically used as protein, extract, or oil ingredient. 19 products were purchased from Danish stores or webshops, while 13 and 7 products, respectively, were purchased from webshops in the rest of the EU and outside the EU.
There are several commercially available ELISA analysis kits that can measure food proteins in complex mixtures. They are validated for use in a diverse range of foods, but not for analysis of food proteins in cosmetic products. Performance of a method validation was therefore necessary. In the method validation, a known quantity of milk, almond, or soy protein is added to determine whether the matrices affect the result. The trials showed that the specific cosmetic matrices affect the recovery rate, limits of detection, and limits of quantification.
The result of the final analyses was that all eight products analysed for cow's milk had measurable quantities of protein from 0.36-250 µg milk protein/g product. Of the 23 products analysed for almond protein, one product had a clear almond content (43 µg almond protein/g). The other products contained protein below or close to the ELISA limit of detection. The results for the 10 products analysed for soy protein were all below or close to the ELISA limit of detection. Overall, the ELISA method can be used to determine the presence of milk protein, almond protein, and soy protein in cosmetic products when the proteins are present in a sufficient quantity. The specific cosmetic matrices affect the recovery rate, limits of detection, and limits of quantification. There is therefore some uncertainty in determining protein concentrations in cosmetic products.

Risk assessment

Compared to contact allergy to chemicals, there is limited knowledge about sensitization to food proteins via the skin. For chemicals, it is known that the unit to be used in risk assessments is dose per unit area, as a certain concentration of the substance must be present to trigger sensitization. It is assumed that this also applies to proteins.

Protein can sensitize via human skin

There are two well-described associations between skin exposure to food allergens and food allergy. One in young children (peanuts) and the other in adults (acid hydrolysis of gluten). The children who had atopic dermatitis and were not tolerant to peanuts have probably been sensitized through low doses of peanut protein, and both the condition of the skin and the lack of oral tolerance have been of decisive importance. Data on the correlation between the dose of peanut protein, frequency, and duration of exposure and the risk of sensitization cannot be derived from the studies. Results from studies on the protein content in peanut oil range from 0.1-10.7 µg/g. Unfortunately, these data cannot be linked to the clinical studies.

The correlation—that skin sensitization can lead to food allergies if oral tolerance has not been established—has been formulated as “the dual allergen exposure hypothesis”, which is assumed to apply to food allergens in general.
Studies of skin exposure to acid hydrolysed gluten come mainly from the use of a facial soap in Japan, where a large number of people developed food allergy to gluten. These are adults who must be assumed to have been tolerant to wheat and thus gluten. It is not possible to calculate an exact dose/area exposure, but the content of acid hydrolysed gluten in the soap suggests an exposure dose that may be 1,000 times higher than the exposure to peanut protein that has sensitized young children with eczema. This suggests that a higher dose is needed to break a previously established tolerance. In addition, the new epitopes formed after acid hydrolysis also play a role.

Protein may sensitize via the skin in animals

There are many studies—especially in mice—that examine which conditions are crucial to skin sensitization with food proteins. Only some of these studies contain data on dose/area. Of these, there are only three studies containing dose response data with low doses, all conducted in animals without tolerance to the allergens being studied. This concerns the following studies with 1. Ovalbumin (OVA) from chicken eggs, 2. Whey protein and whey protein hydrolysates from cow’s milk, and 3. Gluten and modified forms of gluten. 1. and 2. have been conducted with prolonged occlusion, which causes skin changes similar to atopic dermatitis. In 3., the skin changes are minor. In this study, the effective dosage period is 1 hour 3 times per week for 5 weeks, followed by stimulation of the immune system in the gastrointestinal tract.

Although the trials have been conducted differently and the substances are different, it seems that lowest effect level (LOAEL) is of the same magnitude, that is less than 10 µg/cm2.

Exposure based on analysis data

The exposure assessments are based on milk protein analyses for three soaps, three creams, and a shampoo, and the single finding of almond protein in one cream. The creams are all body lotions for babies or people with dry skin/atopic dermatitis. It is assumed that the selected creams are applied to the whole body, including in the diaper area, where the skin is occluded and may be damaged. All exposure scenarios have been calculated for a 4-month-old baby, where the likelihood of a lack of oral tolerance is greatest.
The highest exposures are obtained from body lotion. If body lotion with almond oil containing 43.07 µg almond protein/g is used twice a day, this gives a dose of 0.025 µg almond protein/cm^2.
If a whole body scenario is calculated for a 4.5-month-old baby to whom soap is applied with the highest concentration of milk protein (250.54 µg/g) all over the body followed by a cream with the highest measured concentration of milk protein (121.71 µg/g) all over the body, this gives an exposure from soap of 0.003 µg/cm^2 and from cream of 0.072 µg/cm^2, totalling 0.075 µg milk protein/cm^2. If a worst-case scenario is calculated for cream used solely in the diaper area and using the cream with the highest concentration of milk protein (121.71 µg /cm^2), the dose will be 0.346 µg milk protein/cm^2.

Risk assessment of food proteins in cosmetic products for children

Among children with atopic dermatitis there is a risk of skin sensitization to food proteins, where oral tolerance has not been established. On the basis of data from human studies or from animal studies, it is not possible to determine which doses (µg protein/cm^2) cause sensitization.
The precautionary principle therefore warrants that food proteins or food protein-rich ingredients (e.g. milk powder) should not be added to cosmetic products for children having an age where oral tolerance to the proteins cannot be expected. The oral tolerance is established when the children are introduced to the foods, typically during the first two years of their life.

Studies in animals support the importance of the protective effect of oral tolerance. They also show that food proteins may cause skin sensitization in low doses, down to about 1 µg protein/cm^2.

Conclusion and perspective

The available knowledge does not allow limits to be set for the quantity of food protein that cannot sensitize young children.

Knowledge from animal studies show that the condition of the skin and the duration of the exposure are of importance to sensitization, but there is a lack of knowledge about the influence of these parameters on the sensitizing dose. There is also a lack of knowledge about the correlation between the potency of the allergens and the dose that causes sensitization.

There is high focus on the development of alternative protein sources for human nutrition, so-called novel foods. It should be considered, if these foods should be used in cosmetic products, before being introduced as food, as it is important that oral tolerance is established before the human population is exposed to new proteins via the skin.

1. Introduction

1.1 Use of food-based ingredients in cosmetic products

Food-based ingredients are widely used in the production of cosmetic products. The ingredients can be used to give the products certain physicochemical properties, such as a creamy consistency as well as foaming or emulsifying effects (Day et al 2006, Wu et al 1976). In addition, certain food-based ingredients have anti-inflammatory, anti-oxidizing, or other desirable biological properties that are assumed to give the cosmetic products health-promoting qualities (Dini and Laneri 2021). Finally, the companies may wish to add these ingredients to their products to market them as being 'natural', 'organic', 'vegan', 'sustainable', or similar (Dini and Laneri 2021).

A previous study has mapped the use of natural ingredients in cosmetic products available in the Danish retail trade (Bruusgaard-Mouritsen et al 2020). In that study, 'natural ingredients' were defined as ingredients derived from plants and animals. The study showed that many cosmetic products contain ingredients made from foods that may cause food allergy. In addition, the study found that several of these foods are used in cosmetic products that have caused allergic reactions in some consumers. In the past couple of decades, several studies have indicated that the development of food allergy may occur through skin exposure to foods (Brough et al 2020). It is thus relevant to assess whether the use of cosmetic products containing food-based ingredients poses a risk to consumers of developing food allergy.

1.2 Food allergy

Food allergy is an allergic reaction to normally harmless proteins (allergens) in foods. Allergic individuals who come into contact with the food to which they are allergic may develop a variety of symptoms, including skin reactions (rash and angioedema) and gastrointestinal tract reactions (pain, vomiting, and diarrhoea) (Longo et al 2013). In severe cases, these individuals may develop life-threatening anaphylaxis with an impact on pulmonary and circulatory function. Food allergy is most commonly mediated by allergen-specific IgE antibody, which binds to mast cells (cells in the immune system) in mucous membranes and skin (Yu et al 2016). Here, exposure to allergens activates the mast cells, resulting in allergic tissue inflammation and symptoms. There is currently no curative treatment for food allergy, which means that allergic individuals must avoid exposure to allergens and settle for treatment of symptoms in the event of accidental exposure.

1.3 Allergenic foods

In principle, any food can cause food allergy, as the immune system may potentially recognize all food proteins as a foreign protein entering the body from the outside. However, primarily certain foods typically cause food allergy. This is presumably due to the allergy-causing food proteins having specific physicochemical properties that stimulate the immune system to develop allergy (Bannon 2004). Some food proteins have a high potential to lead to the development of food allergy and therefore have so-called 'high allergenicity'. It has been suggested that food allergens are typically small, heat-labile, and digestion-resistant proteins (Monaci et al 2020). These properties are assumed to reduce allergen breakdown in the gastrointestinal tract, thus increasing the risk of an allergic reaction. In addition to high allergenicity, 'allergenic' foods must be consumed in a quantity and to an extent that cause allergy development in the population. For example, a highly allergenic food that is not consumed will not be a clinical problem, whereas there will be an unknown risk of developing food allergy in connection with a food with unknown allergenicity—such as novel foods—that has not previously been eaten by the population. EU Regulation 1169/2011, Annex II, lists the foods that most frequently cause

allergies and severe allergic reactions (Table 1). Products containing these foods, or elements thereof, require emphasized labelling in connection with sales for human consumption in the EU, so that allergic individuals can avoid allergens from these foods.

TABLE 1. Most allergenic foods, see EU Regulation 1169/2011 Annex II

Foods
Milk
Eggs
Cereals - Wheat - Rye - Barley - Oats
Peanuts
Soy
Lupin
Tree nuts - Almond - Hazelnuts - Walnuts - Cashew nuts - Pecan nuts - Brazil nuts - Pistachio nuts - Macadamia nuts - Queensland nuts
Sesame seeds
Mustard
Celery
Fish
Shellfish
Molluscs

1.4 Prevalence of food allergy

The prevalence of food allergy is dependent on demographic parameters such as geographical region and age. Among countries in Europe, the prevalence of food allergy in children is between 1.9-5.6 per cent (Lyons et al 2020) and between 0.3-5.6 per cent in adults (Lyons et al 2018). In addition, there is variation in relation to which foods primarily cause allergies in children and adults. Allergy to milk and eggs is most common among children in Europe, whereas allergy to fish, shellfish, hazelnuts, and certain fruits is most common among adults. International studies suggest that the prevalence of food allergy has been increasing in the Western world over the past decades (Sichrer and Sampson 2018). The number of treatment-requiring food-induced anaphylaxis is also increasing (Pouessel et al 2018). In addition, foods are the most frequent cause of anaphylaxis among children (Yu and Lin 2018). Both individuals with food allergy and their next-of-kin experience impaired quality of life as a result thereof (Greenhawt 2016).

1.5 Development of food allergy

The development of food allergy (sensitization) is difficult to examine in humans, as, for ethical reasons, allergy obviously cannot be induced in healthy individuals. Therefore, animal models play a key role in understanding the sensitizing mechanisms. Traditionally, studies of allergy development have focused on sensitization via the oral route (Kanagaratham et al 2018, Li et al 1999). The reason for this is presumably that oral ingestion is the natural route of exposure to foods, and most food-induced allergic reactions occur after oral ingestion. Over the past couple of decades, however, several epidemiological studies in humans have indicated that the development of food allergy may occur through skin exposure to foods (Brough et al 2020). One of the first studies found a correlation between the use of skin creams containing peanut oil and the development of peanut allergy in children with eczema (Lack et al 2003). Subsequent studies found that environmental exposure to peanuts is a risk factor for the development of peanut allergy, especially in children with eczema and impaired skin barrier function (Fox et al 2009, Brough et al 2014, 2015). Several experimental studies have shown that foods can sensitize through the skin in animal models of eczema (Hussain et al 2018, Noti et al 2014, Ballegaard et al 2021). But experimental studies also show that eczema is not necessary for sensitization to certain foods via the skin (Tordesillas 2014, Larsen et al 2022). The underlying immunological mechanism involves antigen-presenting cells that take up the food allergen in the skin, after which these cells migrate to draining lymph nodes and initiate a T cell-mediated type-2 immune response (Brough et al 2020). The activated T cells stimulate B cells, which differentiate into IgE-producing plasma cells. A recent study has shown that antigen-presenting cells in human skin have specific properties that promote sensitization (Mayer et al 2021). Together, these studies support the assumption that skin sensitization plays a role in the development of food allergy in humans. However, it is unknown to what extent sensitization via the oral route of exposure also plays a role, and whether there is a difference in the route of sensitization between different foods. In addition, it is uncertain how much the condition of the skin affects sensitization to different foods (e.g. presence of eczema, physical injuries, inflammation conditions, and impaired skin barrier function). Finally, it is uncertain to what extent that environmental exposure to foods via the airways may contribute to the development of food allergy (Kulis et al 2021). Despite these uncertainties, it must be assumed that the skin plays a key role in the development of food allergy, especially in individuals with eczema and impaired skin barrier function.

1.6 Tolerance to foods

Under normal circumstances, the body's reaction to the ingestion of foods is the development of oral tolerance, which is an active immunological process (Tordesillas 2018). The developed tolerance is specific to the proteins present in the ingested food. This means that tolerance is only achieved for the foods eaten. Despite this knowledge having been available for decades, there have been varying recommendations regarding the introduction of allergenic foods in children. Previous clinical guidelines focused on the prevention of food allergy by late introduction of allergenic foods (12-36 months of age)—the so-called 'allergen avoidance' strategy (Zieger 2003). However, recent clinical studies have shown that early introduction (3-4 months of age) of peanuts and eggs protects against the development of peanut allergy and egg allergy, respectively (Du Toit et al 2015, Perkin et al 2016). Based on these studies, the latest guidelines from the European Academy of Allergy and Clinical Immunology (EAACI) recommend the introduction of peanuts and eggs at the age of 4-6 months (Halken et al 2021). Early introduction of allergenic foods for prevention of food allergy in children supports the so-called 'dual allergen exposure' hypothesis. This hypothesis states that if oral ingestion occurs before skin exposure, oral tolerance is developed—whereas allergy is developed if exposure occurs on the skin first (Kulis et al 2021). The hypothesis is further supported by experimental studies in animals showing that oral tolerance protects against sensitization to foods via the skin (Ballegaard et al 2021, Larsen et al 2022). It can therefore be assumed that the risk of developing food allergy via environmental exposure to foods on the skin is greatest in children who have not yet been intro-

duced to the given food via their diet. However, it should be noted that a soap containing hydrolyzed wheat was the cause of several cases of wheat allergy in adults, who are presumed to have been tolerant to wheat before using the soap in question (Yagami et al 2017).

2. Objective

A previous study has found that food-based ingredients are used in cosmetic products available in Danish retail trade. The latest research has shown that the development of food allergy may occur through the skin in connection with exposure to food proteins. It is therefore relevant to assess whether there is a risk of developing food allergy in consumers after use of cosmetic products containing food proteins. The overall objective of this project is to build up knowledge about the use of food proteins in cosmetic products. Mapping is done of the market for cosmetic products with food-based ingredients which are available to Danish consumers in general retail trade and via online purchases in webshops in and outside the EU. As the age for introduction of foods in childhood affects the risk of developing food allergy, the pattern of food introduction in children is examined through analysis of data from the Danish National Diet Survey. Based on the mapping, a selection is made of a number of products marketed for children and/or for a specific skin type for protein content analysis using the ELISA method, and the analysis method is validated for this purpose. Finally, it is examined through literature studies whether a risk assessment can be made of cosmetic products containing food protein in relation to the development of allergy in consumers who have not previously been exposed to the food proteins in question via their diet.

3. Mapping cosmetic products

This mapping establishes the extent to which cosmetic products with food-based ingredients are available to Danish consumers in general retail trade and via online purchases. With focus on the foods that are among the most frequent causes of food allergy, the use of different types of food-based ingredients (including protein, extracts, oils, etc.) is examined as well as the extent to which the ingredients are used after chemical modification. Furthermore, it is assessed to what extent the different types of ingredients contain food protein, as the protein from the food is what causes the allergy.

3.1 Use of foods in cosmetic products in the Danish market

3.1.1 Background and methodology

The database behind the Kemiluppen app was used for mapping cosmetic products in the Danish market. The database has been established and is maintained by the Danish Consumer Council THINK Chemicals (Forbrugerrådet TÆNK Kemi). Via Kemiluppen, consumers can submit pictures of cosmetic products that they find in the retail trade. The Danish Consumer Council THINK Chemicals then collects information about the product ingredients and states whether the product contains contact allergens (type IV allergy), suspected endocrine disruptors, environmentally harmful substances, etc. The information is subsequently made available to consumers via the app. This analysis uses the Kemiluppen database from April 2022, containing information about 13,845 products. Due to the large number of products in the database, it is assumed that the database products constitute a representative selection of cosmetic products available in the Danish market, although there may be a bias in the type of product scanned by consumers and the reason for choosing to scan a product. It is assumed that the products in the database constitute the most accessible cosmetic products on the market, as it is presupposed that these are the products that consumers most often find in the retail trade. In addition, it must be expected that the products are largely available to Danish consumers online, as most Danish retail chains have linked webshops.

3.1.2 Identification of food-based ingredients

A total of 13,845 cosmetic products in the Kemiluppen database were reviewed for ingredients extracted from the foods that most frequently cause food allergy (see foods in Table 1). Information from the CoSIng database (European Commission database for information on Cosmetic Substances and Ingredients) and the SpecialChem database (specialchem.com) were used for the classification of ingredients. Overall, the ingredients could be divided into the following types:

- **Protein:** Protein extracted from the food
- **Whole:** The whole food (e.g. milk) or part thereof (e.g. seeds, shells, or the like)
- **Extract:** Extracts from the food
- **Oil:** Oil or fat extracted from the food

Ingredients based on protein from foods must be assumed to contain food allergens if the protein is extracted from that part of the food which is typically consumed (milk, seeds, nut, or the like). Overall, it must be assumed that the whole ingredient type will typically contain protein from the food. Ingredients of this type based on, for example, milk, seeds, or nuts are also likely to contain food allergens, but it is unknown whether ingredients based on parts of the food that are typically not consumed (e.g. shells, flowers, and straw) contain food allergens.

It is unknown whether ingredients that are extracts of a food contain protein or food allergens, as it is typically not specified how the extract has been produced or possibly further processed. However, it is assumed that these will typically be aqueous extracts, and the extract must therefore be assumed to contain water-soluble food allergens if the extract is produced from that part of the food which is typically consumed (seeds, nut, or the like).

It is unknown whether ingredients consisting of oil or fat extracted from foods contain protein or food allergens. However, it must be assumed to be in tiny quantities relative to protein ingredient extracts, and the whole ingredient type. However, it has previously been found that the use of creams containing peanut oil increases the risk of developing peanut allergy in children [18]. This indicates that even tiny quantities of protein can lead to the development of food allergy via the skin.

The above findings underline that a risk assessment of cosmetic products containing ingredients from foods must take into account the type of ingredient and from which part of the food the ingredient has been produced. Furthermore, it is necessary to measure the food allergen content of the product and/or ingredient used, as there is presumed to be great variation in protein content between the different types of ingredients.

3.1.3 Identification of cosmetic products containing food-based ingredients

The Kemiluppen database was reviewed for identification of products containing food-based ingredients that typically cause food allergy (see foods in Table 1). In total, 3,741 cosmetic products (27 per cent of the total 13,845 products in the database) were identified, and more than 28 per cent of these products contained two or more food-based ingredients. TABLE 2 shows the number of cosmetic products distributed on food and type of ingredient.

It was found that protein ingredients are typically from milk, cereals, and soy. The whole ingredient type typically comes from milk and cereals. Extract ingredients are typically produced from soy and cereals. Oil ingredients are typically based on tree nuts, soy, sesame, and cereals.

The above results show that there is variation in which foods typically form the basis of the different food-based ingredient types used in cosmetic products.

TABLE 2. Number of cosmetic products in the Kemiluppen database containing food-based ingredients.

