Opinion of the Scientific Panel on Dietetic Products, Nutrition and Allergies on a request from the Commission relating to the evaluation of allergenic foods for labelling purposes

(Request Nº EFSA-Q-2003-016)

(adopted on 19 February 2004)

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SUMMARY

Amongst adverse reactions to food there are immune-mediated and non-immune mediated reactions. Food allergies comprise immune-mediated reactions to foods mediated either by IgE antibodies or other immunological pathways. Food intolerance comprises non-immune-mediated responses that are dependent on enzyme deficiencies, pharmacological reactions, or, as is true in the majority of cases, unknown mechanisms.

EU legislation has recently been modified regarding food labelling in order to ensure derogations to the obligatory declaration of food ingredients are not applicable to those ingredients which may induce food allergies and/or food intolerances (Annex IIIa of Directive 2003/89/EC[1]). This pertains to cereals containing gluten, crustaceans, eggs, fish, peanuts, soybeans, milk and dairy products including lactose, nuts, sesame seeds, celery, mustard, and sulphite at concentration of 10 mg/kg and above.

In view of the recent scientific developments in this field and the earlier opinion of the Scientific Committee on Food (SCF) on "Adverse reactions to foods and food ingredients" expressed in 1995, the European Food Safety Authority is asked to advise the Commission on: 1) The scientific basis supporting the identification of foods, food components and food ingredients which induce food allergies and food intolerance for foodstuffs labelling purposes; and 2) The possibility of determining thresholds or of identifying other elements (including food processing) which would establish that a food component or a food ingredient is no longer susceptible of inducing adverse reactions.

In general terms, it can be stated that all allergens and products thereof mentioned in the list can cause adverse health effects, and in some cases exposure to these can be fatal. These are the most common food allergens which are generally resistant to food processing and they have the capacity to trigger an allergic reaction in an allergic consumer if they are added to foods. Some of these allergens are very widely distributed all throughout Europe, while others, such as mustard and celery, are more geographically restricted. This list should be kept under review in the light of changing food practices and emergence of new clinical observations and other kind of scientific information.

There is high variability in sensitivity between different sensitised individuals with respect to the dose of allergens required to trigger an adverse effect. In addition, for ethical reasons, highly sensitive individuals are often not tested in an appropriate way to establish thresholds. Hence, the information available is insufficient to draw firm conclusions regarding the highest dose that would not cause an adverse effect. Thus, a system of risk evaluation based on the assessment of no observed adverse effect levels (NOAEL) does not apply currently.

Processing can influence allergenicity of the foods, as does the food matrix in which the allergens are presented to the consumer. In addition, individuals who suffer from allergies to the same food may react to different components of that foodstuff. The data available do not indicate that food processing predictably influences allergenicity, and also the influence of the matrix cannot be predicted.

[1] Directive 2003/89/EC of the European Parliament and of the Council amending Directive 2000/13/EC as regards indication of the ingredients present in foodstuffs. Official Journal of the European Union L 308, 25.11.2003, p. 15.

To minimise the risks to the consumer, analysis of foods for traces of potential food allergens is desirable. However, while sensitive test systems are coming into use and are commercially available for analysis of some allergens in foods, major problems remain with regard to factors such as: insufficient extraction, detection limits outside the range of clinical sensitivity, insufficient specificity due to cross-reaction and insufficient interlaboratory reproducibility.

The possibility that specific derivatives of the food allergens listed in Annex IIIa of the Directive are unlikely to trigger an allergic reaction needs to be evaluated on a case by case basis.

Summary assessment of allergenic foods included in Annex IIIa of Directive 2003/89/EC

Cereals with regard to coeliac disease

Coeliac disease is an immunologically-based disease caused by gluten. The causal relationship between gluten and its "toxicity" in individuals genetically predisposed to develop coeliac disease is firmly established. Acid hydrolysis may destroy properties of gluten which elicit coeliac disease. However, partial hydrolysis and enzymatic degradation and heat treatment during food processing do not destroy coeliac-triggering peptide units. There are insufficient data to suggest a threshold dose of gluten tolerable for all coeliac patients. The current Codex Alimentarius limit for gluten-free foods of 200 mg gluten/kg food for coeliac patients requires reconsideration.
Detection assays for gluten in foods are available.