Food	Protein	Whole	Extract	Oil
Milk	166	105	3	3
Eggs	0	2	0	0
Cereals				
- Wheat	578	19	215	136
- Oats	40	44	178	50
- Barley	0	0	91	0
- Rye	0	0	7	0
Soy	240	5	760	745
Peanut	0	0	0	36
Tree nuts				

- Almond	6	9	87	962
- Brazil nut	0	0	0	21
- Cashew nut	0	0	1	0
- Hazelnut	4	0	0	30
- Macadamia nut	0	0	0	153
- Pecan nut	0	1	0	0
- Queensland nut	0	1	3	263
- Walnut	0	36	3	2
Sesame	1	2	8	173
Celery	0	0	4	0
Lupin	7	0	2	0
Mustard	0	0	2	0
Shellfish	0	0	17	0
Fish	0	0	6	1

3.1.4 Identification of modified food-based ingredients in cosmetic products

Food-based ingredients for use in cosmetic products can be modified to give the final product specific properties. In addition, hydrolysis and/or chemical modification of proteins can, in most cases, reduce the allergenic properties (reduce the allergenicity of the protein) (Bøgh and Larsen 2021).

Modification of the food-based ingredients identified in the 3,741 cosmetic products in Kemiluppen (3.1.3) was reviewed for each ingredient type (TABLE 3, TABLE 4, TABLE 5, TABLE 6). Overall, the modifications could be divided into the following modification types:

- **Unmodified (UM):** No modification.
- **Hydrolysis (H):** Hydrolysis (degradation) of protein through treatment with acid, base, and/or enzyme.
- **Chemical modification (CM):** Other chemical modification than hydrolysis. Typically binding to carbohydrates with low molecular weight.
- **Hydrolysis and chemical modification (HCM):** Both hydrolysis and other chemical modification.
- **Fermentation (F):** Fermentation typically gives rise to degradation of proteins.

It has been found that protein ingredients based on wheat and soy were typically hydrolyzed or both hydrolyzed and chemically modified (Table 3). Among the protein ingredients, milk was the most frequent ingredient used without modification.

Relative to protein ingredients, whole food-based ingredients, extracts, and oils are modified to a much lower degree (TABLE 4, TABLE 5, TABLE 6). It must therefore be assumed that any food allergens in ingredients of this type will typically have unchanged allergenicity.

The above results show that hydrolysis and other chemical modification are widespread among food-based ingredients for use in cosmetic products, where, especially, protein ingredients are widely used in modified form. A risk assessment of cosmetic products containing ingredients from a food must therefore take into account the chemical modification of the ingredient.

TABLE 3. Modification of food-based ingredients of the protein type.

Food	Unmodified	Hydrolysis	Hydrolysis and chemical	Fermentation
Milk	124	31	8	3
Cereals				
- Wheat	20	356	202	0
- Oats	5	7	28	0
Soy	36	202	2	0
Tree nuts				
- Hazelnut	0	4	0	0
- Almond	6	0	0	0
Sesame	0	1	0	0
Lupin	0	7	0	0

TABLE 4. Modification of food-based ingredients of the whole type.

Food	Unmodified	Hydrolysis	Chemical	Fermented
Milk	104	0	0	1
Eggs	2	0	0	0
Cereals				
- Wheat	5	1	13	0
- Oats	39	5	0	0
Soy	0	3	0	2
Tree nuts				
- Almond	9	0	0	0
- Pecan nut	1	0	0	0
- Queensland nut	1	0	0	0
- Walnut	36	0	0	0
Sesame	2	0	0	0

TABLE 5. Modification of food-based ingredients of the extract type.

Food	Unmodified	Hydrolysis	Chemical	Hydrolysis and chemical	Fermented
Milk	3	0	0	0	0
Cereals					

- Wheat	129	44	37	5	0
- Oats	178	0	0	0	0
- Barley	89	0	0	0	2
- Rye	7	0	0	0	0
Soy	757	0	0	0	3
Tree nuts					
- Cashew nut	1	0	0	0	0
- Almond	87	0	0	0	0
- Queensland nut	3	0	0	0	0
- Walnut	3	0	0	0	0
Sesame	8	0	0	0	0
Celery	4	0	0	0	0
Lupin	1	1	0	0	0
Mustard	2	0	0	0	0
Shellfish	17	0	0	0	0
Fish	6	0	0	0	0

TABLE 6. Modification of food-based ingredients of the oil/fat type.

Food	Unmodified	Chemical
Milk	3	0
Cereals		
- Wheat	130	6
- Oats	50	0
Soy	595	150
Peanut	35	1
Tree nuts		
- Hazelnut	30	0
- Macadamia nut	152	1
- Almond	960	2
- Brazil nut	21	0
- Queensland nut	263	0
- Walnut	2	0
Sesame	173	0

3.1.5 Foods in cosmetic products in the Danish market aimed at children

Children are the population group most at risk of developing food allergy. This may be because children have an immature immune system, greater prevalence of atopic eczema, and/or have not developed oral tolerance. Therefore, exposure to food proteins through cosmetic products may be particularly problematic for this group. The 3,741 cosmetic products with food-based ingredients identified in the Kemiluppen database (Chapter 3.1.3) were reviewed for products aimed at infants and children. The review was based on product names that could indicate marketing towards this group as well as subsequent inspection of the packaging. In total, 76 products aimed at children were identified containing food-based ingredients that typically cause food allergy. TABLE 7 shows the number of products, broken down by product category, ingredient type, and food with indication of modification. The products were found typically to contain oil, especially extracted from almonds, soy, and sesame. In addition, protein, whole food, and extract ingredients based on soy, oats, and wheat were found.

TABLE 7. Number of cosmetic products aimed at children identified in the Danish market, broken down by product category, ingredient type, and food.

	Ingredient type				
Category	**Protein**	**Whole**	**Extract**	**Oil**	**Number**
Oil	-	-	-	Almond (UM)	7
Oil	-	-	-	Soy (UM)	3
Oil	-	-	-	Almond (UM) + Oats (UM)	1
Oil	-	-	-	Soy (UM) + Wheat (UM)	1
Oil	-	-	-	Oats (UM)	1
Oil	-	-	-	Sesame (UM)	1
	-				
Cream	-	-	-	Almond (UM)	17
Cream	-	-	-	Almond (UM) Sesame (UM)	9
Cream	-	-	-	Soy (UM)	5
Cream	-	-	Soy (UM)	-	4
Cream	-	-	-	Oats (UM)	3
Cream	-	Oats (UM)	-	Oats (UM)	2
Cream	-	-	Oats (CM)	-	2
Cream	-	Oats (UM)	-	-	1
Cream	-	-	Oats (UM)	Almond (UM)	1
Cream	-	-	Soy (UM)	Sesame (UM)	1
	-				
Shampoo	-	-	Soy (UM)	-	3
Shampoo	-	-	Oats (UM)	-	2
Shampoo	Soy (H)	-	-	-	1
Shampoo	-	-	Oats (UM)	Almond (UM)	1
Shampoo	-	-	-	Almond (UM)	1
Shampoo	-	-	-	Almond (UM) Sesame (UM)	1

Shampoo	-	-	-	Sesame (UM)	1
Soap	-	Oats (UM)	-	-	5
Soap	Wheat (HCM)	-	-	-	1
Soap	-	-	-	Almond (UM) Sesame (UM)	1

Cream: Creams, lotions, and ointments. Shampoo: Shampoo and shampoo+body wash products. Soap: Hand soap, body soap, bath soap, and other wash products. UM: Unmodified. H Hydrolyzed. HCM: Hydrolyzed and other chemical modification. F. Fermented.

3.1.6 Foods in cosmetic products in the Danish market aimed at specific skin types

Atopic dermatitis is a risk factor for the development of food allergy. The probable reason for this is that the disease has been associated with increased skin permeability and skin inflammation, which, in animal studies, has been shown to increase sensitization to food allergens in connection with skin exposure. Therefore, cosmetic products containing food allergens marketed for use on sensitive, inflamed, damaged, itchy, and dry skin may pose a specific risk to this population. Children have atopic dermatitis to a greater extent than adults, and we expect that parents regard infants' and children's skin as sensitive. Therefore, it is assumed that children are also particularly exposed to products aimed at specific skin types.

The 3,741 cosmetic products with food-based ingredients identified in the Kemiluppen database (Chapter 3.1.3) were reviewed for products aimed at specific skin types. The review was based on product names that could indicate marketing aimed at a specific skin type (sensitive, eczema, itching, dry, etc.) and a subsequent inspection of the packaging. A total of 154 products—aimed at specific skin types—were identified that contained ingredients based on foods which typically cause food allergy. TABLE 8 shows the number of products broken down by product category, ingredient type, and food with indication of modification. The products were found typically to contain oats and almond, with especially oats being added as extract. Protein, whole food, and extract ingredients based on milk, soy, and wheat were found. In addition, oil from almond and macadamia nut were found as ingredients in some products.

TABLE 8. Number of cosmetic products aimed at specific skin types identified in the Danish market, broken down by product category, ingredient type, and food.

	Ingredient type				
Category	**Protein**	**Whole**	**Extract**	**Oil**	**Number**
Oil	-	-	Oats (UM)	-	4
Oil	-	-	Soy (UM)	-	2
Oil	-	-	-	Almond (UM)	1
Oil	-	-	-	Almond (UM) Oats (UM)	1
Cream	-	-	Oats (UM)	-	35
Cream	-	-	-	Almond (UM)	28
Cream	-	-	-	Oats (UM)	10
Cream	-	-	Soy (UM)	-	8
Cream	-	-	-	Soy (UM)	5
Cream	-	-	-	Almond (UM) Soy (UM) +	4

				Soy (CM)	
Cream	-	-	-	Macadamia nut (UM)	2
Cream	-	-	-	Macadamia nut (UM) + Soy (UM)	2
Cream	Milk (UM) + Soy (UM)	-	Soy (UM)	-	2
Cream	-	-	Wheat (CM)	-	2
Cream	Milk (H)	-	-	-	1
Cream	Milk (UM) + Soy (UM)	-	Soy (UM) + Wheat (UM) + Barley (UM)	-	1
Cream	Milk (UM) + Soy (UM)	-	Soy (UM)	Soy (CM)	1
Cream	Wheat (H)	-	Wheat (H)	-	1
Cream	Wheat (HCM) + Oats (H)	-	-	-	1
Cream	Soy (H)	-	-	Soy (UM)	1
Cream	-	Milk (UM)	-	-	1
Cream	-	Oats (UM)	-	-	1
Cream	-	Milk (UM)	-	Almond (UM)	1
Cream	-	Oats (UM)	-	Almond (UM)	1
Cream	-	-	Wheat (UM)	-	1
Cream	-	-	Soy (UM)	Soy (UM)	1
Cream	-	-	Oats (UM)	Soy (UM)	1
Cream	-	-	-	Wheat (UM)	1
Cream	-	-	-	Wheat (UM) + Oats (UM)	1
Cream	-	-	-	Almond (UM) Macadamia nut (UM)	1
Cream	-	-	-	Milk (UM)	1
Cream	-	-	-	Sesame (UM)	1
Cream	-	-	-	Sesame (UM) + Macadamia nut (UM)	1
Shampoo	-	-	-	Almond (UM)	2
Shampoo	Wheat (H)	-	-	-	1
Shampoo	-	-	Almond (UM)	-	1
Soap	-	-	Oats (UM)	-	9
Soap	Milk (UM)	-	-	-	5
Soap	-	-	Almond (UM)	-	3
Soap	-	-	Soy (UM)	-	3
Soap	-	-	-	Almond (UM)	3
Soap	-	-	-	Wheat (UM) + Oats (UM)	1
Soap	-	-	-	Soy (UM)	1

Cream: Creams, lotions, and ointments. Shampoo: Shampoo and shampoo+body wash products. Soap: Hand soap, body soap, bath soap, and other wash products. UM: Unmodified. H Hydrolyzed. HCM: Hydrolyzed and other chemical modification.

3.2 Food-based ingredients in cosmetic products available on the Internet

3.2.1 Identification of cosmetic products in non-Danish EU webshops

This mapping entailed an identification of the range of cosmetic products available to Danish consumers on the Internet via commercial webshops that dispatch products to Denmark from non-Danish addresses in the EU. Products aimed at children and containing food-based ingredients that typically cause food allergy were identified through systematic searches on Google, webshops, and producers' websites. A total of 151 cosmetic products were identified. TABLE 9 shows the number of products, broken down by product category, ingredient type, and food with an indication of modification. The products were typically found to contain oil, especially extracted from almonds and soy. Protein, whole food, and extract ingredients based on milk, oats, wheat, and soy were found. In addition, oil from macadamia nut was found as an ingredient in some products.

TABLE 9. Number of cosmetic products aimed at children identified in non-Danish webshops in the EU, broken down by product category, ingredient type, and food.

	Ingredient type				
Category	**Protein**	**Whole**	**Extract**	**Oil**	**Number**
Oil				Almond (UM)	38
Oil				Almond (UM) Soy (UM)	3
Oil				Macadamia nut (UM)	2
Oil				Sesame (UM)	2
Oil				Soy (UM)	2
Oil			Soy (UM)	Almond (UM)	1
Oil				Oats (UM)	1
Oil				Almond (UM) Macadamia nut (UM)	1
Oil				Almond (UM) Sesame (UM)	1
Cream				Almond (UM)	17
Cream				Soy (UM)	12
Cream			Oats (UM)		6
Cream		Milk (UM)			4
Cream			Oats (UM)	Almond (UM)	4
Cream				Soy (UM) + Macadamia nut (UM)	4
Cream		Oats (UM)			3
Cream			Almond (UM)	Almond (UM)	3
Cream		Oats (UM)		Oats (UM)	2
Cream			Almond (UM)		2
Cream			Oats (UM)	Soy (UM)	2
Cream				Almond (UM)	2

				Sesame (UM)	
Cream				Almond (UM) Soy (UM)	2
Cream				Sesame (UM)	2
Cream		Oats (UM)		Almond (UM)	1
Cream		Oats (UM + H)			1
Cream			Oats (UM)	Sesame (UM)	1
Cream				Macadamia nut (UM)	1
Cream				Soy (UM) + Brazil nut (UM)	1
Cream				Soy (UM) + Sesame (UM)	1
Shampoo	Wheat (H)				4
Shampoo	Oats (HCM)				2
Shampoo				Almond (UM)	2
Shampoo	Wheat (UM)			Almond (UM)	1
Shampoo	Wheat (UM)				1
Shampoo	Oats (H)				1
Shampoo	Wheat (H)	Milk (UM)			1
Shampoo	Wheat (HCM)			Almond (UM)	1
Shampoo	Wheat (HCM)				1
Shampoo			Oats (UM)	Almond (UM)	1
Shampoo			Oats (UM)		1
Shampoo				Sesame (UM)	1
Shampoo				Soy (UM)	1
Shampoo				Soy (UM) + Sesame (UM)	1
Soap			Oats (UM)		3
Soap		Milk (UM)			2
Soap	Wheat (H)				1
Soap		Oats (UM)		Sesame (UM)	1
Soap			Almond (UM)		1
Soap				Almond (UM)	1
Soap				Soy (UM)	1

Cream: Creams, lotions, and ointments. Shampoo: Shampoo and shampoo+body wash products. Soap: Hand soap, body soap, bath soap, and other wash products. UM: Unmodified. H Hydrolyzed. HCM: Hydrolyzed and other chemical modification.

3.2.2 Identification of cosmetic products in webshops outside the EU

A mapping was done of the cosmetic product range available to Danish consumers on the Internet via webshops that dispatch products to Denmark from addresses outside the EU. Products aimed at children and containing food-based ingredients that typically cause food allergy were identified through systematic searches on Google, webshops, and producers' websites. Webshops in the United States and the UK generally had a list of ingredients, and they were therefore the largest source of purchased products from outside the EU. Large webshops in, for example, China and India rarely had a list of ingredients, were not in English, and often required

the shipment of large quantities. These webshops were excluded, as Danish consumers are not expected to use them to a significant extent. A total of 91 cosmetic products were identified. TABLE 10 shows the number of products, broken down by product category, ingredient type, and food with an indication of modification. The products were typically found to contain oil, especially extracted from almonds and soy. Protein, whole food, and extract ingredients based on oats, milk, wheat, and soy were found. In addition, oil from macadamia nut was found as an ingredient in some products.

TABLE 10. Number of cosmetic products aimed at children identified on webshops outside the EU, broken down by product category, ingredient type, and food.

	Ingredient type				
Category	**Protein**	**Whole**	**Extract**	**Oil**	**Number**
Oil				Soy (UM)	3
Oil		Oats (UM)	Oats (UM)	Oats (UM)	1
Oil				Oats (UM) + soy (UM)	1
Oil				Wheat (UM)	1
Oil				Almond (UM)	1
Oil				Almond (UM) Sesame (UM)	1
Cream				Almond (UM)	8
Cream		Oats (UM)			8
Cream				Macadamia nut (UM)	4
Cream				Almond (UM) Macadamia nut (UM)	4
Cream				Almond (UM) Soy (UM)	4
Cream		Milk (UM)		Soy (UM)	3
Cream		Milk (F)			3
Cream				Soy (UM)	3
Cream		Oats (UM)			2
Cream			Oats (UM)	Oats (UM)	2
Cream			Oats (UM)		2
Cream	Oats (H + HCM)		Oats (UM)		1
Cream		Oats (UM)	Oats (UM)		1
Cream				Oats (UM)	1
Shampoo			Oats (UM)		3
Shampoo	Soy (HCM)				2
Shampoo	Oats (H)		Oats (UM)		2
Shampoo		Oats (UM)			2
Shampoo				Soy (UM)	2
Shampoo	Wheat (?)				1
Shampoo	Wheat (H)				1
Shampoo	Oats (H)				1

Shampoo	Oats (HCM)				1
Shampoo			Oats (UM)	Wheat (UM)	1
Soap			Oats (UM)		5
Soap		Milk (UM)		Almond (UM)	4
Soap		Oats (UM)			4
Soap		Oats (UM)	Oats (UM)		3
Soap	Wheat (H)				2
Soap	Soy (HCM)				1
Soap	Oats (HCM)				1
Soap				Almond (UM)	1

Cream: Creams, lotions, and ointments. Shampoo: Shampoo and shampoo+body wash products. Soap: Hand soap, body soap, bath soap, and other wash products. UM: Unmodified. H Hydrolyzed. HCM: Hydrolyzed and other chemical modification. F. Fermented.

3.3 Subsidiary conclusion

Food-based ingredients are widely used in cosmetic products in both unmodified form and modified form. It was found that cosmetic products aimed at children and specific skin types in particular contained oils from almond or soy. In addition, oats were found as an additive in many products as whole ingredient or extract. Finally, it was found that milk is added as protein or whole food ingredient in certain products.

4. Consumption of allergenic foods in children

Studies suggest that early introduction of foods in children protects against the development of food allergy. This means that, in early childhood, there is a temporal window for allergy development before the child has been introduced to a given food via the diet. Based on dietary studies conducted by DTU Food, this chapter examines when Danish children are introduced to the various foods that typically cause food allergy.

4.1 Dietary study: Introduction to foods in children aged 0-24 months

In the period April 2014 to April 2015, DTU Food performed a national dietary survey among infants and young children in which a personal interview with the parents was conducted. This included questions aimed at establishing the time of introduction of different foods in children aged 0-24 months. A total of 437 parents of children aged 12-24 months were asked at what age their child was introduced to a given food. 427 of these parents answered the questions. Based on these data, it is possible to assess the introduction of certain foods that typically cause food allergy **(**stated in TABLE 1. Most allergenic foods, see EU Regulation 1169/2011 Annex II

4.1.1 Breastfeeding and introduction of infant formula and drinking milk

Most mothers started breastfeeding at birth (95 per cent) and about 75 per cent responded full breastfeeding at 1 month of age, 55 per cent at 4 months, and 11 per cent at 6 months. Infant formula was thus introduced by 5 per cent early after birth and by 25 per cent after 1 month.