Cereals with regard to food allergy

Cereals can cause food allergy. Allergy to cereals in the general population is not very frequent, as few cases are reported in relation to the widespread consumption; in children, however, wheat is a frequent cause of food allergy. Cereal allergens cross-react with pollen allergens.
Since wheat is mostly consumed cooked or heat-treated, it is evident that its allergenicity normally survives thermal treatment. Some wheat allergens can be destroyed by heating, while others are thermostable.
The lowest reported amount of wheat able to provoke an allergic reaction is 500 mg.
No immunochemical method to analyse foods for non-gluten cereal allergen has been reported.

Fish and crustaceans

Fish and crustaceans are common food allergens. All major fish allergens cross-react and no fish has been found to be safe in allergic patients. Food processing may affect the allergenicity, but is not a reliable method to reduce allergenicity. Doses of fish provoking an allergic reaction have been reported to be in the milligram range, and for shrimp as a member of crustaceans in the gram range. Thresholds doses have not been established.
Radioimmunoassays for detection of fish allergens have been described but have not been validated for detection of fish allergens in food. For crustaceans immunochemical detection methods are available but are not sufficiently sensitive to detect the lowest amount demonstrated to elicit an allergic reaction.

Egg

Egg proteins are frequent triggers of allergic reactions. There are possible clinical cross-reactivities between hen eggs and eggs from other species. Heat denaturation and other food

processing treatments do not reliably reduce the allergenicity. Doses reported to trigger allergic reactions in clinical studies range from microgram to low milligram levels of orally administered egg proteins.
Assays to detect egg allergens in foods are available.

Peanut
Peanut is a common cause of food allergic reactions, and it is a member of the legume family. It cross-reacts with other members of the legume family, such as soy and lupin. It is the most common cause of reported fatal food-induced anaphylaxis. Peanuts are widely used as ingredients of food. Heat treatment may increase its allergenicity. Reactions can be triggered by doses in the microgram range. It is not possible to determine a reliable threshold dose.
Sensitive detection methods for peanut allergens are commercially available but are not appropriate for detection of low levels in processed foods.

Soy
Soy is a food allergen and soy protein is widely used in processed foods. As a legume, soy may cross-react with other legumes, including peanuts. Cross-reaction with cows' milk allergens has been described.
As for many food allergens, heat denaturation and enzymatic digestion of soy affect allergenicity and may reveal new allergenic epitopes. Levels for triggering adverse reaction in soy allergic individuals are variable and are in the low microgram range, although studies that address these questions have not been performed in a satisfactory way.
Immunochemical and PCR detection methods for analysis of soy and soy allergens have been described, but seem to be inappropriate for detection in food.

Milk
Most cows' milk proteins are potential food allergens. Numerous milk allergens have been identified, and some remain active during food preparation and during digestion. Data available show that a substantial proportion of allergic individuals reacts to very low (in the range of micrograms) amounts of allergens, but are insufficient to establish validated threshold doses nor to derive a level of exposure which could protect allergic consumers against a reaction to milk products present in their food in trace amounts. These considerations may be applied to milk of species other than cows, such as buffalos, goats and ewes.
Immunochemical detection methods for major milk allergens have been described but may not be appropriate for processed foods.
Lactose intolerance is not an allergic or an immune-mediated disease, and does not provoke anaphylactic reactions. It results from a reduced capacity to digest lactose due to a reduced lactase activity in the small intestine. Doses less than 10 g (corresponding to 200 mL of milk) per day are often tolerated by most adults with reduced lactase levels. Residual amounts of cows' milk proteins that can still be present in lactose as contaminants from the production process of lactose might be harmful for milk allergic patients.

Nuts
Nuts are a common cause of allergic reactions. Multiple nut sensitivities are frequent and often associated with peanut allergies, but cross-reacting allergens have not been identified. Birch pollen sensitised individuals may react to hazelnut allergens. Roasting may reduce but not abolish hazelnut allergenicity. No such data are available for other nuts. A few micrograms may cause reactions in sensitised individuals, but threshold doses have not been established.

Several assay systems to detect nut allergens in foods have been developed.

Celery
Celery is often found in prepacked food as it is widely used in the food industry because of its aromatic flavour. Allergic reactions occur predominantly to raw celery and less frequently to cooked celery, but allergenicity of celery powder is comparable to that of raw celery. Celery allergic patients may react to doses of allergen in the milligram range, but there are insufficient data to determine threshold levels.
There is currently no detection assay available.

Mustard
The major allergens of mustard are resistant to heat and other food processing procedures. Allergen doses causing allergic reactions in mustard allergic patients can be in the high microgram range, although threshold doses have not been established. No specific detection method for mustard allergens has been described.