Approximately 20 per cent of the parents of 1-year-old children responded that infant formula had not been introduced and nearly 70 per cent had not received gruel or infant formula (follow-on formula, which is infant formula for children aged from 6 months and up), see FIGURE 1. Date of introduction of infant formula, gruel, or the like, as well as drinking milk, stated by parents of children aged 12 to 24 months (n = 427). Approx. 70 per cent had not been given infant formula as a cup drink, while less than 10 per cent had not been given ordinary drinking milk. Of the approximately 80 per cent who were given infant formula during their first year, 44 per cent were introduced to it between the age of 0-3 months. A few were given drinking milk before they were 6 months old, but most during their first year and a few only after 1 year. This picture must be assumed to have changed because the recommendations from the Danish Health Authority were changed in March 2015, so that introduction of drinking milk is not recommended until the age of 1 year, while it is recommended that infant formula as drinking milk in a cup could be commenced at the age of 9 months. Ordinary milk in the food can be given in small quantities (under 100 ml) from the age of 9 months.

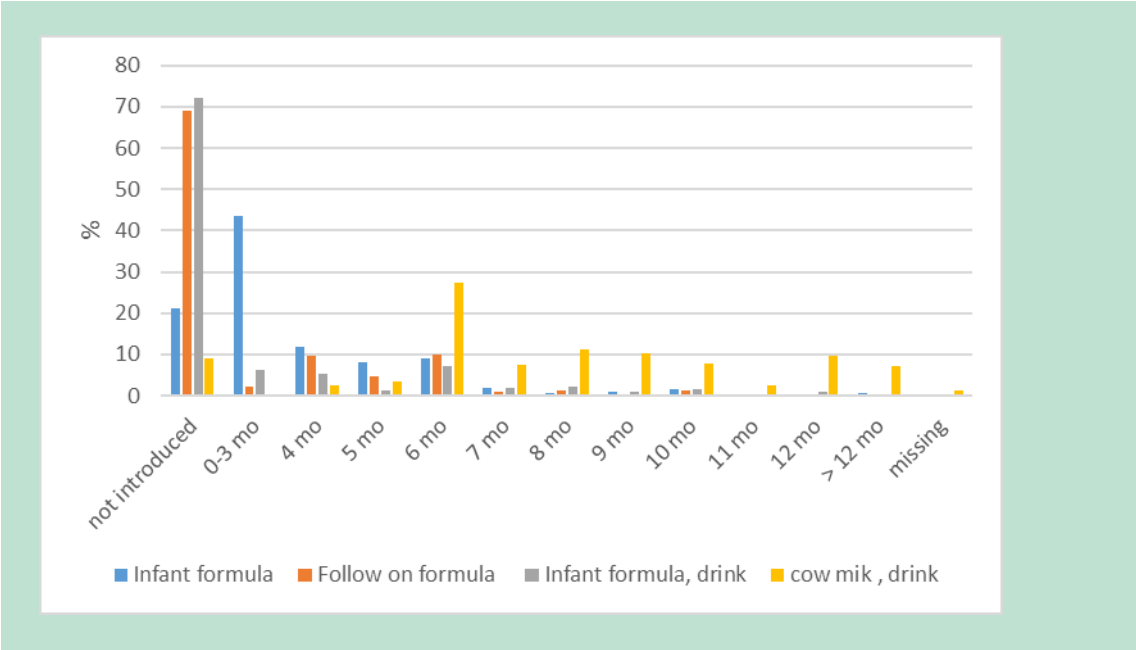

FIGURE 1. Date of introduction of infant formula, gruel, or the like, as well as drinking milk, stated by parents of children aged 12 to 24 months (n = 427).

4.1.2 Introduction of porridge and mash of various foods

A few parents of children aged 12-24 months responded that they had introduced porridge before 4 months (FIGURE 2. Time of introduction of porridge, mash, and fish, stated by parents of children aged 12-24 months (n = 427). The first porridge was typically corn, rice, or millet porridge. It is currently not recommended to the same extent to use rice in young children due to inorganic arsenic content. Most were introduced to these types of porridge at the age of 4-6 months, most at 4 months. Correspondingly, introduction to oatmeal and wholemeal porridge as well as 'øllebrød' (rye porridge) also started at 4 months, where it was given to less than 10 per cent, while approx. 50 per cent were introduced to these porridge types at the age of 6 months. Mashed vegetables/potatoes were also introduced to approx. 80 per cent of the children at the age of 6 months; approx 40 per cent were introduced to this before the age of 4 months, and approx. 20 per cent at the age of 4 and 5 months, respectively. A few introduced fish in mash at 4 and 5 months, but most from 6 months, while approx. 20 per cent were not introduced to mash with fish. Fish in whole pieces were introduced to a very few at the age of 5 months, but most from the age of 8 months and upwards. Only approx. 5 per cent were not introduced to fish.

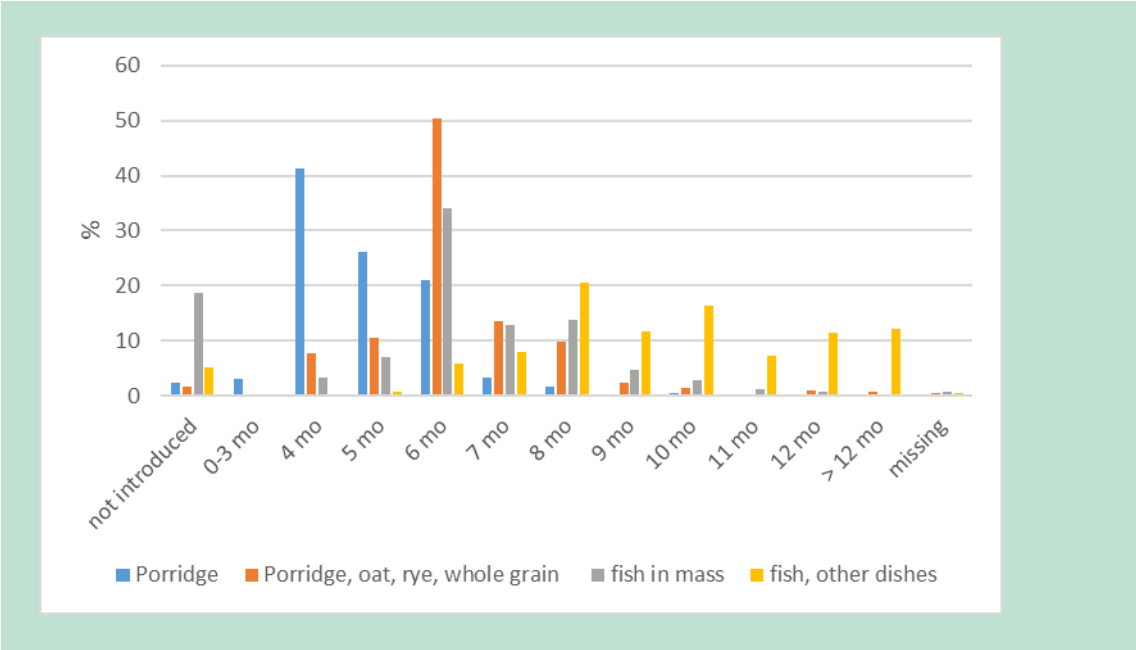

FIGURE 2. Time of introduction of porridge, mash, and fish, stated by parents of children aged 12-24 months (n = 427).

4.2 Dietary survey: Diet registration in children aged 6-36 months

In the national dietary survey of infants and young children, DTU Food implemented a diet registration among children aged 6-36 months in the period April 2014 to April 2015. A total of 1,364 parents registered the foods and beverages that their child ate and drank every day over a one-week period. In the dietary survey, the children were divided into different age groups: 6-7 months, 8-9 months, 10-11 months, 12-23 months, and 24-36 months. Based on these data, we can calculate the proportion of children who have eaten the food in question for each age group. The proportion of children who have eaten the food in question for each age group (stated as a percentage of the total number of children in the age group) can provide an estimate of when and of how many children were introduced to certain of the foods that typically cause food allergy (stated in TABLE 1. Most allergenic foods, see EU Regulation 1169/2011 Annex II
).

4.2.1 Proportion who ingest milk products and breast milk

The diet registration showed that nearly all children aged 6-7 months (91 per cent) had received a milk-based product, including infant formula or the like (FIGURE 3. The proportion of children who had an intake of milk and dairy products during the week of diet registration, broken down by age group. For comparisons, infant formula and breast milk are shown.Both the proportion who were given ordinary milk products (drinking milk and sour milk products as well as milk on porridge and mash) and the proportion who were given infant formula were high in this age group, 73 per cent and 77 per cent, respectively, while the proportion who were given breast milk was 61 per cent in this age group. The proportion given infant formula and breast milk decreases in subsequent age groups, and the consumption of ordinary milk products increases to nearly 100 per cent in these groups. Consumed milk products originate primarily from cows. In comparison with food introduction (Chapter 4.1), it can be seen that milk was first introduced as infant formula with subsequent introduction of ordinary milk products.

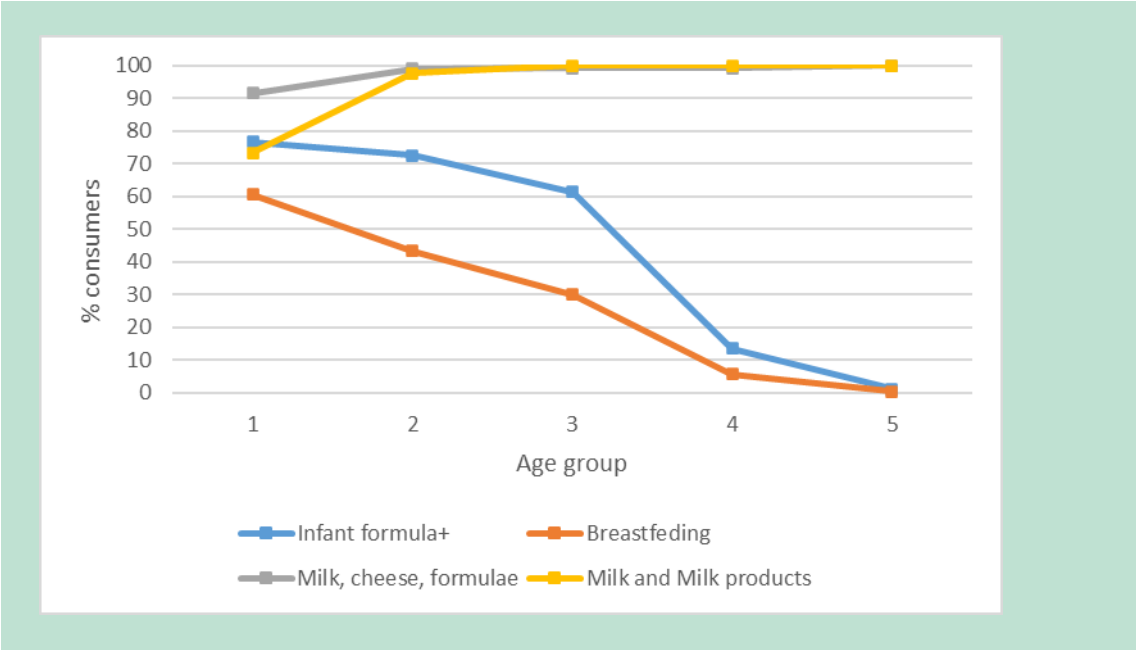

FIGURE 3. The proportion of children who had an intake of milk and dairy products during the week of diet registration, broken down by age group. For comparisons, infant formula and breast milk are shown.

4.2.2 Cereals: Wheat and oats

The proportion who ingested foods with wheat was high (>70 per cent users) in the age group 6-7 months (Figure 4). In the older age groups, the proportion who eat wheat bread increases, while the proportion who eat porridge with wheat decreases. This shows a shift in wheat protein source with age. The proportion of oat-containing products was >80 per cent in the age group 6-7 months, with a slightly decreasing proportion of users in older age groups.

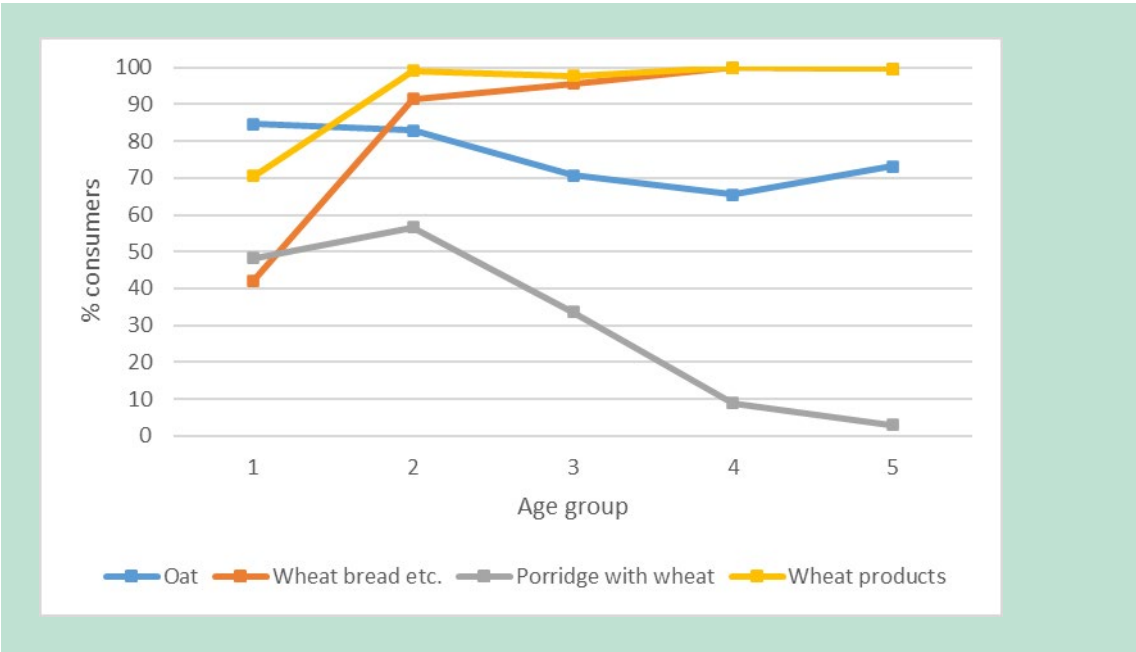

FIGURE 4. Proportion of children who had an intake of oats, wheat bread, porridge with wheat, and products with wheat in total during the diet registration week, broken down by age group.

4.2.3 Eggs, fish, and shellfish

The proportion who ate eggs, fish, or shellfish was <50 per cent in the age group 6-7 months in the diet registration survey (FIGURE 5). The proportion who eat these foods increased in the subsequent age groups, with egg eaters reaching a proportion close to 100 per cent. The slightly lower proportion of children who had eaten fish may be due to this food not being eaten every week among the study participants. Shellfish consumption is generally low in all age groups (<20 per cent), but this level may also be due to this food category not being consumed every week among the study participants.

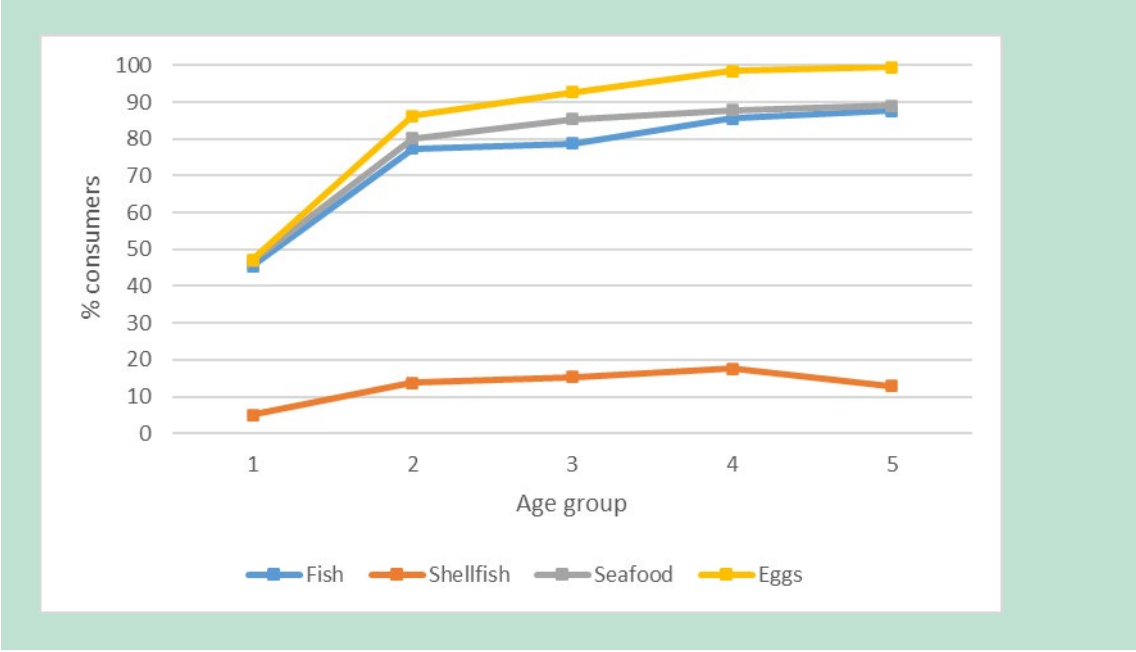

FIGURE 5. The proportion of children who had an intake of eggs, fish, or shellfish during the diet registration week, broken down by age group.

4.2.4 Peanuts, tree nuts, and sesame

The proportion who ate peanuts, tree nuts, and sesame was low (<10 per cent) in the age group 6-7 months in the survey (FIGURE 6). The proportion increases in subsequent age groups, but only reaches >50 per cent around the age of 12 months. These findings indicate that peanuts and tree nuts are introduced much later than milk, cereals, eggs, and fish.

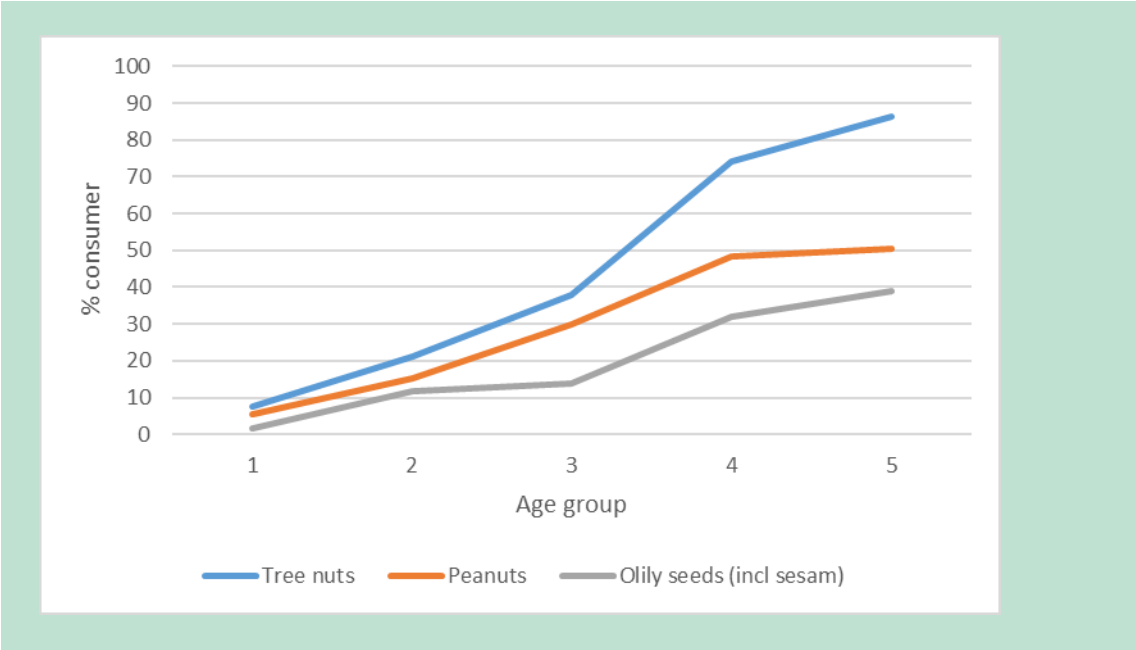

FIGURE 6. The proportion of children who had an intake of tree nuts, peanuts (including peanut butter), and fatty seeds (including sesame) during the diet registration week, for each age group.

4.3 Strengths and weaknesses

It is a strength of the above dietary surveys that they include both questions about the introduction of foods going back in time among children aged 12-24 months, as well as registration of food intake over a period of one week among children aged 6-36 months. However, there may be some uncertainty connected within the interpretation of the composition of the registered intake, which is due to the level of detail in the registration method. For example, it is not registered whether the wheat bread contains seeds and grains, or whether eggs and milk are contained in composite products. Due to a lack of details, no information can be found about the introduction of certain allergenic foods, such as soy, celery, and lupine.

The surveys were conducted in 2014-2015. The culture and dietary guidelines for the introduction of foods in children may have changed since then. For example, it is assumed that the introduction of milk has changed following new guidelines for milk and infant formula in the transitional diet issued by the Danish Health Authority in 2015.

4.4 Subsidiary conclusion

The dietary survey from 2014-2015 shows variation in the introduction and consumption of foods that typically cause food allergy. The results indicate early introduction of milk and cereals (around the age of 4-6 months), followed by eggs, fish, and shellfish (around the age of 6-9 months), and then peanuts, tree nuts, and sesame (age of 1-2 years).

5. Analysis of food proteins in cosmetic products

Danish consumers' access to cosmetic products containing food-based ingredients that typically cause allergy (stated in TABLE 1. Most allergenic foods, see EU Regulation 1169/2011 Annex II
) was mapped in Chapter 3. As the proteins from these foods are those which primarily cause food allergy, it is necessary to examine whether the cosmetic products contain proteins from the ingredients used.

5.1 Focus on selection of foods

The results of the mapping in Chapter 3 were that cosmetic products aimed at children and specific skin types typically contain ingredients based on almond, soy, oats, cow's milk, wheat, and/or macadamia nuts (among the foods that typically cause food allergy). Based on the mapping, it was decided to focus on analysis of proteins from the following foods:

- **Cow's milk:**
  The most common food allergy in children
  Typically used as protein or whole ingredient in cosmetic products

- **Almond:**
  Almond oil finds frequent use in cosmetic products
  Some products contain almond extract

- **Soy:**
  Typically used as protein, extract, or oil ingredient in cosmetic products

5.2 Selection of cosmetic products for analysis

Based on the mapping in Chapter 3, a representative selection of cosmetic products containing ingredients based on milk, almond, and/or soy were purchased for analysis (see TABLE 11). Purchases were made of a total of 39 products available to Danish consumers in stores and/or webshops with dispatch in or to Denmark. A total of 19 products were purchased in Danish stores or webshops, while 13 and 7 products, respectively, were purchased from webshops in the rest of the EU and outside the EU.

TABLE 11. Cosmetic products purchased for analysis of food protein content with indication of ingredient type and content of focus allergen.

Product ID	Place of purchase*	Category**	Ingredient type Protein***	Ingredient type Whole***	Ingredient type Extract***	Ingredient type Oil***
MST-01	DK	Cream	-	-	-	Almond (UM)
MST-03	DK	Cream	-	-	-	Almond (UM) Sesame (UM)
MST-04	DK	Cream	Milk (H)	-	-	-
MST-05	DK	Cream	-	-	-	Almond (UM)
MST-06	DK	Cream	-	-	-	Almond (UM)

MST-07	DK	Cream	-	-	-	Almond (UM)
MST-08	DK	Cream	Soy (H)	-	-	Soy (UM)
MST-09	DK	Cream	-	-	Oats (UM)	Soy (UM)
MST-11	DK	Oil	-	-	-	Almond (UM)
MST-12	DK	Oil	-	-	-	Almond (UM)
MST-13	DK	Oil	-	-	-	Almond (UM)
MST-14	DK	Oil	-	-	-	Soy (UM)
MST-15	DK	Oil	-	-	-	Soy (UM)
MST-16	DK	Oil	-	-	-	Almond (UM)
MST-17	DK	Shampoo	-	-	Oats (UM)	Almond (UM)
MST-18	DK	Shampoo	-	-	-	Almond (UM)
MST-19	DK	Soap	Milk (UM)	-	-	-
MST-20	DK	Soap	-	-	-	Almond (UM)
MST-21	DK	Soap	-	-	-	Almond (UM)
MST-22	Rest of the EU	Cream	-	-	Almond (UM)	Almond (UM)
MST-23	Rest of the EU	Cream				Almond (UM)
MST-24	Rest of the EU	Cream				Soy (UM)
MST-25	Rest of the EU	Cream		Milk (UM)		
MST-26	Rest of the EU	Oil				Almond (UM)
MST-27	Rest of the EU	Oil				Almond (UM)
MST-28	Rest of the EU	Oil				Almond (UM) Soy (UM)
MST-29	Rest of the EU	Shampoo	Wheat (H)	Milk (UM)		
MST-30	Rest of the EU	Shampoo				Almond (UM)
MST-31	Rest of the EU	Soap			Almond (UM)	
MST-32	Rest of the EU	Soap		Milk (UM)		
MST-33	Rest of the EU	Soap		Milk (UM)		
MST-34	Rest of the EU	Cream				Almond (UM) + Soy (UM)
MST-35	Outside the EU	Cream				Soy (UM)
MST-36	Outside the EU	Cream		Milk (UM)		
MST-37	Outside the EU	Cream		Milk (F)		
MST-38	Outside the EU	Oil				Almond (UM)
MST-39	Outside the EU	Oil				Soy (UM)
MST-40	Outside the EU	Shampoo	Soy (HCM)			
MST-41	Outside the EU	Cream				Almond (UM)

*Where the cosmetic product was purchased. **Cream: Creams, lotions, and ointments. Shampoo: Shampoo and shampoo+body wash products. Soap: Hand soap, body soap, bath soap, and other wash products. ***Ingredient types. UM: Unmodified. H Hydrolyzed. HCM: Hydrolyzed and other chemical modification. F. Fermented.

5.3 Choice of analysis method

The enzyme-linked immunosorbent assay (ELISA) method is used for the analysis of food proteins in cosmetic products. This antibody-based method is widely used for protein analysis due to its generally high specificity (ability to detect only one specific protein) and sensitivity (detection of miniscule quantities of protein). There are several commercially available analysis kits that can measure food proteins. These are used in the food industry to check cross-contamination of foods with food allergens. The method is used by the industry to ensure that processed foods do not contain allergens that can cause allergic reactions in consumers with food allergy. This project uses commercially available analysis kits from Eurofins Technologies. These analysis kits have been validated by the producer for use in analysis of complex and diverse foods (e.g., cakes, meat products, chocolate, etc.). In addition, the analyses have been tested for the absence of cross-reactivity with other food allergens than those the analysis kit is intended to detect. Below are the specifications of the analyses, including standard series, Limit of Detection (LOD) and Limit of Quantification (LOQ).

- **Cow's milk:**
  - ELISA kit: SENSISpec ELISA Milk 96 Wells
  - Producer: Eurofins Technologies (www.eurofins-technologies.com)
  - Standard series: 0, 0.4, 1, 4, and 10 ppm
  - LOD: 0.05 ppm
  - LOQ: 0.4 ppm

- **Almond:**
- ELISA kit: SENSISpec ELISA Almond 96 Wells
- Producer: Eurofins Technologies (www.eurofins-technologies.com)
  - Standard series: 0, 0.4, 1, 4, and 10 ppm
  - LOD: 0.06 ppm
  - LOQ: 0.4 ppm

- **Soy:**
  - ELISA kit: SENSISpec ELISA Total Soy Protein
  - Producer: Eurofins Technologies (www.eurofins-technologies.com)
  - Standard series: 0, 2, 6, 18, and 36 ppm
  - LOD: 0.2 ppm
  - LOQ: 2 ppm

The producer of the above analysis kits has not examined whether these ELISA assays can be used for analysis of food proteins in cosmetic products. In the research literature, there are only two previous studies that have used commercially available ELISA assays for protein analysis of cosmetic products (both for wheat protein analysis) (Thomson and Grace 2012, Sharma et al 2016). It is therefore necessary to perform a method validation to establish whether the selected ELISA assays are useful for analysis of food proteins in cosmetic products.

5.4 Method validation

Cosmetic products are a mixture of different substances that give the product the desired properties (e.g., fats and soaps). These substances can affect the analysis of food proteins, as they may influence the function of the analytical reagents. Therefore, a number of cosmetic products—primarily aimed at children— were selected for method validation. The selected products (matrices) are shown in TABLE 12 with an indication of the type and content of the product. Only products containing no ingredients based on milk, almond, or soy were selected. In the method validation, a known quantity of milk, almond, or soy protein is added so that it can be determined whether the matrices affect the result. This type of trial is called recovery trial, where a known quantity of protein is added (by so-called ‘spiking’ of the sample).

TABLE 12. The cosmetic products (matrices) selected for method validation.

Matrix no.	Description
1	Baby oil & bath
2	Baby oil
3	Baby cream
4	Baby cream
5	Baby shampoo & bath
6	Baby shampoo

5.4.1 Validation parameters

The limit of detection and limit of quantification of the analyses are determined by analysis of 6 samples that do not contain food protein (so-called blank samples). The limit of detection and limit of quantification are determined as the mean value of the analysis with addition of 3 and 10 times the standard deviation of the samples, respectively. The measurement range and correctness of the analyses (the ability to determine the correct quantity of protein) are determined by spiking with a known quantity of food protein in the concentrations used in the standard series of analyses. Correctness is determined as the slope of the linear correlation curve between added (spike) concentration vs. measured concentration. I.e., a slope of 1 indicates optimal correctness with good coherence, whereas a value greater or lower than 1 shows an overestimation or underestimation in the analysis, respectively.

5.4.2 Validation results: Cow's milk ELISA assay

Virtually all matrices (TABLE 13) gave rise to a higher value for the limit of detection and limit of quantification of the cow's milk ELISA relative to the limits stated by the producer (LOD: 0.05 ppm and LOQ: 0.4 ppm, respectively). The results from the recovery trial (TABLE 14 and Appendix 1.1) were that the correctness of analyses with the oil matrices was good. In turn, however, the correctness was only adequate for one of the cream products, whereas the other cream product led to an overestimation of the protein content. This finding suggests that different compositions of cream products have different impacts on the correctness of the cow's milk ELISA. It was also found that the shampoo products led to an underestimation of the protein content. Therefore, the conclusion is that cosmetic matrices affect the function of the cow's milk ELISA assay.

TABLE 13. LOD and LOQ for matrices analysed with cow's milk ELISA assay.

Matrix no.	Description	LOD*	LOQ**
1	Baby oil & bath	0.92 ppm	3.02 ppm
2	Baby oil	0.49 ppm	1.03 ppm
3	Baby cream	0.84 ppm	2.22 ppm
4	Baby cream	0.56 ppm	2.60 ppm
5	Baby shampoo & bath	0.32 ppm	0.79 ppm
6	Baby shampoo	< 0.05 ppm	1.35 ppm

*Limit of Detection. **Limit of Quantification.

TABLE 14. Results from recovery trials for matrices analysed with cow's milk ELISA assay.

Matrix no.	Description	Range of measurement	Correctness*
1	Baby oil & bath	0 – 4 ppm	0.996x
2	Baby oil	0 – 10 ppm	0.943x
3	Baby cream	0 – 4 ppm	1.409x
4	Baby cream	0 – 10 ppm	0.803x
5	Baby shampoo & bath	0 – 10 ppm	0.352x
6	Baby shampoo	0 – 10 ppm	0.134x

*Slope of curve for linear correlation between spike concentration vs. measured concentration.

5.4.3 Validation results: Almond ELISA assay

The different matrices have a limited impact on the almond ELISA's limit of detection and limit of quantification (TABLE 15), as most were below the kit limits of 0.06 and 0.4 ppm, respectively. However, the results from the recovery trial (TABLE 16 and Appendix 1.2) were that analyses based on the oil and cream matrices led to an overestimation of the protein content. This overestimation meant that the 10 ppm sample was outside the measurability of the analysis. Therefore, 10 ppm was deleted in the determination of the range of measurement, which means that the analyses based on the oil and cream matrices have a range of measurement of 0 - 4 ppm. In turn, there was good correctness for the shampoo products, which had a correlation close to 1 and a range of measurement of 0 - 10 ppm.

TABLE 15. LOD and LOQ for matrices analysed with almond ELISA assay.

Matrix no.	Description	LOD*	LOQ**
1	Baby oil & bath	<0.06 ppm	<0.40 ppm
2	Baby oil	0.09 ppm	<0.40 ppm
3	Baby cream	<0.06 ppm	<0.40 ppm
4	Baby cream	0.14 ppm	0.41 ppm
5	Baby shampoo & bath	<0.06 ppm	0.40 ppm
6	Baby shampoo	<0.06 ppm	<0.40 ppm

*Limit of Detection. **Limit of Quantification.

TABLE 16. Results from recovery trial for matrices analysed with almond ELISA assay.

Matrix no.	Description	Range of measurement	Correctness*
1	Baby oil & bath	0 – 4 ppm	1.459x
2	Baby oil	0 – 4 ppm	1.451x
3	Baby cream	0 – 4 ppm	1.312x
4	Baby cream	0 – 4 ppm	1.243x
5	Baby shampoo & bath	0 – 10 ppm	0.988x
6	Baby shampoo	0 – 10 ppm	0.943x

*Slope of curve for linear correlation curve between spike concentration vs. measured concentration.

5.4.4 Validation results: Soy ELISA assay

Several of the matrices (TABLE 17) resulted in a higher value for the limit of detection and limit of quantification of the soy ELISA relative to the limits stated by the producer (LOD: 0.2 ppm and LOQ: 2 ppm, respectively). The results from the recovery trial (TABLE 18 and Appendix 1.3)

were that the matrices gave rise to varying correctness, with several having an underestimation of the protein content. This finding therefore suggests that the diverse composition of cosmetic products will have a different impact on the correctness of the soy ELISA.

TABLE 17. LOD and LOQ for matrices analysed with soy ELISA assay.

Matrix no.	Description	LOD*	LOQ**
1	Baby oil & bath	0.92 ppm	3.02 ppm
2	Baby oil	0.49 ppm	< 2 ppm
3	Baby cream	0.84 ppm	2.22 ppm
4	Baby cream	0.56 ppm	2.60 ppm
5	Baby shampoo & bath	0.31 ppm	< 2 ppm
6	Baby shampoo	0.03 ppm	< 2 ppm

*Limit of Detection. **Limit of Quantification.

TABLE 18. Results from recovery trial for matrices analysed with soy ELISA assay.

Matrix no.	Description	Range of measurement	Correctness*
1	Baby oil & bath	0 – 36 ppm	0.859x
2	Baby oil	0 – 36 ppm	0.339x
3	Baby cream	0 – 36 ppm	0.830x
4	Baby cream	0 – 36 ppm	0.941x
5	Baby shampoo & bath	0 – 36 ppm	0.597x
6	Baby shampoo	0 – 36 ppm	0.361x

*Slope of curve for linear correlation between spike concentration vs. measured concentration.

5.5 Analysis results: Cow's milk

The ELISA analyses showed measurable milk protein in all purchased products with milk-based ingredients (TABLE 19). Especially products with whole and protein ingredient types had the highest levels of milk protein. It is important to note that the method validation results show that the specific composition of the products may lead to an overestimation or underestimation of the protein content. However, it must be concluded that the products analysed contain measurable levels of milk protein. Several of the samples had low added spike recovery (TABLE 19). This may be due to a varying effect of the cosmetic matrices, as was also seen in the validation analyses of the ELISA assay for milk (Chapter 5.4.2). Therefore, there is significant uncertainty about the exact milk protein content in the analysis. Therefore, a different method should be used if a more precise determination of the quantity of milk protein content is desired.

TABLE 19. Analytical results for cosmetic products containing milk.

Product ID	Place of purchase*	Category**	Content***	Concentration	Spike****
MST-04	DK	Cream	Milk: Protein (H)	0.36 ppm	55.93 %
MST-19	DK	Soap	Milk: Protein (UM)	36.09 ppm	-
MST-25	Rest of the EU	Cream	Milk: Whole (UM)	10.07 ppm	-2.11 %
MST-29	Rest of the EU	Shampoo	Milk: Whole (UM)	3.91 ppm	-10.82 %
MST-32	Rest of the EU	Soap	Milk: Whole (UM)	250.54 ppm	-
MST-33	Rest of the EU	Soap	Milk: Whole (UM)	101.66 ppm	-

MST-36	Outside the EU	Cream	Milk: Whole (UM)	4.36 ppm	48.43 %
MST-37	Outside the EU	Cream	Milk: Whole (F)	121.71 ppm	-

*Where the cosmetic product was purchased. **Cream: Creams, lotions, and ointments. Shampoo: Shampoo and shampoo+body wash products. Soap: Hand soap, body soap, bath soap, and other wash products. ***Ingredient type. UM: Unmodified. H Hydrolyzed. F. Fermented. ****Per cent recovery of 5 ppm spike added during the analysis of the cosmetic product.

5.6 Analysis results: Almond

Almond oil was the most frequent ingredient in the cosmetic products analysed for almond protein (TABLE 20). Product MST-06 was the product with the highest ascertained almond protein content. The remaining products were below or close to the limit of detection (0.06 ppm). In the products MST-21, MST-26 and MST-41, small quantities of almond protein were found. In general, there was good recovery of almond protein in the spike samples (TABLE 20), indicating that the almond ELISA can be used on cosmetic products with a varied product composition.

TABLE 20. Analysis results for cosmetic products containing almond.

Product ID	Place of purchase*	Category**	Content***	Concentration	Spike****
MST-01	DK	Cream	Almond: Oil (UM)	0.03 ppm	107 %
MST-03	DK	Cream	Almond: Oil (UM)	0.02 ppm	109 %
MST-05	DK	Cream	Almond: Oil (UM)	0.00 ppm	93 %
MST-06	DK	Cream	Almond: Oil (UM)	43.07 ppm	-
MST-07	DK	Cream	Almond: Oil (UM)	0.00 ppm	101 %
MST-11	DK	Oil	Almond: Oil (UM)	0.00 ppm	111 %
MST-12	DK	Oil	Almond: Oil (UM)	0.00 ppm	109 %
MST-13	DK	Oil	Almond: Oil (UM)	0.00 ppm	113 %
MST-16	DK	Oil	Almond: Oil (UM)	0.00 ppm	108 %
MST-17	DK	Shampoo	Almond: Oil (UM)	0.00 ppm	94 %
MST-18	DK	Shampoo	Almond: Oil (UM)	0.00 ppm	106 %
MST-20	DK	Soap	Almond: Oil (UM)	0.00 ppm	55 %
MST-21	DK	Soap	Almond: Oil (UM)	0.08 ppm	63 %
MST-22	Rest of the EU	Cream	Almond: Extract (UM) + oil (UM)	0.04 ppm	89 %
MST-23	Rest of the EU	Cream	Almond: Oil (UM)	0.02 ppm	85 %
MST-26	Rest of the EU	Oil	Almond: Oil (UM)	0.08 ppm	119 %
MST-27	Rest of the EU	Oil	Almond: Oil (UM)	0.01 ppm	99 %
MST-28	Rest of the EU	Oil	Almond: Oil (UM)	0.00 ppm	103 %
MST-30	Rest of the EU	Shampoo	Almond: Oil (UM)	0.00 ppm	83 %
MST-31	Rest of the EU	Soap	Almond: Extract (UM)	0.00 ppm	81 %
MST-34	Rest of the EU	Cream	Almond: Oil (UM)	0.03 ppm	81 %
MST-38	Outside the EU	Oil	Almond: Oil (UM)	0.03 ppm	97 %
MST-41	Outside the EU	Cream	Almond: Oil (UM)	0.20 ppm	75 %

*Where the cosmetic product was purchased. **Cream: Creams, lotions, and ointments. Shampoo: Shampoo and shampoo+body wash products. Soap: Hand soap, body soap, bath soap, and other wash products. ***Ingredient type. UM: Unmodified. ****Per cent recovery of 5 ppm spike added during the analysis of the cosmetic product.