Sesame seed
Sesame seeds are widely, and increasingly, used in many processed foods. A few milligrams of sesame protein are able to cause allergic symptoms.
Assays for detection of sesame allergens are available.

Sulphites
Sulphites are used as food additives and may cause severe reactions in sensitive individuals mostly in asthmatic patients. The pathogenesis of adverse reactions to sulphites has not been clearly documented but it is unlikely that sulphite reactions are allergic or immune-mediated or produce anaphylactic reactions. Most sulphite-sensitive individuals will react to ingested metabisulphite in quantities ranging from 20 to 50 mg of sulphites in the food. The smallest concentration of sulphites able to provoke a reaction in sensitive individuals has not been established. Labelling of foods containing sulphiting agents in concentrations of 10 mg/kg or more is required in the EU, though the threshold for sensitivity reactions may be even lower.

KEYWORDS

Food allergy, allergic reaction, allergen, threshold dose, food labelling, food safety.

BACKGROUND

In its report of 22 September 1995 the Scientific Committee on Food (SCF) identified the most common food allergens as well as the principal sources of food intolerance. For persons suffering from food allergy or food intolerance, the first requirement is information on food composition, in order to be able to choose a product with full knowledge of the facts and to eliminate the risk of adverse reactions.

New legislation (Directive 2003/89/EC) lays down compulsory labelling of allergenic ingredients in foodstuffs to address the issue of food allergies and intolerances. To this end, it amends the rules for labelling ingredients set out in Directive 2000/13/EC and mainly comprises the following points:

- It removes with the exemption on labelling components of compound ingredients accounting for less than 25% of the finished product and so establishes the principle that all the ingredients must be listed.

- It contains a list of ingredients (Annex IIIa) responsible for most food allergies or intolerances, for which no exemptions are permitted.

The new legislation thus provides that the ingredients listed in the following list (Annex IIIa of the Directive), or those manufactured from these ingredients, will always have to be declared in the label of foodstuffs which contain them, notwithstanding the exemptions provided in Directive 2000/13/EC:

- Cereals containing gluten and products thereof
- Crustaceans and products thereof
- Eggs and products thereof
- Fish and products thereof
- Peanuts and products thereof
- Soybeans and products thereof
- Milk and products thereof (including lactose)
- Nuts and products thereof
- Celery and products thereof
- Mustard and products thereof
- Sesame seeds and products thereof
- Sulphur dioxide and sulphites at concentrations of more than 10 mg/kg or 10 mg/litre expressed as SO_2.

The doses of allergens likely to induce food allergies can be very small. Consequently, all the derived products from an ingredient appearing in the list of food allergens (Annex IIIa) shall be included in the label with a clear reference to the name of the allergenic source.

It is noted that certain food allergens can be destroyed by cooking or conservation while others are resistant. Also, transformation of the foodstuffs and the introduction of new food technologies could help to create allergens. In addition, some studies mention allergenicity thresholds for certain allergens.

TERMS OF REFERENCE

In view of the recent scientific developments in this field and the earlier SCF opinion on "Adverse reactions to foods and food ingredients" expressed in 1995, the European Food Safety Authority is asked to advise the Commission on:

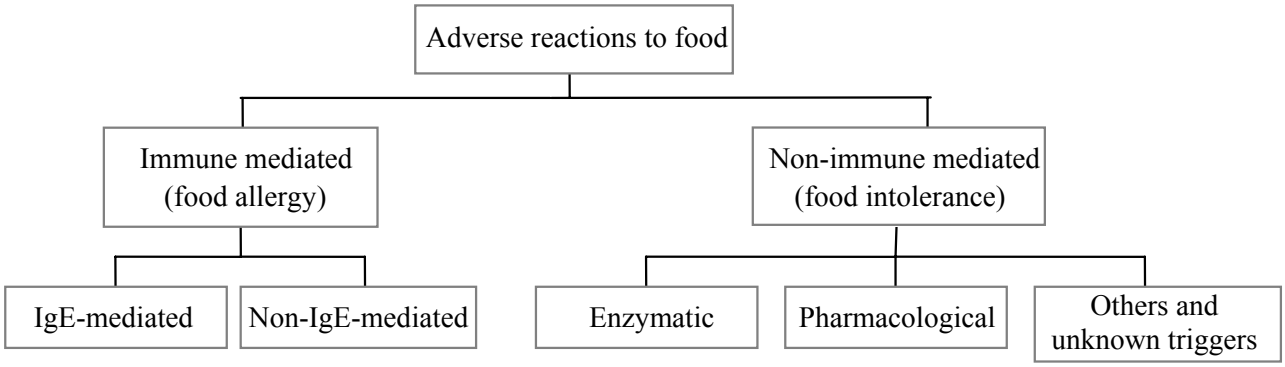
- The scientific basis supporting the identification of foods, food components and food ingredients which induce food allergies and food intolerance for foodstuffs labelling purposes;

- The possibility of determining thresholds or of identifying other elements (including food processing) allowing to establish that a food component or a food ingredient is no longer susceptible to induce adverse reactions.