5.7 Analysis results: Soy

Soy oil was the most frequent ingredient in the cosmetic products analysed for soya protein (TABLE 21). All products were below or close to the ELISA limit of detection (0.2 ppm). In the method validation, it was found that the limit of detection and limit of quantification were a lot higher when analysed on cosmetic matrices. A limit of detection of around 1 ppm was generally found in the validation of cosmetic matrices (see TABLE 17). It must therefore be assumed that soy protein cannot be detected in the analysed products, as the measured concentrations are far below 1 ppm (TABLE 21). Analysis of the spike samples shows a reasonable recovery (i.e., a spike recovery close to 100 per cent) of soy protein in the cosmetic products (TABLE 21). This indicates that the analysis can be performed on the selected cosmetic products.

TABLE 21. Analysis results for cosmetic products containing soy.

Product ID	Place of purchase*	Category**	Content***	Concentration	Spike****
MST-08	DK	Cream	Soy: Protein (H)	0.21 ppm	2 %
MST-09	DK	Cream	Soy: Oil (UM)	0.30 ppm	75 %
MST-14	DK	Oil	Soy: Oil (UM)	0.32 ppm	51 %
MST-15	DK	Oil	Soy: Oil (UM)	0.13 ppm	125 %
MST-24	Rest of the EU	Cream	Soy: Oil (UM)	0.28 ppm	96 %
MST-28	Rest of the EU	Oil	Soy: Oil (UM)	0.00 ppm	50 %
MST-34	Rest of the EU	Cream	Soy: Oil (UM)	0.00 ppm	99 %
MST-35	Outside the EU	Cream	Soy: Oil (UM)	0.08 ppm	79 %
MST-39	Outside the EU	Oil	Soy: Oil (UM)	0.22 ppm	44 %
MST-40	Outside the EU	Shampoo	Soy: Protein (HCM)	0.00 ppm	118 %

*Where the cosmetic product was purchased. **Cream: Creams, lotions, and ointments. Shampoo: Shampoo and shampoo+body wash products. Soap: Hand soap, body soap, bath soap, and other wash products. ***Ingredient type. UM: Unmodified. H Hydrolyzed. HCM: Hydrolyzed and other chemical modification. ****Per cent recovery of 10 ppm spike added during the analysis of the cosmetic product.

5.8 Subsidiary conclusion

Overall, the ELISA method can be used to determine the presence of milk protein, almond protein, and soy protein in cosmetic products when the proteins are present in sufficient quantity. The specific cosmetic matrices affect the recovery rate, limits of detection, and limits of quantification. There is therefore some uncertainty in determining low protein concentrations in cosmetic products. In particular, milk protein could be found in the cosmetic products where milk is a whole or protein ingredient. Almond protein was found in a single cream product containing almond oil. It is uncertain whether cosmetic products containing oil from almond or soy contain protein from these foods, as the levels are very close to the limit of detection of the analyses.

6. Risk assessment

Cosmetic products containing food-based ingredients are available to Danish consumers via purchases in stores and on the Internet. Several studies indicate that food allergy may be developed through skin exposure to food proteins. A literature review will be done for the purpose of establishing whether there is currently sufficient knowledge to perform an assessment of the risk associated with the use of cosmetic products containing food proteins in relation to food allergy in children.

Compared to contact allergy to chemicals, knowledge about sensitization to food proteins via the skin is limited. For chemicals, it is known that the unit to be used in risk assessments is dose per unit area (Api et al 2008). This means that it is not the total dose that is used. The background is that a certain concentration of the substance must be present to trigger a sensitization. It is not known whether this also applies to proteins, but as the mechanisms of the two forms of sensitization resemble each other, it is assumed that concentration per unit area is decisive for sensitization. This assumption is used in the following.

In the analyses of the cosmetic products, measurable content of milk protein and almond protein was found, and it is being examined whether it is possible to find sensitization data that can be used in a risk assessment. Exposure data will be based on the analysis results.

6.1 Data from humans: Elicitation

Contact allergy to chemicals—a type IV reaction—is frequent in the human population. The diagnosis is made on the basis of patch testing—i.e. elicitation after exposure—where the dose used is high enough to induce an allergic response and low enough not to trigger an irritation. Typically, one standard dose is used. Fisher et al (2011) have examined the possibility of using human data for elicitation to determine a secure dose for sensitization. For this purpose, they have examined data from dose-response studies with eight different contact allergens and determined the dose that gave a positive response after patch testing (elicitation) in 10 per cent of the studied population (ED10). They found that the median value for ED10 was 0.835 $\mu g/cm^2$. They compared ED10 for elicitation with sensitization data (EC3 from Local Lymph Node Assay see section 6.7) and found no obvious correlation between sensitization doses and elicitation doses. Once the contact allergy has been established, there does not seem to be much difference between the doses that trigger symptoms (Fisher et al 2011).

It is not known whether the same applies to sensitization to proteins—a type I allergy—and there are no data for examining this. In addition, the most relevant exposure in relation to elicitation in this project is not the skin, but the gastrointestinal tract. There is a large volume of dose-response data on elicitation of food allergy after oral exposure used to calculate ED01, ED05, etc. (Remmington et al 2020), but there is no knowledge of the correlation with sensitization data, either oral sensitization or sensitization via skin or airways.

As the above shows, elicitation data from humans cannot be used for an assessment of the risk of sensitization.

6.2 Data from humans: Sensitization

It has been known for many years that proteins may sensitize via the skin in connection with occupational skin exposure, so-called ‘food handler dermatitis’ (Hjorth and Roed-Petersen 1976, Lukács et al 2016).

The recognition that the skin may be a route of sensitization for food allergens in non-occupational skin exposure is relatively new. The objective of this section is to examine studies with a well-documented correlation between skin sensitization and food allergy.

6.2.1 Protein from peanuts may sensitize children with atopic dermatitis

Historically, it has been difficult to explain how children could develop allergic symptoms the first time they ate a food. A possible explanation came in 2003 with a study that showed that allergy to peanuts in young children correlated with the use of peanut oil-based skin cream in children with skin inflammation (atopic dermatitis). 91 per cent of the children who developed symptoms of food allergy after intake of peanuts had been exposed to peanut oil-based skin cream in their first six months of life, to a great extent during periods in which the child had eczema (Lack et al. 2003).

Later studies have shown allergens from peanuts in household dust. Studies of the correlation between the occurrence of peanut allergens in household dust and sensitization to peanuts have shown that exposure to peanut protein in dust (13-151 µg peanut protein/g dust) correlated with sensitization to peanuts and probable food allergy. The correlation was strongest among children with atopic dermatitis. There was no association between exposure to peanut dust and sensitization with peanuts in the group of children without atopic dermatitis, indicating that the sensitization occurs through an impaired skin barrier. The study also indicates that an impaired skin barrier—such as in children with filaggrin loss-of-function mutation that impacts the skin barrier—is not sufficient for sensitization, but that an active inflammatory condition must also be present (Brough et al 2013, 2014, 2015).

The hypothesis that sensitization to peanuts in young children may occur via skin with an active inflammatory condition was supported by subsequent studies of oral tolerance. It was found that early introduction of peanuts into the diet reduced the frequency of peanut allergy in high-risk children i.e. children with severe eczema, allergies to eggs, or both (Du Toit et al 2015).

Based on available studies, it is not possible to say anything about which doses of protein have caused sensitization, as there are no analyses of, for example, creams for peanut protein that correlate quantity with sensitization. The quantity of protein in house dust cannot be translated into exposure doses.

Teuber et al (1997) examined peanut oils for protein content and showed that two highly refined oils had a content of 3.0 and 5.7 µg protein/ml, respectively. Two low-grade refined oils contained 10.5 and 10.7 µg protein/ml. The quantities correlated with the degree of binding to IgE from patients with peanut allergy. In a similar study, Olszewski et al (1998) measured the protein concentration in commercial peanut oils and found 0.1-0.2 µg protein/g. Blom et al (2017) cite results from an unpublished study in which highly refined peanut oil that meets FEDIOL (the European Vegetable Oil and Proteinmeal Association) standards was examined. The results were an average protein concentration in refined peanut oil of 0.69 mg peanut protein/kg (0.69 µg/g) based on 22 samples containing 0.070 to 1.756 µg/g.

Data on the protein content of peanut oil thus vary from 0.1-10.7 µg/g. Unfortunately, these data cannot be linked to the clinical studies.

6.2.2 Acid hydrolyzed wheat gluten can break oral tolerance to wheat

Gluten can be hydrolyzed with enzymes or by treatment with acid during heating. Acid hydrolysis causes different degrees of deamidation and changes the physicochemical properties in a way that makes the product suitable as an emulsifier usable in the cosmetics and food industries (Kroghsbo et al 2014, Ballegaard et al 2021).

In 2002, Pecquet et al. described a case of urticaria (hives) in an atopic woman who had used a skin cream containing hydrolysed wheat protein. The woman subsequently developed generalized urticaria after eating a food with hydrolysed wheat protein from the same producer. The woman could tolerate food products with ordinary wheat. Although the route of sensitiza-

tion is not known, the authors suggest that the chronology of reactions is in favour of cutaneous sensitization. Other descriptions of cases followed, but it was not entirely clear how the sensitization had occurred or what type of hydrolysed gluten was involved (Leduc et al 2003, Lauriére et al 2006). Cases have subsequently been reported of allergic reactions to foods with acid hydrolysed gluten from Finland (Pelkonen et al 2011) and Denmark (Christensen et al 2018). The route of sensitization was not known in these cases either, but it has been characteristic of the European cases that the persons could tolerate ordinary wheat products. IgE from people who are allergic to acid hydrolysed wheat can bind to deamidated epitopes (glutamine to glutamic acid) in gluten, and the binding rate increases with increased degree of deamidation (Denery-Papini et al. 2012).

Reports from Japan described IgE-mediated reactions to a facial soap containing acid-hydrolysed gluten and food allergy to common wheat in adults (Fukutomi et al 2011, Chinuki et al 2011, Nakamura et al 2013). The soap had been used by a large number of people, and an epidemiological study showed a correlation between use of the soap and wheat allergy (Fukutomi 2014). In the Japanese cases, it was clear that the sensitization had occurred via the skin. The combination of acid hydrolysed gluten and detergent in the soap may have enhanced the sensitization, but the length of exposure is also a contributing factor. The patients described by Fukutumi et al (2011) had been using the soap for 1-3 years before onset of the food-related symptoms. It is characteristic of the Japanese cases—relative to the European cases—that the tolerance to common wheat is broken.

Cosmetic Ingredient Review, CIR (2014), describes the content of hydrolysed wheat gluten in cosmetic products up to 0.09 per cent (900 µg/g). The Japanese soap contained 0.3 per cent (3 mg/g) acid hydrolysed gluten (Fukutomi et al 2014), i.e. 1 g of soap gave an exposure of 3 mg of acid hydrolysed gluten.

6.2.3 Milk

Bruusgaard-Mouritsen et al (2020) have reviewed the literature describing reactions to food proteins in the skin. They describe four cases of type I reactions to milk proteins where the previous history suggests that sensitization to milk has occurred via the skin. Two of the cases concern infants (aged 11 and 12 months). The other two cases are women aged 16 and 35.

6.2.4 Oats

Creams containing oats improve the skin barrier and are used in the treatment of eczema. They may cause both types I and IV reactions after skin exposure (Bruusgaard-Mouritsen et al 2020). Bruusgaard-Mouritsen et al (2020) describe two cases of type I sensitization and four cases of combined types I and IV sensitization. One of the cases described—a woman of 33 years—developed symptoms in her mouth after oral consumption of oats.

6.2.5 Soy

Soy protein is used in various cosmetic products. Two cases of Type I allergy to soy protein after skin exposure have been described. In one of these cases, a 30-year-old woman developed severe allergic symptoms after eating soy products (Bruusgaard-Mouritsen et al 2020).

6.2.6 Subsidiary conclusion

There are two well-described associations between skin exposure to food allergens and food allergy. One in young children (peanuts) and the other in adults (acid hydrolysed gluten). The children—who had atopic dermatitis and did not have oral tolerance to peanuts—have probably been sensitized by low doses of peanut protein, and both the condition of their skin and the lack of oral tolerance have been of essential importance (Brough et al 2015, Du Toit et al 2015). Data on the correlation between the dose of peanut protein, frequency, and duration of exposure and the risk of sensitization cannot be derived from these studies.

That skin sensitization may lead to food allergy if oral tolerance has not been established has been formulated as “the dual allergen exposure hypothesis" (Lack 2012), which is assumed to apply to food allergens in general.
In the Japanese studies of skin exposure to acid hydrolysed gluten, the subjects were adults who are assumed to have been tolerant to wheat and thus gluten. The descriptions indicate that the skin had not been damaged before use of the soap began, which means that the initial contact had been on intact skin. It is not possible to calculate an exact dose/unit area, but the content of acid hydrolysed gluten in the soap suggests an exposure dose that may be 1,000 times higher than the exposure with protein from peanut oil that has sensitized young children with eczema. This suggests that a higher dose is needed to break a previously established tolerance. In addition, the new epitopes formed after acid hydrolysis also play a role (Ballegaard 2021).

6.3 Animal studies: Elicitation

Data on elicitation—which will typically be anaphylactic shock after oral exposure—are discussed in the section on sensitization. In keeping with the text on elicitation in humans, it is unlikely that data on elicitation after skin exposure in animals will help us understand sensitization doses.

6.4 Animal studies: Sensitization

By way of introduction, it is important to establish that the animal studies that examine skin sensitization with food allergens are not designed for risk assessment purposes. They are typically conducted to examine whether skin sensitization with proteins is possible, which conditions are important, and the consequences for food allergy. Therefore, there are many thoughts on how such experiments can be designed, and this makes it difficult to compare them. The recognition that skin sensitization may have consequences that extend beyond the skin and the importance of oral tolerance is relatively new (Lack et al. 2003). This may explain why science is still preoccupied with mechanisms rather than with considering how to perform risk assessments.

6.4.1 Factors to be included in assessment of animal models for skin sensitization with proteins

6.4.1.1 Animal models for skin sensitization with food proteins

The first study to show that the application of food protein to the skin of mice could lead to the formation of specific IgE—a so-called Th2 response—dates from 1996 (Wang et al. 1996). Since then, there have been a large number of studies on animals that examine sensitization with proteins via the skin. Primarily mice have been used in trials, but there has also been use of rats, guinea pigs, and dogs (Spergel et al. 1998; Fallon et al. 2009; Dunkin, Berin and Mayer, 2011; Marsella, 2015; Matsunaga et al., 2015; Ballegaard et al., 2019). Many different experimental designs have been used by many different research groups. The individual groups have usually conducted a limited number of trials with the same design, which makes it difficult to compare the conditions needed for sensitization to occur.

6.4.1.2 Food—food proteins

There are only trials with three different foods where we have information about the dose response. These are: 1. Ovalbumin (OVA) from chicken eggs, which has been used as a model substance by many research groups, 2. whey protein concentrate and hydrolysates of whey protein from cow’s milk, and 3. gluten from wheat and hydrolysates of gluten (see section 6.4.2).
Not all proteins appear to sensitize equally effectively. Comparison of different foods has shown that extract from peanuts or cashew nuts is better at sensitizing than extracts from soy or green beans (Tordesillas et al 2014).

Both pure proteins and protein extracts can thus sensitize, but the importance of matrix is not clear. In one study, Wawrin et al (2014) showed that exposure to milk protein resulted in beta lactoglobulin (BLG)-specific IgE, but exposure to pure BLG did not, meaning that this involved an effect caused by the other components in the milk.

6.4.1.3 Animals

The dose-response studies use Balb/c mice, which is the mouse strain that gives the highest response in oral trials with food allergens (Smit et al 2011), or Brown Norway rats, which are also used to study food allergy in other contexts, as this is a so-called high IgE responder strain (Ballegaard 2019).

6.4.1.4 Feed

The animals' feed is an important parameter in terms of knowing whether the animals are naive or tolerant to the food being used for sensitization. There is no information about the feed in the trials with OVA, but as eggs are not included in ordinary feed for mice, it can be assumed with a high probability that the animals have not been given eggs in their feed and that they are therefore naive in relation to eggs. In the study with whey proteins, it is specified that the animals are given milk-free feed. In the trials with gluten proteins, the animals are either on gluten-free feed or on gluten-containing feed, and they are thus either naive or tolerant in relation to gluten. The trials showed that oral tolerance protected against the development of allergy to gluten, which supports the importance of being exposed to a food by mouth, so that oral tolerance can be established before skin exposure to the food (Ballegaard et al 2019, 2021, Larsen et al 2022).

6.4.1.5 Condition of the skin

Atopic dermatitis in humans is a known risk factor for the development of food allergy (Martin et al 2015), just as mutations in the gene that codes for filaggrin—a protein essential in maintaining a normal skin barrier—have also been associated with the development of food allergy (Brown et al. 2011).

The condition of the skin is therefore a decisive factor in the animal trials. It is not always clear in the descriptions of the trials what has been the condition of the animals' skin before and during exposure to allergens.

In the trials in which the substance is applied to the body, it is necessary to cut off the hair and repeat this regularly, for example once a week. This is typically done with an electric cutter. Depilatory cream has also been used in some studies.

Damage to the skin may increase sensitization. So-called 'tape stripping'—where ordinary tape is used 3-10 times to remove the outer layer of the skin—affects the condition of the skin and promotes cytokine production in the skin. It is used as a surrogate for scratching with nails, which occurs frequently in atopic dermatitis (Oyoshi et al., 2009).

Both tape stripping and the use of depilatory cream may damage the skin and cause inflammation with increased production of cytokines (Wavrin et al 2014, Galand et al 2016, Pazos-Castro et al 2022).

Another way to affect the skin is to apply the Vitamin D analogue MC903, which causes atopic dermatitis-like skin changes (Noti et al 2014).

The use of patches for application of foods may also affect the skin. Spergel et al (1998) found that—after three weeks with patches—the skin had thickened with inflammation in the animals dosed with OVA, but not in the animals dosed only with saline. Iwamoto et al (2019) also find histological changes in the skin after tape stripping and occlusion. They assume that tape stripping activates Langerhans cells in the skin that promote the absorption of protein from the surface in parallel to what occurs in atopic dermatitis (for further elaboration, see section 1.5).

6.4.1.6 Application and occlusion

It is important that the animals cannot ingest the food.. This probably explains why many studies use patches that are attached for up to weeks at a time. When the substance is applied using patches that remain on the skin for a long time, the permeability of the skin—and thus the absorption of the substance—will be affected (see section 6.4.1.5).

6.4.1.7 Duration and dose response

Both the dose and dosage duration are of importance. In a study with sesame in mice, Navuluri et al (2006) showed that 5 µg/mouse (0.05 mg/ml) could not measurably induce specific IgG1 and IgE. 50 µg/mouse (0.5 mg/ml) only induced IgG1, and 500 µg/mouse induced both an IgG1 and an IgE response (5 mg/ml). In addition, there were clear signs that duration played a role. Others have found similar results (Birmingham et al. 2007, Parvataneni et al. 2009).

6.4.1.8 Post-immunization

Immunization after skin sensitization can be used to further stimulate the immune system and thus make it easier to measure the skin sensitization effect. In various trials, post-immunization has been done in airways and gastrointestinal tract (oral) (Morita et al 2012, Tordesillas et al 2014, Ballegaard et al 2019). Oral post-immunization is most relevant in the current context.

6.4.1.9 Detection of sensitization

Most studies use ELISA methods for detection of specific IgE and IgG1 in the blood as measures of sensitization. In addition, for example Th2 cytokines are measured after stimulation of cells from lymph nodes or spleen. In some studies, anaphylactic shock is measured after provocation to determine that the measured specific IgE is functional, i.e. can trigger an allergic reaction.

6.4.2 Assessment of selected studies

Below are included studies for which information has been provided about doses per area unit and where several doses have been examined, thus constituting information about dose response. This concerns studies with 1. OVA from chicken eggs, 2. whey protein and whey protein hydrolysates from cow's milk and 3. gluten and modified forms of gluten. The three studies examining the lowest doses are reviewed below.

6.4.2.1 Ovalbumin (OVA)

Wang et al (1996) are the first to use a food to examine skin sensitization with protein in mice. The underlying question is whether proteins in latex may cause skin sensitization. Until then, the assumption had been that the hydrophobic barrier of the skin and the size of the proteins mean that proteins cannot cause skin sensitization.

The study was conducted in Balb/c mice, which were shaved on their back. OVA was applied with a patch that remained for a week at a time and was renewed after 4 days. This procedure was repeated 2x at an interval of 2 weeks or 5x. The patch was kept in place with a bandage. The doses were 10,000, 100, or 10 µg/cm^2 in the 2x trial. Specific IgE was detected in the blood as well as T cell and cytokine production after stimulation of cells from the regional lymph node, which showed that this is a type I allergy. The IgE level was the same in the three dose groups. In the 5x trial, the animals were dosed with 10,000, 100, or 1 µg/cm^2. The animals dosed with 10,000 and 100 µg/cm^2 formed specific IgE. 1 in 6 animals dosed with 1 µg/cm^2 developed a low, but measurable IgE response. There is no statistical analyses of the results, but the response in the low group is most likely not statistically significant. The authors conclude that the duration of the exposure period is of significance to which dose causes sensitization. They demonstrate that there is lymphocyte infiltration in the skin after exposure, which is in conformity with current knowledge that long-term exposure with occlusion induces atopic dermatitis -like changes in the skin (Iwamoto et al 2019).

The result of this study is that OVA may induce a specific IgE response in animals with a condition of the skin similar to atopic dermatitis. The dose 10 µg/cm^2 gives a clear positive response after dosage for 2 weeks. The dose of 1 µg/cm^2 is on the edge of sensitizing after dosage for 5 weeks. Based on this study, it is therefore not possible to determine a No Expected Sensitizing Induction Level (NESIL). The lowest effect level (LOAEL) is between 1 and 10 µg/cm^2.

6.4.2.2 Whey protein concentrate (VPC) and hydrolysates of VPC

Iwamoto et al (2019) have examined the sensitizing effect of whey protein and two whey protein hydrolysates. The study was conducted in Balb/c mice on dairy-free feed, 8 animals per group, which were shaved on their backs and tape stripped six times. The substances were applied to two 1 cm^2 large patches kept in place with tape that remained on for one week at a time with an interval of 2 weeks either 2x or 3x. The dose was 3 µg in total, 1.5 µg/cm^2. The results of the main trial with 2x dosage were increased BLG-specific IgE in the blood, basophilic activation, cytokine production, and increased concentration of mast cell protease in the blood and anaphylaxis after provocation. Histology after 3x dosage showed an increased number of eosinophils in the skin, and an analysis of the blood showed further increased BLG-specific IgE relative to the 2x dosage. All these changes were only seen in the group dosed with whey protein concentrate and not in the groups with the 2 hydrolysates. The authors conclude that the tape stripping used has damaged the skin's outer layer—the stratum corneum—and induced migration of Langerhans cells similar to that seen in atopic dermatitis.

The result of this trial is that whey protein concentrate (WPC) may induce a BLG-specific IgE response in animals where the condition of the skin is similar to atopic dermatitis. The dose of 1.5 µg/cm^2 gives a clearly positive response after 2 weeks of dosage. BLG accounts for 33 per cent of the WPC used, and the dose has therefore been 0.5 µg BLG/cm^2. As it cannot be derived from the trial whether it is BLG alone that has triggered the response or whether the other whey proteins contribute to the sensitization, the dose of 1.5 µg WPC/cm^2 is used in the further analysis. Whey makes up 20 per cent of the protein fraction in milk (Maryniak et al 2022). If the exposure with WPC is to be converted to the corresponding exposure with milk protein, it will be 5 x higher = 7.5 µg milk protein/cm^2. This dose does not take into account any sensitizing effect of casein, which constitutes 80 per cent of the proteins in milk. There is no effect of dosing with the hydrolysates. We do not know the quantity of intact protein in the hydrolysates, but PHW1 contains 2 per cent protein with a molecular weight > 5000 Dalton and PHW2 4 per cent protein with a molecular weight > 5000 Dalton. Based on this information, it is not possible to calculate the quantity of intact protein and thus a NESIL for intact protein. It is therefore not possible to establish a NESIL on the basis of this study. LOAEL is 7.5 µg milk protein/cm^2.

6.4.2.3 Gluten and hydrolysates of gluten

Ballegaard et al (2019, 2021) have used Brown Norway (BN) rats naive to gluten to examine sensitization with gluten and hydrolysates of gluten. The animals were shaved on their belly and the skin was scraped lightly with sandpaper before the substance was applied and covered with a bandage for an hour to avoid that the animals were exposed to the substance orally. After an hour, the dressing was removed, and the dosed piece of skin was washed. The dosage is thus the dose absorbed into the skin over the course of an hour. This was repeated 3 days a week for 5 weeks. In Ballegaard et al. (2021), the animals were dosed with 5, 50, or 500 µg/cm^2. After skin dosage, the animals were dosed with a stomach tube with gluten 2x with an interval of 1 week.
Gluten: Specific serum IgE after 50 or 500 µg/cm^2 with dose response.
Enzyme hydrolyzed gluten: Specific serum IgE after 5, 50, or 500 µg/cm^2 with dose response. 5 in 8 animals dosed with 5 µg/cm^2 have low, but measurable, IgE.
Acid hydrolyzed gluten 1: Specific serum IgE after 50 or 500 µg/cm^2 with dose response.

Acid hydrolyzed gluten 2: Specific serum IgE after 50 or 500 µg/cm^2 with dose response.
Acid hydrolyzed gluten 3: Specific serum IgE after 50 or 500 µg/cm^2 with dose response.

There is increased fluid loss over the skin right after scraping with sandpaper—as also seen in atopic dermatitis —but no cell infiltration, i.e. no inflammatory changes (Larsen et al 2022).

Based on this study, NESIL for gluten and the three acid hydrolyzed glutens is 5 µg/cm^2.
LOAEL for enzyme hydrolyzed gluten is 5 µg/cm^2.

6.4.2.4 Summary animal studies

The three dose response studies have all been conducted in animals that are naive in relation to the allergens being studied. Two of these have been performed with prolonged occlusion that causes skin changes similar to atopic dermatitis. In the third study, the skin changes are minor. In this study, the effective dosage period is one hour 3x per week for 5 weeks, followed by stimulation of the immune system in the gastrointestinal tract.
Larsen et al (2022) have compared the influence of different skin conditions on sensitization in the same model as Ballegaard (2019, 2021). The conclusion drawn in this study is that skin scraped with sandpaper and skin with changes similar to irritative contact allergy (dosage with SLS) or atopic dermatitis (dosage MC903) do not affect the degree of sensitization relative to intact skin. 500 µg/cm^2 is dosed in this study. It is not to be known whether the conclusion would be the same at lower doses.

Although the trials have been conducted differently and the substances are different, it seems that LOAEL is of the same magnitude, i.e. less than 10 µg/cm^2.
Gluten and the acid hydrolyzed glutens have a NESIL of 5 µg/cm^2 after postimmunization with gluten.

Data that can be incorporated in the risk assessment are:
LOAEL for milk protein of 7,5 µg/cm^2 and no NESIL.
There are no data on almond protein. If we use data from OVA and whey in the risk assessment of almond protein, we have a LOAEL of between 1 and 10 µg/cm^2 and no NESIL.

Data from the trials with gluten are not used for risk assessment of almond as gluten consists of a group of proteins that are very large and have very special physicochemical properties that do not resemble the proteins in almond.

6.5 Conclusion on analysis results for use in exposure assessment

6.5.1 Milk

The MST-04 sample is a cream with hydrolysed milk protein. As the ELISA method has been developed to test intact protein and the degree of hydrolysis is not known, the result cannot be used to determine the quantity of milk protein.
Several of the products showed low recovery of the added spike protein (see TABLE 19). It is therefore difficult to generalize about the extent to which it has been possible to determine the quantity of the milk protein present by means of the ELISA method used. It is therefore assumed that the values found are minimum values. However, it should be noted that the results from the recovery trial (Table 14) showed good recovery of milk protein in oil and cream.
The analysis results (TABLE 19) from three soaps (MST-19, MST-32, MST-33), three creams (MST-25, MST-36, MST-37) and one shampoo (MST-29) are included in the risk assessment.

6.5.2 Almond

There is a high to very high recovery of almond protein in all product types, which makes the results credible in terms of the possibility of detecting almond protein and the measured content. All results except one are below the limit of detection (TABLE 20). There is a product MST-06—a cream—with a measurable almond protein content, and this result is included in the risk assessment.

6.5.3 Soy

The sample MST-08 is a cream with hydrolysed soy protein and MST-40 is a shampoo with modified protein (hydrolysed and other chemical modification). As the ELISA method has been developed to test intact protein and the degree of hydrolysis is not known, the result cannot be used for a quantitative assessment.

There is high to very high recovery of soy protein in all other product types, which makes the results credible in terms of the possibility of detecting soy protein and the measured content. All samples are below the limit of quantification (2 ppm) and close to the limit of detection of the method (0.2 ppm) (TABLE 21). All samples are therefore regarded as not containing measurable quantities of soy protein and are therefore not included in the risk assessment.

6.6 Exposure based on analysis data

6.6.1 Exposure scenarios for the selected products:

Table 22 below shows an assessment of infants' exposure to the products analysed which contained a measurable concentration of food protein (see TABLE 19). As no measurable quantities of soy protein could be detected in the selected products, a risk assessment has only been performed for milk or almond content.

TABLE 22 Overview of products used in the risk assessment

Product ID	Category	Contents	Protein concentration µg/g (ppm)
MST-06	Cream (whole body)	Almond: Oil (UM)	43.07
MST-19	Soap (shower gel)	Milk: Protein (UM)	36.09
MST-25	Cream (whole body)	Milk: Whole (UM)	10.07
MST-29	Shampoo	Milk: Whole (UM)	3.91
MST-32	Soap (shower gel)	Milk: Whole (UM)	250.54
MST-33	Soap (shower gel)	Milk: Whole (UM)	101.66
MST-36	Cream (whole body)	Milk: Whole (UM)	4.36
MST-37	Cream (whole body)	Milk: Protein (F)	121.71

The above creams are all body lotions aimed at babies or people with dry skin/atopic dermatitis. It is assumed that it is most likely that the selected creams are used on the whole body, including in the diaper area, where the skin is occluded and may be damaged (see SCCS' Notes of Guidance).

6.6.2 Exposure calculation method

The exposure assessment for the selected cosmetic products is based on the latest edition of the SCCS' Notes of Guidance from 2021 (SCCS/1628/21).

Exposure to food proteins through the skin is calculated as dose/area exposed skin.
The external dermal exposure is calculated per product using the following formula:
$E_{dermal}\ x = C_x\ X\ q_x\ X\ f_{ret}\ x$

E_{dermal} x (µg/day):	Daily dermal exposure from product x
X:	product
C_x (µg/g):	The concentration of ingredient in product x
q_x (g/day):	Amount of product used per day
f_{ret} x:	Retention factor for product x

For leave-on products such as cream, a retention factor of 1 is used. For rinse-off products (shampoo and soap), a retention factor of 0.01 is used in accordance with to the SCCS' Notes of Guidance.
The daily dermal exposure is divided by the exposed skin area.
The exposure scenarios have been created for children who have not previously been exposed orally to cow's milk or almond, respectively, and where the dermal exposure thus constitutes the first exposure. The intake of cow's milk for Danish children is described in section 4 of the report and shows that 55 per cent of infants at 4 months of age are fully breastfed. Just over half of the infants have therefore not yet been exposed to cow's milk via their diet (infant formula or foods) at 4 months of age. Therefore, all exposure scenarios have been calculated for a child aged 4 months.
According to the dietary survey, tree nuts (including almonds) become part of the diet much later. As can be seen from section 4.2.4 Figure 5, less than 10 per cent of infants aged 6-7 month ingest tree nuts. At 12 months of age, the proportion of tree nut eaters is >50%.
It is presumed that the group of infants who are at the highest risk of developing allergy to either milk or almond are those who are exposed to the highest total dose of milk or almond/area exposed skin, respectively. The estimated dose is dependent on the concentration in the analysed products as well as on the quantities of cosmetic products used on the children.

6.6.3 Data basis for infants' exposure to the selected cosmetic products.

An attempt has been made to estimate, using relevant guidance documents, the quantity of the selected cosmetic products used on infants. According to SCCS' Notes of Guidance, comprehensive exposure data on infants' exposure to cosmetic products representative of the entire European population are not available in the open literature. References to recent studies (from 2015-2020) examining how large the quantities are that are used on infants, within specific cosmetic product categories are referenced in the SCCS' Notes of Guidance in Table A.7. There are, for example, original studies on French children (Ficheux et al. 2016 and 2019, Gomez-Berrada et al. 2017a) as well as a meta-study on young children from countries in and outside Europe (Gomez-Berrada et al. 2017b). In addition, there are a small number of studies from the USA and Asia. For the majority of the studies, the young children cover an age group range from 0-23/24 months, and it is therefore difficult or impossible to derive specific data for infants.
It is assessed that the most representative values regarding the quantity and frequency of the selected product categories shampoo, soap, and cream (for the whole body) used on young children can be found in the study conducted by Gomez-Berrada et al. 2017a because this study describes European conditions and because it is possible to derive used quantities of the cosmetic products on young children. The study examined the exposure of young French children to 7 frequently used cosmetic products aimed at young children under realistic conditions. 78 young children participated in the study, which was conducted in 2010-2011. The products were dispensed at the start of the trial and the families themselves reported the frequency of use, while the quantity of the products was measured. Based on this study, it is possible to deduce how large quantities of the relevant product categories soap, shampoo, and cream were used on the 50 per cent percentile of infants aged 0-5 months.

Table 23: Quantities of cosmetics used for children aged 0-5 months*

Product	Percentile	quantity	age:	comment
Soap (shower gel, body)	P50	4.5 g	0-5 months	Per time
Shampoo	P50	2.7 g	0-5 months	Per time
Cream	P50	1.0 g	0-5 months	Per time

*From Gomez-Berrada et al. 2017a

The 95th percentile (P95) of the children studied had their hair washed and were washed with soap (shower gel) every day. Half (P50) had cream applied every day, while the 95th percentile had cream applied twice a day. It is assumed in the exposure assessments that cream is applied to the children twice daily because some of the products are meant for dry or red skin, and 2 daily applications are recommended for children with atopic dermatitis (atopiskeksemforening.dk).

6.6.4 Exposure scenario for use of the selected products in the diaper area.

The skin in the diaper area is particularly prone to becoming red and irritated. In connection with the transition to solid food from the age of 6-12 months, the risk may be greater, and this could be relevant in connection with dermal exposure to almond, but redness and irritation in the diaper area also occur in younger children.
One of the products (MST-25) is meant for use on damaged skin/atopic dermatitis, and it must be assumed as realistic that it may be used exclusively in the diaper area (see scenario 5).
The ECHA's consumer exposure guidance (ECHA Guidance, Chapter R.15) recommends the digital consumer exposure program ConsExpo, developed by RIVM. Data used in the program can be found in RIVM's Cosmetics Fact Sheet (RIVM report 320104001/2006), among other sources, and here a default value is given for the quantity of baby cream/ointment for use in the diaper area of 0.27 g per application and a frequency of 2 applications per day. These values have been used in exposure scenario 4. There are no data in RIVM's Cosmetics Fact Sheet on the quantities of cream used on the whole body of young children.

Anatomical data for infants:
Full body area and head:
Default data from RIVM's general fact sheet (Biesebeek et al 2014) for an infant aged 3-6 months are used based on the size of a 4.5-month-old baby corresponding to a total body area of 3,400 cm^2 and head (incl. neck) corresponding to 18.2 per cent of total body area (620 cm^2) (Biesebeek et al. 2014).
The diaper area:
RIVM's general fact sheet (Biesebeek et al 2014) states a default value of 190 cm^2 for the diaper area of a 4.5-month-old baby.

Exposure scenario 1:
Cream containing Almond (4.5-month-old baby):
Body lotion with almond oil containing 43.07 µg almond protein/g
1 g of cream with 43.07 µg/g almond protein with a retention factor of 1 is applied twice daily to the whole body corresponding to 3400 cm^2. (1g x43.07 µg/g x1 RF x 2)/3400 cm^2.
This gives a dose of 0.025 µg/cm^2.
Exposure scenario 2:
Soap + cream with the highest measured concentrations of milk (4.5-month-old baby):
A worst-case scenario for the whole body has been calculated for a 4.5-month-old baby who has soap with the highest concentration of milk protein (250.54 µg/g) applied all over the body followed by cream with the highest measured concentration of milk protein (121.71 µg/g) all over the body.

Soap: (4.5 g x 250.54 µg/g x0.01 RFx1)/3400 cm^2= 0.003 µg/cm^2
Cream: (1 g x 121.71 µg/g x1 RFx2)/3400 cm^2 = 0.072 µg/cm^2
Total exposure: 0.003 µg/cm^2+ 0.07 µg/cm^2= 0.075 µg/cm^2.
Exposure scenario 3:
Shampoo with the highest measured concentration of milk.
The scenario has been calculated for a 4.5-month-old baby. As the skin on the scalp is not exposed to either soap or cream, the calculated local exposure has not been added up with the calculated whole-body exposure for exposure scenario 2. The exposed skin area is calculated as half the skin area on the head (RIVM Cosmetics Fact Sheet). That is half of 620 cm^2.
Shampoo: (2.7 g x 3.91 µg/g x0.01 RFx1) /310 cm^2= 0.0003 µg/cm2
Exposure scenario 4:
Exposure exclusively in the diaper area
For this scenario, the product (MST-25) has been used, which is the most likely product to be used solely in the diaper area. 0.27 g cream with 10.07 µg/g whole milk (UM) is used twice a day in the diaper area corresponding to 190 cm^2. This gives a dose of 0.029 µg /cm^2.
If a worst-case scenario is prepared for cream that is used solely in the diaper area and using the cream with the highest concentration of milk protein (121.71 µg/cm^2), the dose would be 0.346 µg /cm^2.

6.7 Assessment of the sensitizing properties of cosmetic products

Many chemical substances can be sensitizing in connection with skin exposure, and some of these are used in cosmetics such as preservatives and fragrance substances. These are small molecules (haptens) that are to bind to the skin's proteins in order to be sensitizing. This results in a so-called type IV reaction, where the sensitization occurs in the regional lymph node. Antibodies are not developed, but T cells, which are responsible for the subsequent allergic reaction. Risk assessment of new chemicals typically uses data from an animal model—Local Lymph Node Assay (LLNA) (OECD Guideline TG 429)—which has been developed to predict the ability of a chemical to sensitize and its potency. The chemical (3 doses) is applied to the ears of mice on day 0, 1, and 2, and radioactively labelled thymidine is injected on day 5. The regional lymph node is removed, and the degree of proliferation in the lymph node, which is a measure of sensitization, is measured using the incorporation of radioactively labelled thymidine. The dose that results in proliferation that is 3x higher than the background is referred to as LC3. The choice of LC3 as the dose used to assess potency is empirical, based on knowledge of the potency of chemicals from human data. LLNA thus gives a picture of the potency of the chemical, but the result cannot be translated into which dose will sensitize humans. The QRA2 method is an attempt to translate these data—based on short-term exposure of a few mice—for use in assessing the risk from long-term human exposure from various sources.
In 2017, SCCS published a scientific assessment of the QRA2 method (SCCS/1589/17). Their conclusion was that it is not yet possible to use the QRA2 method to determine a NESIL for contact allergy to fragrance substances, because several aspects of the method are not clear and as the scientific rationale behind the method should be described better. According to SCCS, the QRA2 method can, if revised, be used for safety assessment of perfume allergens, and potentially also for other cosmetic ingredients.
One of SCCS's points of criticism of the QRA2 method was that there was no rationale for the size of the uncertainty factors used (in QRA2 called SAFs) for taking into account the duration of exposure and variability in the population (intraspecies variation). The QRA2 method uses a 'default value' of 10 for intraspecies variation, which is also used in many other regulatory contexts, including in ECHA's guidelines (Guidance on information requirements and chemical safety assessment Chapter R.8: Characterisation of dose[concentration]-response for human health). However, the data basis for QRA2 showed indications that the intraspecies variation in people who developed contact allergy to fragrance substances was higher than 10.