ASSESSMENT

I. INTRODUCTION

Amongst adverse reactions to food there are immune-mediated and non-immune mediated reactions. Immune-mediated reactions to food are mediated either by IgE antibodies or other immunological pathways. Food intolerances comprise non-immune-mediated responses that are dependent on enzyme deficiencies, pharmacological reactions, or, as is true in the majority of cases, arise by unknown mechanisms.



Adverse health consequences due to allergic reactions to food including food intolerances (Table 1) occur in about 1-3% of the population and about 4-6% of children. Allergic reactions can affect the life of a considerable number of people, whether it is in a benign or life-threatening way.

Table 1. Common clinical features of adverse reactions to foods

Organ system	Clinical features
Skin	Atopic dermatitis Urticaria Angio-oedema
Gastrointestinal tract	Oral allergy syndrome Abdominal pain Colic, nausea Vomiting Diarrhoea Constipation Bloating Gastro-oesophageal reflux (heart burn) Enteropathies Failure to thrive
Respiratory tract	Asthma Rhinitis
Eyes	Conjunctivitis Watering eyes
Central nervous system	Headache Abnormal behaviour (ADHD), rare
Generalised (systemic)	Anaphylaxis (with all its complications)

II. CLINICAL SYMPTOMS DUE TO FOOD ALLERGY[2]

Adverse reactions to food have been classified into different groups on the basis of the pathogenetic mechanism (see Figure in page 11). Adverse reactions to food include immunologically-mediated reactions, which may be IgE and non-IgE-mediated; and non-immunological reactions, such as intolerance and idiosyncrasy (Ortolani, 1995).

IgE-mediated reactions are represented by well-defined clinical entities as demonstrated by a number of double-blind placebo-controlled food challenge (DBPCFC) studies.

For an allergic reaction to take place, a two-step process is required. First, the capacity must be established to respond with an allergic reaction when exposed to the particular allergen. This requires an immune response to take place, in which the immune system responds with IgE antibody production against the allergen. This phase is called the induction phase, or sensitisation. Once an individual has become sensitised to a particular allergen, the individual may develop a symptomatic allergic reaction when again exposed to the allergen in question. This is called the provocation or triggering phase.

The food allergic nature of some clinical syndromes such as migraine, attention deficit hyperactivity disorder, irritable bowel syndrome and others is still controversial. There is some published evidence that in a small proportion of individuals exposure to certain foods may be the underlying trigger (Taylor, 1999; Rowe and Rowe, 1994; Carter *et al.*, 1993; Egger *et al.*, 1983; Grant, 1979; Jones *et al.*, 1982).

1. SKIN

1.1 Urticaria and angio-edema

Urticaria is an intensely itchy rash which results from inflammation and leakage of fluid from the blood into superficial layers of the skin in response to various mediators. Synonyms are “hives” or “nettle rash”. Angio-edema is the presence of fluid in subcutaneous tissues, particularly in the face, and in addition in the sub mucosa of eyes, lips, and sometimes tongue and throat. Urticaria occurs in acute and chronic forms, acute being defined as lasting for less than six weeks, whereas chronic urticaria lasts for longer periods. In childhood, urticaria is more commonly of the acute type.

Urticaria due to food ingestion generally occurs within hours of ingestion, and often fades within 3 hours. Initially there are localised symptoms of itching and burning, which progresses to erythema and urticaria. Immune (IgE) mediated contact urticaria to foods is common and may progress to more widespread urticaria, angio-edema, and eventually anaphylaxis. Very rarely, urticaria and angio-edema can be induced by taking exercise soon after eating a food, such as wheat, shellfish, nuts, or celery, whereas neither the food nor the exercise alone causes any reaction.

Chronic urticaria is only associated with food allergy in a small proportion of the patients.

[2] Section based in parts on COT, 2000.