It is not possible to use the QRA2 method in the risk assessment of food proteins in cosmetics. The same applies to being able to perform a classic risk assessment with determination of DNEL.
No validated experimental methods have been developed for assessment of the ability of proteins to sensitize via the skin. There are thus no comparable data that take into account the condition of the skin, the duration of exposure, and dose.

6.8 Risk assessment of food proteins in cosmetic products for children

As can be seen from the above, there is a risk of skin sensitization to food proteins among children with atopic dermatitis, where oral tolerance has not been established. Based on data from human studies or data from animal studies, it is not possible to determine which doses (µg protein/cm^2) have a sensitizing effect. The precautionary principle (EU 2000) therefore warrants that food proteins or food protein-rich ingredients (e.g. milk powder) should not be added to cosmetic products for children having an age where oral tolerance to the proteins cannot be expected. Oral tolerance has been established when the children are introduced to the foods, typically during the first two years of their life (see section 4).

Studies in animals support the importance of the protective effect of oral tolerance. They also show that food proteins can sensitize over the skin in low doses, down to about 1 µg protein/cm^2. As mentioned above, it is not possible to assess how low a dose will be sensitizing for people without oral tolerance, who will typically be young children. The results from this report have shown that it is possible to detect food proteins (milk, almond) in cosmetic products marketed for children or where it is likely that the products will be used for children. Based on the calculated exposure scenarios, it can be seen that the doses to which young children may be exposed from the analysed products are in the magnitude of 0.0003-0.075 µg protein/cm^2 for shampoo, and soap and cream used for the whole body. If we compare with the worst case exposure in scenario 4 for cream used solely in the diaper area, the estimated dose of milk protein is 0.346 µg /cm^2, which is close to the dose that has a sensitizing effect in animal trials.

Oils extracted from foods contain varying quantities of protein depending on the extraction method used. Oils for use in cosmetic products for young children should contain as little protein as possible. Highly refined oils have the lowest protein content. Highly refined soy oil contains 0.062-0.265 µg soy protein/g (Rigby et al 2011). In comparison, highly refined peanut oil (data from the same laboratory) contains 0.070 to 1.756 µg peanut protein/g (Blom et al 2017). The project found 36 cosmetic products having peanut oil as ingredient. None of these were aimed at children or specific skin types.

Not all proteins are equally effective at sensitizing over the skin. In a trial on mice, the mice could be sensitized with protein from peanuts or cashew nuts, but not with protein from soybeans or peas (Tordesillas 2014). These studies indicate that the consequence of protein residues in oils will be different and depend on whether the oil originates from a food with potent food allergens. In addition, it is also crucial whether the protein residues consist of allergens.

6.9 Conclusion and perspectives

The available knowledge does not make it possible to set limits on the quantity of food protein that cannot sensitize young children.

Animal studies show that food proteins can sensitize in low doses. It has not been possible to determine NESIL for the relevant foods. Even if it had been possible to determine NESIL, it would not have been possible to translate this knowledge into risk to humans. There are no data to support this.

Oral tolerance or lack thereof is the most crucial parameter for sensitization with foods via the skin. This knowledge is based on studies of sensitization of young children and is supported by results from experimental studies in animals.

It is known that the condition of the skin and the duration of the dosage are of importance to sensitization, but there is a lack of knowledge about the influence of these parameters on the sensitizing dose. In addition, there is a lack of knowledge about the relationship between potency and dose. For example, does it take 50x as much soy protein to sensitize via the skin relative to protein from peanuts?

It is important that small children are not exposed to proteins on the skin in early life. The plant oils used in cosmetic products should have as low a protein content as possible and be derived from foods where the risk of developing allergy is the least. The project found a measurable amount of almond protein in one of 23 products with almond oil. None of the ten products with soy oil had a measurable amount of soy protein. This shows that it is possible to produce cosmetic products with low residues of protein from food.

If food proteins are modified—as seen in acid hydrolysis of gluten—this may result in sensitization to the new epitopes, but also breach of an established oral tolerance to non-modified epitopes. Whether this is a unique history that can only occur in connection with acid hydrolysis of gluten proteins is not known, but this knowledge should be taken into account in the assessment of the risk of modification of food proteins used in cosmetics.

There is great focus on the development of alternative protein sources for human nutrition, so-called novel foods. It should be considered, if these foods should be used in cosmetic products, before being introduced as food, as it is important that oral tolerance is established before the human population is exposed to new proteins via the skin.

7. References

Ballegaard ASR, Madsen CB, Bøgh KL. An animal model for wheat allergy skin sensitisation: a comparative study in naive versus tolerant Brown Norway rats. Int Arch Allergy Immunol. 2019;178:106-118.

Ballegaard, A. R., Castan, L., Larsen, J. M., Piras, C., Villemin, C., Andersen, D., Madsen, C. B., Roncada, P., Brix, S., Denery-Papini, S., Mazzucchelli, G., Bouchaud, G. & Bøgh, K. L. Acid Hydrolysis of Gluten Enhances the Skin Sensitising Potential and Drives Diversification of IgE Reactivity to Unmodified Gluten Proteins. Mol. Nutr. Food Res. 2100416 (2021) doi:10.1002/mnfr.202100416.

Bannon, G. A. What makes a food protein an allergen? Curr. Allergy Asthma Rep. 4, 43–46 (2004).

Biesebeek et al 2014: General Fact Sheet General default parameters for estimating consumer exposure - Updated version 2014 RIVM report 090013003/2014

Birmingham, N. P. et al. (2007) 'An adjuvant-free mouse model of tree nut allergy using hazelnut as a model tree nut.', International archives of allergy and immunology, 144(3), pp. 203–10. doi: 10.1159/000103993.

Blom MW, Kruizinga AG, Rubingh CM, Remington BC, Crevel RWR, Houben GF Assessing food allergy risks from residual peanut protein in highly refined vegetable oil Food and Chemical Toxicology 106 (2017) 306-313

Brough HA, Santos AF, Makinson K, et al. Peanut protein in household dust is related to household peanut consumption and is biologically active. J Allergy Clin Immunol 2013;132:630-8

Brough, H. A., Simpson, A., Makinson, K., Hankinson, J., Brown, S., Douiri, A., Belgrave, D. C. M., Penagos, M., Stephens, A. C., McLean, W. H. I., Turcanu, V., Nicolaou, N., Custovic, A. & Lack, G. Peanut allergy: effect of environmental peanut exposure in children with filaggrin loss-of-function mutations. J. Allergy Clin. Immunol. 134, 867-875. (2014).

Brough, H. A., Liu, A. H., Sicherer, S., Makinson, K., Douiri, A., Brown, S. J., Stephens, A. C., Irwin McLean, W. H., Turcanu, V., Wood, R. A., Jones, S. M., Burks, W., Dawson, P., Stablein, D., Sampson, H. & Lack, G. Atopic dermatitis increases the effect of exposure to peanut antigen in dust on peanut sensitization and likely peanut allergy. J. Allergy Clin. Immunol. 135, 164-170 (2015).

Brough, H. A., Nadeau, K. C., Sindher, S. B., Alkotob, S. S., Chan, S., Bahnson, H. T., Leung, D. Y. M. & Lack, G. Epicutaneous sensitization in the development of food allergy: What is the evidence and how can this be prevented? Allergy 75, 2185–2205 (2020)

Brown, S. J. et al. (2011) 'Loss-of-function variants in the filaggrin gene are a significant risk factor for peanut allergy', Journal of Allergy and Clinical Immunology. Elsevier Ltd, 127(3), pp. 661–667. doi: 10.1016/j.jaci.2011.01.031.

Bruusgaard-Mouritsen, M. A., Johansen, J. D., Zachariae, C., Kirkeby, C. S. & Garvey, L. H. Natural ingredients in cosmetic products—A suggestion for a screening series for skin allergy. Contact Dermatitis 83, 251–270 (2020).

Bøgh, K. L. & Larsen, J. M. Reducing Allergenicity by Proteolysis. Food Eng. Ser. 499–523 (2021) doi:10.1007/978-3-030-55482-8_19/FIGURES/1.

Chinuki Y, Murata S, Morita E. A case of wheat-dependent exercise-induced anaphylaxis sensitized with hydrolysed wheat protein in a soap. Contact Dermatitis 2011;65:55-7.

Christensen MJ, Skov PS, Poulsen LK, Bindslev-Jensen C, Mortz C (2018) Clinical relevance of sensitization to hydrolyzed wheat protein in wheat-sensitized subjects. J Allergy Clin Immunol;141, 802-805

CIR (2014) Cosmetic ingredient review. Safety assessment of hydrolysed wheat protein and hydrolysed wheat gluten as used in cosmetics. Tentative report March/June 2014.
Day, L., Augustin, M. A., Batey, I. L. & Wrigley, C. Wheat-gluten uses and industry needs. Trends Food Sci. Technol. 17 82–90 (2006).

Denery-Papini S, Bodinier M, Larré C, Brossard C, Pineau F, Triballeau S, Pietri M, Battais F, Mothes T, Paty E, Moneret-Vautrin DA. Allergy to deamidated gluten in patients tolerant to wheat: specific epitopes linked to deamidation. Allergy 2012;67:1023-32.

Dini, I. & Laneri, S. The New Challenge of Green Cosmetics: Natural Food Ingredients for Cosmetic Formulations. Molecules 26, (2021).

Du Toit, G., Roberts, G., Sayre, P. H., Bahnson, H. T., Radulovic, S., Santos, A. F., Brough, H. A., Phippard, D., Basting, M., Feeney, M., Turcanu, V., Sever, M. L., Gomez Lorenzo, M., Plaut, M. & Lack, G. Randomized Trial of Peanut Consumption in Infants at Risk for Peanut Allergy. N. Engl. J. Med. 372, 803–813 (2015).

Dunkin, D., Berin, M. C. and Mayer, L. (2011) 'Allergic sensitization can be induced via multiple physiologic routes in an adjuvant-dependent manner.', The Journal of allergy and clinical immunology, 128(6), p. 1251–1258.e2. doi: 10.1016/j.jaci.2011.06.007.

ECHA Guidance on Information Requirements and Chemical Safety Assessment Chapter R.8: Characterisation of dose [concentration]-response for human health (version 2.1 2012).

ECHA Guidance on Information Requirements and Chemical Safety Assessment Chapter R.15: Consumer exposure assessment (Version 3.0 July 2016).

EU Commission. Communication from the Commission on the precautionary principle (COM (2000) 1 final of 2 February 2000.

Fallon, P. G., Sasaki, T., Sandilands, A., Campbell, L. E., Saunders, S. P., Mangan, N. E., Callanan, J. J., Kawasaki, H., Shiohama, A., Kubo, A., Sundberg, J. P., Presland, R. B., Fleckman, P., Shimizu, N., Kudoh, J., Irvine, A. D., Amagai, M. & McLean, W. H. I. A homozygous frameshift mutation in the mouse Flg gene facilitates enhanced percutaneous allergen priming. Nat. Genet. 41 602–608 (2009).

Ficheux AS., Dornic N., Bernard A., Chevillotte G., Roudot AC. Probabilistic assessment of exposure to cosmetic products by French children aged 0-3 years, Food Chem Toxicol 94:85-92 (2016a). Doi: 10.1016/J.FCT.2016.05.02

Ficheux, AS., Gomez-Berrada, M. P., Roudot, A. C. & Ferret, P. J. Consumption and exposure to finished cosmetic products: A systematic review. Food and Chemical Toxicology vol. 124 280–299 (2019). doi: 10.1016/j.fct.2018.11.060

Fischer LA, Menné T, Voelund A, Duus Johansen J. (2011) Can exposure limitations for well-known contact allergens be simplified? An analysis of dose–response patch test data. Contact Dermatitis, 64, 337–342.

Fox, A. T., Sasieni, P., du Toit, G., Syed, H. & Lack, G. Household peanut consumption as a risk factor for the development of peanut allergy. J. Allergy Clin. Immunol. 123, 417–423 (2009).

Fukutomi Y, Itagaki Y, Taniguchi M, Saito A, Yasueda H, Nakazawa T, Hazegawa M, Nakamura H, Akiyama K. Rhinoconjunctival sensitization to hydrolysed wheat protein in facial soap can induce wheat-dependent excercise-induced anaphylaxis. J Allergy Clin Immunol 2011;127:531-3.

Fukutomi,Y, Taniguchi, M, Nakamura H, Akiyama, K. 2014 Epidemiological link between wheat allergy and exposure to hydrolyzed wheat protein in facial soap Allergy 69, 1405–1411 Galand, C. et al. (2016) 'IL-33 promotes food anaphylaxis in epicutaneously sensitized mice by targeting mast cells', Journal of Allergy and Clinical Immunology. Elsevier Inc., 138(5), pp. 1356–1366. doi: 10.1016/j.jaci.2016.03.056.

Gomez-Berrada MP., Ficheux S., Guillou S., Berge C., de Javel D., Roudot AC., Ferreta PJ., Consumption and exposure assessment to cosmetic products for children under 2 years old. Food Chem Toxicol 105:151-160 (2017). Doi: 10.1016/J.FCT.2017.04.011

Greenhawt, M. Food allergy quality of life and living with food allergy. Curr. Opin. Allergy Clin. Immunol. 16, 284–290 (2016).

Halken, S., Muraro, A., de Silva, D., Khaleva, E., Angier, E., Arasi, S., Arshad, H., Bahnson, H. T., Beyer, K., Boyle, R., du Toit, G., Ebisawa, M., Eigenmann, P., Grimshaw, K., Hoest, A., Jones, C., Lack, G., Nadeau, K., O'Mahony, L., Szajewska, H., Venter, C., Verhasselt, V., Wong, G. W. K. & Roberts, G. EAACI guideline: Preventing the development of food allergy in infants and young children (2020 update). Pediatr. Allergy Immunol. 32 843–858 (2021).

Hjorth N, Roed-Petersen J. Occupational protein contact dermatitis in food handlers. Contact Dermatitis 1976; 2:28–42.

Hussain, M., Borcard, L., Walsh, K. P., Pena Rodriguez, M., Mueller, C., Kim, B. S., Kubo, M., Artis, D. & Noti, M. Basophil-derived IL-4 promotes epicutaneous antigen sensitization concomitant with the development of food allergy. J. Allergy Clin. Immunol. 141, 223-234. (2018).

Höglund, L., Räisänen, J., Hämäläinen, A-M., Warholm, M., van der Hagen, M., Suleiman, A., Kristjánsson, V., Nielsen, E., & Kopp, T. I. (2012). Existing Default Values and Recommendations for Exposure Assessment - A Nordic Exposure Group Project 2011. Nordic Council of Ministers. TemaNord Vol. 2012 No. 505 https://doi.org/10.6027/TN2012-505

Iwamoto H, Matsubara T, Okamoto T, et al. Epicutaneous immunogenicity of partially hydrolyzed whey protein evaluated using tape-stripped mouse model. Pediatr Allergy Immunol. 2020;31:388–395. https ://doi.org/10.1111/pai.13211

Kanagaratham, C., Sallis, B. F. & Fiebiger, E. Experimental Models for Studying Food Allergy. Cell. Mol. Gastroenterol. Hepatol. 6, 356-369.e1 (2018).

Kroghsbo S, Andersen NB, Rasmussen TF, Jacobsen S, Madsen CB (2014) Acid hydrolysis of wheat gluten induces formation of new epitopes but does not enhance sensitizing capacity by the oral route: a study in “gluten free” Brown Norway rats. PLoS ONE 9 (9): e107137

Kulis, M. D., Smeekens, J. M., Immormino, R. M. & Moran, T. P. The airway as a route of sensitization to peanut: An update to the dual allergen exposure hypothesis. J. Allergy Clin. Immunol. 148, 689–693 (2021).

Lack G, Fox D, Northstone K, Golding, J. (2003) Factors Associated with the Development of Peanut Allergy in Childhood. N Engl J with 348, 977-985

Lack G. Update on risk factors for food allergy. J Allergy Clin Immunol 2012; 129:1187–97.

Larsen, J. M., Ballegaard, A. S. R., Dominguez, A. S., Kristoffersen, N. J., Maryniak, N. Z., Locke, A. V., Kazemi, S., Epstein, M., Madsen, C. B. & Bøgh, K. L. The role of skin inflammation, barrier dysfunction, and oral tolerance in skin sensitization to gluten-derived hydrolysates in a rat model. Contact Dermatitis (2022) doi:10.1111/COD.14233.

Lauriére M, Pecquet C, Bouchez-Mahiout I, Snégaroff J, Bayrou O, Raison-Peyron N, Vigan M. Hydrolysed wheat proteins present in cosmetics can induce immediate hypersensitivities. Contact Dermatitis 2006;54:283-9.

Leduc V, Moneret-Vautrin D-A, Guerin L, Morisset M, Kanny G. Anaphylaxisto wheat isolates: immunochemical study of a case proved by means of double-blind, placebo-controlled food challenge. J Allergy Clin Immunol 2003;111:897-9.

Li, X. M., Schofield, B. H., Huang, C. K., Kleiner, G. I. & Sampson, H. A. A murine model of IgE-mediated cow’s milk hypersensitivity. J. Allergy Clin. Immunol. 103, 206–214 (1999).

Longo, G., Berti, I., Burks, A. W., Krauss, B. & Barbi, E. IgE-mediated food allergy in children. Lancet 382, 1656–1664 (2013).

Lukács J, Schliemann S, Elsner P 2016 Occupational contact urticaria caused by food – a systematic clinical review Contact Dermatitis, 75, 195–204

Lyons, S. A., Burney, P. G. J., Ballmer-Weber, B. K., Fernandez-Rivas, M., Barreales, L., Clausen, M., Dubakiene, R., Fernandez-Perez, C., Fritsche, P., Jedrzejczak-Czechowicz, M., Kowalski, M. L., Kralimarkova, T., Kummeling, I., Mustakov, T. B., Lebens, A. F. M., van Os-Medendorp, H., Papadopoulos, N. G., Popov, T. A., Sakellariou, A., Welsing, P. M. J., Potts, J., Mills, E. N. C., van Ree, R., Knulst, A. C. & Le, T. M. Food Allergy in Adults: Substantial Variation in Prevalence and Causative Foods Across Europe. J. Allergy Clin. Immunol. Pract. 7, 1920-1928.e11 (2019).

Lyons, S. A., Clausen, M., Knulst, A. C., Ballmer-Weber, B. K., Fernandez-Rivas, M., Barreales, L., Bieli, C., Dubakiene, R., Fernandez-Perez, C., Jedrzejczak-Czechowicz, M., Kowalski, M. L., Kralimarkova, T., Kummeling, I., Mustakov, T. B., Papadopoulos, N. G., Popov, T. A., Xepapadaki, P., Welsing, P. M. J., Potts, J., Mills, E. N. C., van Ree, R., Burney, P. G. J. & Le, T. M. Prevalence of Food Sensitization and Food Allergy in Children Across Europe. J. Allergy Clin. Immunol. Pract. 8, 2736-2746.e9 (2020).

Marsella, R. (2015) 'Experimental model for peanut allergy by epicutaneous sensitization in atopic beagle dogs', Experimental Dermatology, 24(9), pp. 711–712.

Martin, P. E. et al. (2015) 'Which infants with eczema are at risk of food allergy? Results from a population-based cohort', Clinical and Experimental Allergy, 45(1), pp. 255–264. doi: 10.1111/cea.12406.

Maryniak, N. Z., Sancho, A. I., Hansen, E. B., & Bøgh, K. L. (2022). Alternatives to Cow's Milk-Based Infant Formulas in the Prevention and Management of Cow's Milk Allergy. Foods, 11(7), [926]. https://doi.org/10.3390/foods11070926

Matsunaga, K. et al. (2015) 'Anaphylactic augmentation by epicutaneous sensitization to acid-hydrolyzed wheat protein in a guinea pig model.', The Journal of toxicological sciences, 40(6), pp. 745–52. doi: 10.2131/jts.40.745.

Mayer, J. U., Hilligan, K. L., Chandler, J. S., Eccles, D. A., Old, S. I., Domingues, R. G., Yang, J., Webb, G. R., Munoz-Erazo, L., Hyde, E. J., Wakelin, K. A., Tang, S. C., Chappell, S. C., von Daake, S., Brombacher, F., Mackay, C. R., Sher, A., Tussiwand, R., Connor, L. M., Gallego-Ortega, D., Jankovic, D., Le Gros, G., Hepworth, M. R., Lamiable, O. & Ronchese, F. Homeostatic IL-13 in healthy skin directs dendritic cell differentiation to promote T H 2 and inhibit T H 17 cell polarization. Nat. Immunol. 22, 1538–1550 (2021).

Monaci, L., Pilolli, R., De Angelis, E., Crespo, J. F., Novak, N. & Cabanillas, B. Food allergens: Classification, molecular properties, characterization, and detection in food sources. Adv. Food Nutr. Res. 93 113–146 (2020).

Morita, H. et al. (2012) 'ST2 requires Th2-, but not Th17-, type airway inflammation in epicutaneously antigen- sensitized mice.', Allergology international : official journal of the Japanese Society of Allergology, 61(2), pp. 265–73. doi: 10.2332/allergolint.11-OA-0379

Nakamura R, Nakamura R, Sakai S, Adachi R, Hachisuka A, Urisu A, Fukutomi Y, Teshima RJ. Tissue transglutaminase generates deamidated epitopes on gluten, increasing reactivity with hydrolysed wheat protein-sensitized IgE. Allergy Clin Immunol 2013;132:1436-8.

Navuluri, L. et al. (2006) 'Allergic and anaphylactic response to sesame seeds in mice: identification of Ses i 3 and basic subunit of 11s globulins as allergens', Int Arch Allergy Immunol, 140(3), pp. 270–276. doi: IAA2006140003270 [pii]\n10.1159/000093284.

Noti, M., Kim, B. S., Siracusa, M. C., Rak, G. D., Kubo, M., Moghaddam, A. E., Sattentau, Q. A., Comeau, M. R., Spergel, J. M. & Artis, D. Exposure to food allergens through inflamed skin promotes intestinal food allergy through the thymic stromal lymphopoietin-basophil axis. J. Allergy Clin. Immunol. 133, 1390–9, 1399.e1–6 (2014).

Olszewski A, Pons L, Moutete F, et al. Isolation and characterization of proteic allergens in refined peanut oil. Clin Exp Allergy. 1998;28 (7):850-859.

Oyoshi, M. K. et al. (2009) 'Chapter 3 Cellular and Molecular Mechanisms in Atopic Dermatitis', in Advances in Immunology, pp. 135–226. doi: 10.1016/S0065-2776(09)01203-6.

Parvataneni, S. et al. (2009) 'Development of an adjuvant-free cashew nut allergy mouse model.', International archives of allergy and immunology, 149(4), pp. 299–304. doi: 10.1159/000205575.

Pazos-Castro D et al 2022 NLRP3 priming due to skin damage precedes LTP allergic sensitization in a mouse model Scientific Reports, 2022 12 3,329 https://doi.org/10.1038/s41598-022-07421-y

Pecquet C, Lauriere M, Huet S, Leynadier F. Is the application of cosmetics containing protein-derived products safe? Contact Dermatitis 2002;46:123.

Pelkonen AS, Mäkinen-Kiljunen S, Hilvo S, Siltanen M, Mäkelä MJ. Severe allergic reaction to gluten hydrolysate without reaction to wheat. Ann Allergy Asthma Immunol 2011;106:343-4.

Perkin, M. R., Logan, K., Tseng, A., Raji, B., Ayis, S., Peacock, J., Brough, H., Marrs, T., Radulovic, S., Craven, J., Flohr, C. & Lack, G. Randomized Trial of Introduction of Allergenic Foods in Breast-Fed Infants. N. Engl. J. Med. 374, 1733–1743 (2016).

Pouessel, G., Turner, P. J., Worm, M., Cardona, V., Deschildre, A., Beaudouin, E., Renaudin, J. M., Demoly, P. & Tanno, L. K. Food-induced fatal anaphylaxis: From epidemiological data to general prevention strategies. Clinical and Experimental Allergy vol. 48 1584–1593 (2018).

Remington, B.C., et al., 2020. Updated population minimal eliciting dose distributions for use in risk assessment of 14 priority food allergens. Food Chem. Toxicol. 139 111259.

RIVM report 320104001/2006 Cosmetics Fact Sheet To assess the risks for the consumer: Updated version for ConsExpo 4 H.J. Bremmer, L.C.H. Prud'homme de Lodder, J.G.M. van Engelen

'SCCS Notes of Guidance for the testing of Cosmetic Ingredients and their safety evaluation', 11th Revision SCCS Guidance SCCS/1628/21. (SCCS, Scientific Committee for Consumer Safety).

SCCS (Scientific Committee on Consumer Safety), Opinion on Skin Sensitisation Quantitative Risk Assessment for Fragrance Ingredients (QRA2), preliminary version of 24-25 October 2017 final version of 30 July 2018 SCCS/1589/17

Sharma, G. M., Rallabhandi, P., Williams, K. M., Herrmann, M. & Sadrieh, N. Gluten Quantitation in Cosmetic Products by Enzyme-Linked Immunosorbent Assay. J. AOAC Int. 99 586–590 (2016).

Sicherer, S. H. & Sampson, H. A. Food allergy: A review and update on epidemiology, pathogenesis, diagnosis, prevention, and management. J. Allergy Clin. Immunol. 141 41–58 (2018).

Spergel, J. M. et al. (1998) 'Epicutaneous sensitization with protein antigen induces localized allergic dermatitis and hyperresponsiveness to methacholine after single exposure to aerosolized antigen in mice', Journal of Clinical Investigation, 101(8), pp. 1614–1622. doi: 10.1172/JCI1647.

Smit, J. et al. (2011) 'Contribution of classic and alternative effector pathways in peanut-induced anaphylactic responses', Plos One, 6(12). Available at: http://findit.dtu.dk/en/catalog/2305820417

Teuber SS, Brown RL, Haapanen LAD. Allergenicity of gourmet nut oils processed by different methods J Allergy Clin Immunol 1997;99:502-7

Thompson, T. & Grace, T. Gluten in cosmetics: is there a reason for concern? J. Acad. Nutr. Diet. 112, 1316 (2012).

Tordesillas, L., Goswami, R., Benedé, S., Grishina, G., Dunkin, D., Järvinen, K. M., Maleki, S. J., Sampson, H. A. & Berin, M. C. Skin exposure promotes a Th2-dependent sensitization to peanut allergens. J. Clin. Invest. 124 4965–75 (2014).

Tordesillas, L. & Berin, M. C. Mechanisms of Oral Tolerance. Clin. Rev. Allergy Immunol. 55 107–117 (2018).

Wang, L.-F. et al. (1996) 'Epicutaneous exposure of protein antigen induces a predominant Th2-like response with high IgE production in mice', Journal of Immunology, 156(11), pp. 4079–4082.

Wavrin, S. et al. (2014) 'Cutaneous or respiratory exposures to peanut allergens in mice and their impacts on subsequent oral exposure.', International archives of allergy and immunology, 164(3), pp. 189–99. doi: 10.1159/000363444.

Wu CH, Nakai S, Powrie WD. Preparation and properties of acid-solubilized gluten. J. Agric. Food Chem. 24 504–510 (1976).

Yagami, A., Aihara, M., Ikezawa, Z., Hide, M., Kishikawa, R., Morita, E., Chinuki, Y., Fukutomi, Y., Urisu, A., Fukushima, A., Itagaki, Y., Sugiura, S.-I., Tanaka, H., Teshima, R., Kato, Z., Noguchi, E., Nakamura, M., Saito, H. & Matsunaga, K. Outbreak of immediate-type hydrolyzed wheat protein allergy due to a facial soap in Japan. J. Allergy Clin. Immunol. 140, 879-881.e7 (2017).

Yu, W., Freeland, D. M. H. & Nadeau, K. C. Food allergy: immune mechanisms, diagnosis and immunotherapy. Nat. Rev. Immunol. 16 751–765 (2016).

Yu, J. E. & Lin, R. Y. The Epidemiology of Anaphylaxis. Clinical Reviews in Allergy and Immunology vol. 54 366–374 (2018).

Zeiger, R. S. Food allergen avoidance in the prevention of food allergy in infants and children. Pediatrics 111, 1662–1671 (2003).

Appendix 1 Validation: recovery trials

Appendix 1.1 Recovery trials: Milk ELISA assay

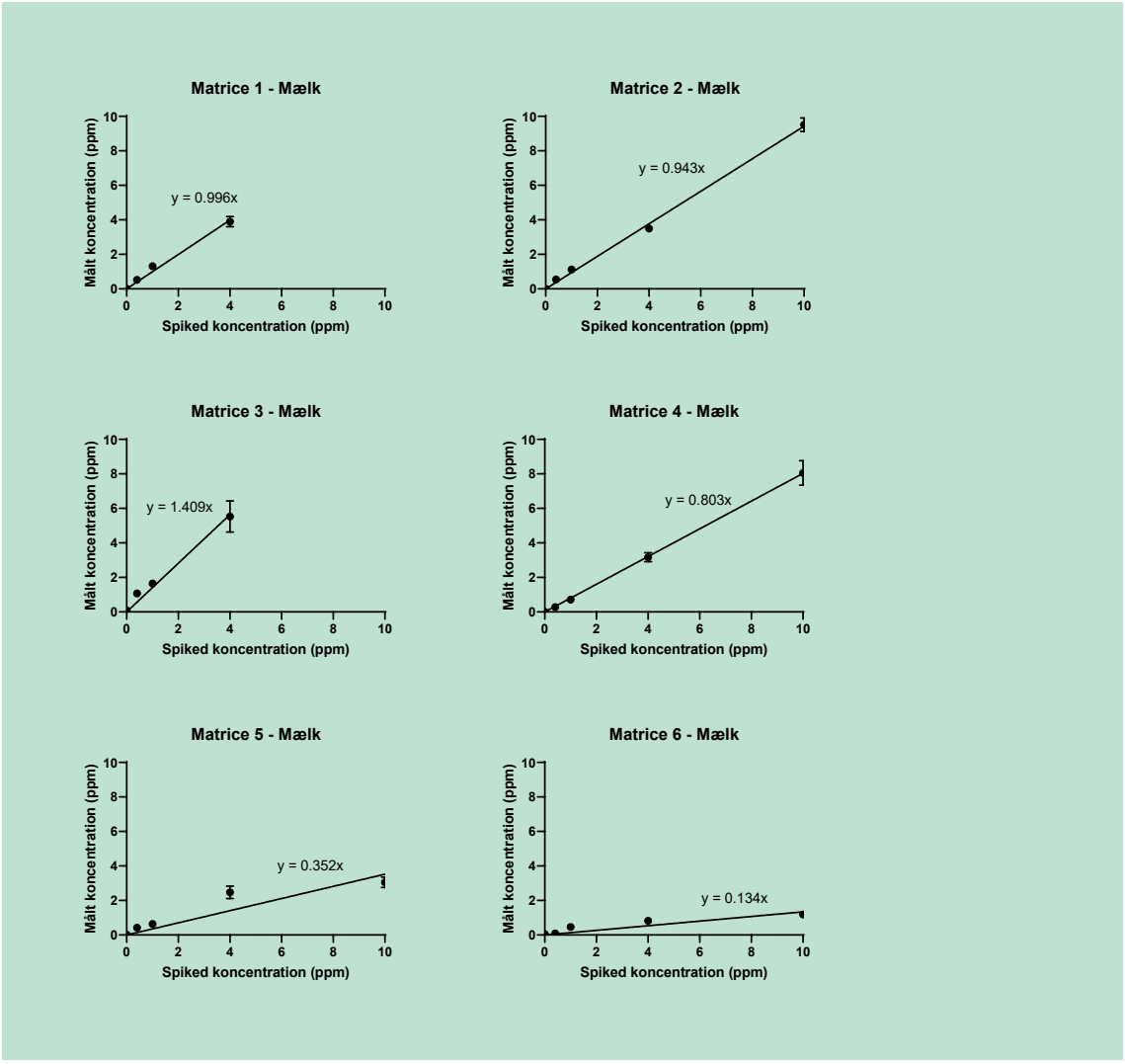

FIGURE 7. Correlation between added spike concentration and measured concentration in measurement of milk protein with ELISA. A slope of 1 indicates optimal correctness with good correlation, whereas a value above or below 1 demonstrates an overestimation or underestimation, respectively, in the analysis.

Appendix 1.2 Recovery trials: Almond ELISA assay

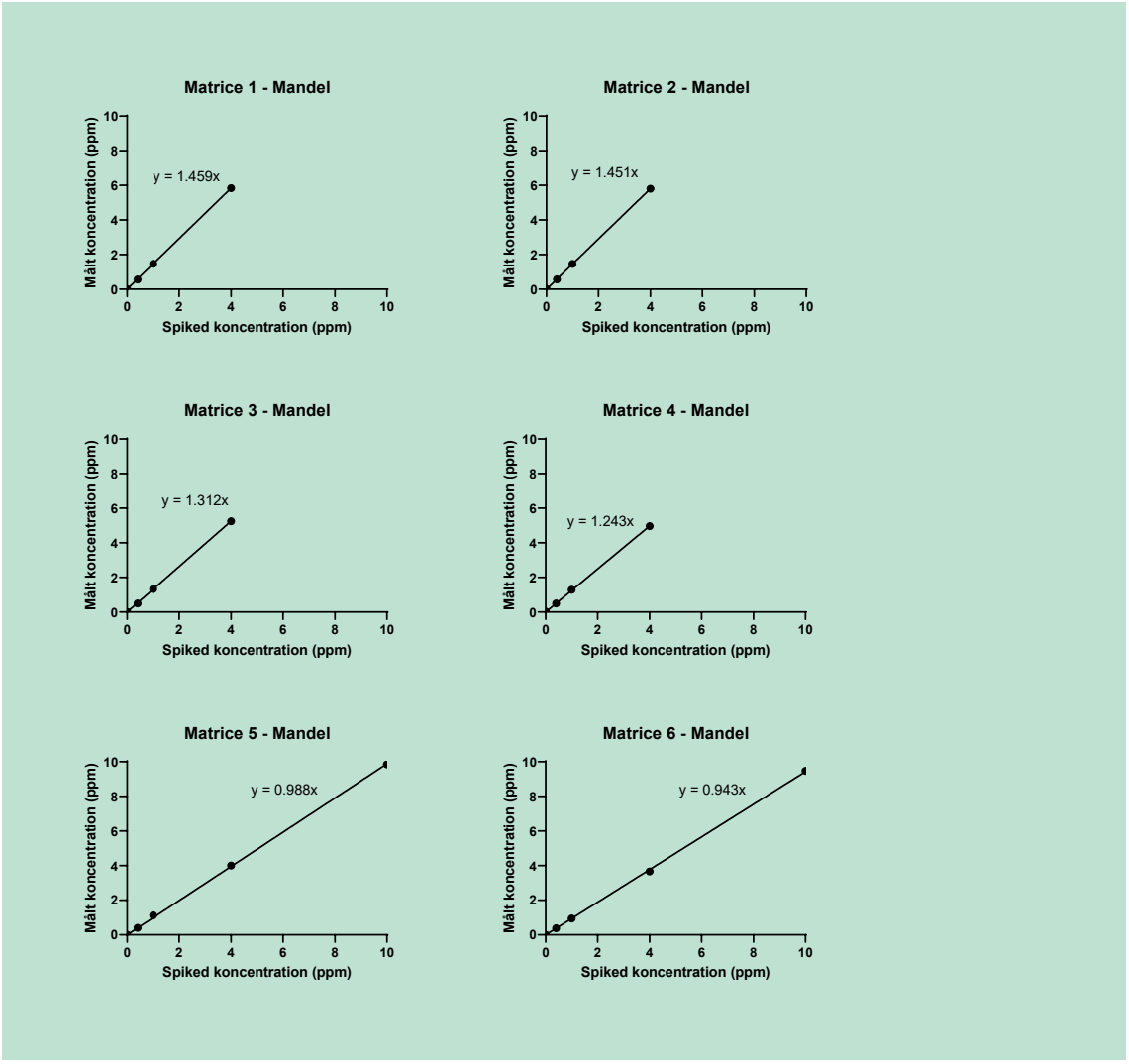

FIGURE 8. Correlation between added spike concentration and measured concentration in measurement of almond protein with ELISA. A slope of 1 indicates optimal correctness with good correlation, whereas a value above or below 1 demonstrates an overestimation or under-estimation, respectively, in the analysis.

Appendix 1.3 Recovery trials: Soy ELISA assay

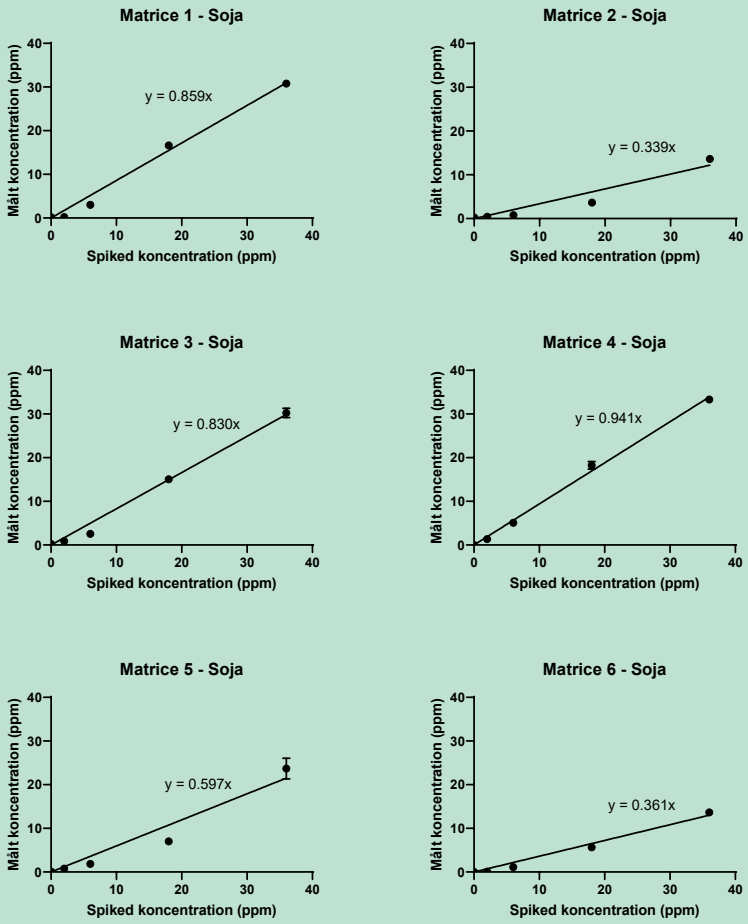

FIGURE 9. Correlation between added spike concentration and measured concentration in measurement of soy protein with ELISA. A slope of 1 indicates optimal correctness with good correlation, whereas a value above or below 1 demonstrates an overestimation or underestimation, respectively, in the analysis.

Mapping and risk assessment of food proteins in cosmetic products
It is popular to use food-based ingredients in cosmetic products. Howev-er, several studies have indicated that food allergy may be developed through skin exposure to foods.

Young children develop tolerance to foods when they are introduced to them, which typically occurs during the first two years of their life. This tolerance protects against sensitization via the skin. If you are exposed to a food via the skin before oral toler-ance has been established, you risk developing food allergy. Children whose skin barrier is not intact, as seen in atopic dermatitis (infantile eczema), is especially vul-nerable.

The market was mapped, partly by use of the database behind the app ”Kemiluppen” from the Danish Consumer Council THINK Chemicals, and partly by searches in webshops. The aim was to identify cosmetic prod-ucts with ingredients based on foods that typically cause food allergy.

Based on the mapping, it was decided to focus on analysis of proteins from milk, al-mond and soy. A number of products were purchased in Dan-ish stores and web-shops, and in webshops in the rest of the EU and out-side the EU. The purchased products were either aimed at children or at specific skin types.

Overall, it was found that the analysis method ELISA can be used to de-termine the presence of milk protein, almond protein and soy protein in cosmetic products, when the proteins is present in a sufficient quantity.

It was not possible to set limits on the quantity of food protein, under which young children cannot be sensitized.

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