1.2 Atopic dermatitis

Atopic dermatitis is an extremely pruritic form of chronic inflammatory skin disease, usually presenting in early infancy, and sometimes persisting in adulthood. Atopic dermatitis often begins in early infancy and represents the first clinical allergic manifestation in a child who will go on to develop other atopic diseases. In the majority of atopic children, asthma will develop later and subsequently allergic rhinitis. This progression is often named atopic march (Spergel and Paller, 2003). The patients with atopic dermatitis have often high IgE levels and positive immediate skin test for several allergens. Patients with atopic dermatitis have a genetic predisposition, as there is a 2-3-fold increased risk for a child to develop the disease if one or both of the parents are affected. Several epidemiological studies are beginning to identify genes involved in atopic predisposition (Lee *et al.*, 2000; Walley *et al.*, 2001; Tsunemi *et al.*, 2002).

Other characteristic features of atopic dermatitis are ichthyosis, keratosis pilaris, white dermographism, atopic folds and orbital darkening. They often develop anterior subcapsular cataracts and keratoconus. Acute atopic dermatitis is an acute rash represented by an erythematous, papulovesicular eruption. Chronic dermatitis is characterised by lichenification, excoriation and dyschromic lesions. The acute rash is typical of the first, infantile, stage up to 2 years of age. This eczematous lesion is highly pruritic and usually involves both cheeks and the extensor part of the extremities. Lesion of the scalp and wheal formation may also be associated.

Infantile atopic dermatitis may be difficult to distinguish from seborrhoeic dermatitis. The second or childhood stage, from 2 to 12 years, is characterised by papular lesions and rash that occur in the flexural areas, such as the antecubital and popliteal ones, hands and feet (Rudikoff and Lebwohlm, 1998). The adult stage is characterized by diffuse lichenification in facial areas such as the periorbital and perioral areas. Chronic lesions and remission periods may characterise the life of older atopic patients. Atopic dermatitis can be divided into two distinct variants: the extrinsic, allergic form which occurs with sensitisation towards foods or aeroallergens and high levels of total IgE antibodies; and the intrinsic, non-allergic variant, with a low level of IgE, in which no sensitisation to foods or aeroallergens can be detected.

The diagnosis of atopic dermatitis is based on well-accepted international criteria, and takes into consideration different clinical and laboratory parameters such as the kind of skin manifestation, distribution, age of onset, frequency of relapses, association of other atopic diseases, total serum IgE, specific IgE, blood eosinophilia and so on. On this basis it is also possible to distinguish between the intrinsic and the extrinsic form of atopic dermatitis. The standard for diagnosis of food hypersensitivity in chronic atopic dermatitis is DBPCFC.

Several clinical studies have addressed the role of food allergy in atopic dermatitis demonstrating also the significant effect of food elimination on the improvement of the lesions (Burks *et al.*, 1998a; Sampson, 1997 and 2003; Chu and Morris, 1997; Niggemann *et al*., 1999; Lever *et al.*, 1998).

Egg allergy is the most frequent cause of severe atopic dermatitis in children (Sampson, 1997), and egg, together with milk, peanut, soy and wheat account for about 90% of food allergy in children with atopic dermatitis. The role of food allergy in patients with atopic dermatitis is linked to the age of the sufferer and the severity of the disease. It is thought that food hypersensitivities do not play a major role in adults with atopic dermatitis. On the

contrary, approximately 40% of children with moderate to severe atopic dermatitis who attended an university paediatric dermatology clinic were found to have food allergy (Heigenmann *et al.*, 1998). Patients who are allergic to peanuts, tree nuts, fish and shellfish are much less likely to outgrow their food-related atopic dermatitis (Skolnick *et al.,* 2001).

2. GASTROINTESTINAL TRACT

Adverse reactions affecting the gastrointestinal tract range from mild oral discomfort after allergen exposure to severe diarrhoea and failure to thrive. Any part of the gastrointestinal tract can be involved and the clinical features may occur alone or together as part of a syndrome.

2.1 Vomiting and gastro-oesophageal reflux

Vomiting occurring alone or in combination with acute or chronic diarrhoea is a common feature of allergic reactions to food (Walker-Smith *et al.*, 1978; Hill *et al.*, 1984). Vomiting may result from irritation or inflammation of the mucosa of the stomach and oesophagus. The inflammatory response may cause bleeding, with blood in the vomit. Gastro-oesophageal reflux can occur as an adverse reaction to food, particularly in infants, with or without development of eosinophilic oesophagitis (Hill *et al.*, 1984; Ford and Walker-Smith, 1987; Moon and Kleinman, 1995).

2.2 Diarrhoea and enteropathies

The passage of frequent loose stools can result from impaired absorption of nutrients and water or from intestinal secretion of fluid as part of an inflammatory response, or from a combination of both (Ford and Walker-Smith, 1987). Bacterial overgrowth syndromes and fermentative diarrhoea may arise from functional gastrointestinal disturbances caused by gastroenteritis and surgery.

In infancy and childhood food adverse reactions to proteins may cause severe diarrhoeas ultimately leading to failure to thrive (Savilahti, 2000; Walker-Smith, 2003).

The major feature of the enteropathies is a loss of the normal structure of the intestinal mucosa which reduces its mucosal digestive and absorptive function (Walker-Smith *et al.*, 1978; Kuitunen *et al.*, 1975). In young children transient enteropathies to cows' milk, soya, eggs, and other foods may occur.

2.2.1 Coeliac disease

Coeliac disease is caused in susceptible subjects by exposure to gluten in wheat. It has a wide range of clinical presentation. The most severe cases may present with diarrhoea and cachexia; less severely affected patients may present with a more insidious malabsorption with weight loss and, in children, failure to thrive. In some childhood cases impaired weight or height gain or delayed puberty is the only clinical evidence of illness (Jewell, 1996).

Patients with coeliac disease usually respond to dietary exclusion of gluten. Poor compliance with a gluten-free diet is associated with deterioration in health and an increased risk of

gastrointestinal, particularly small bowel, lymphoma and other malignancies (Beckett and Ciclitira, 1997)

2.2.2 Allergic eosinophilic gastroenteropathy

Allergic eosinophilic gastroenteropathy comprises a spectrum of conditions, which can occur at any age but which predominantly affect infants and young children, and in which there is eosinophilic inflammation of the gastrointestinal mucosa (Kelly, 2000). Any part of the gastrointestinal tract can be affected and the symptoms and signs reflect the site and extent of the damage. Loss of blood and exudation of serum into the intestinal lumen may result. Involvement of the stomach or oesophagus may present with vomiting. Damage to the small intestine and colon can cause significant loss of endogenous protein and nutrients as well as impaired digestion and absorption.

The causes and mechanisms of these conditions are not well understood. Some cases are associated with atopic clinical features and positive IgE RAST and skin prick tests to milk allergens, but others do not have these features (Moon and Kleinman, 1995; Min and Metcalfe, 1991). Allergic eosinophilic gastroenteropathy is a rare disease.

2.3 Infant colic

Sometimes known as three-month colic, this is a common problem in babies. However, the actual prevalence is uncertain because the condition is often managed by the parents rather than by health professionals. Its aetiology is multifactorial; it is often attributed to adverse reactions to foods such as cows' milk or proteins excreted in maternal breast milk or to a disturbed maternal-infant interaction (Hill and Hosking, 2000). A study in babies and older children with allergy to cows' milk proven by challenge found that 75% reacted with colic during a challenge with capsules containing whey protein (Lothe and Lindberg, 1989). Possible mechanisms have not been fully investigated and the relationship of infant colic to cows' milk and other foods or food components which might be present in maternal breast milk or formulae needs further study. A systematic review concluded that reduction of stimulation and introduction of dietary measures may be helpful in managing this condition (Lucassen *et al.*, 1998).

2.4 Abdominal pain and distension

Abdominal pain, distension and flatulence sometimes occur in instances of adverse reactions to foods. They may be associated with impaired digestion and absorption resulting from damage to the functional integrity of the intestine. Impaired sugar absorption may also be responsible for these symptoms.

2.5 Constipation

Up to 10% of children with cows' milk allergy may suffer from constipation. The underlying mechanisms are as yet unknown (Iacono *et al.*, 1998).

2.6 Oral allergy syndrome

Oral allergy syndrome is an IgE-mediated immediate type allergic reaction characterised by symptoms within several minutes after contact with food, involving the mouth and the

pharynx (Amlot *et al.*, 1987; Ortolani *et al.*, 1988). The direct contact of the offending food triggers oral and pharyngeal itching, oral papule or blisters, lip irritation and swelling, labial angio-edema, and glottis oedema. In some instances these symptoms are followed by a more complex clinical picture, involving several organs, and may lead to life-threatening reactions like anaphylactic shock (Ortolani *et al.*, 1993).

Oral allergy syndrome is frequently associated to selectively labile allergens contained in fresh fruits and vegetables. Oral symptoms are less frequent in patients allergic to foods of animal origin such as milk, eggs, fish, and shrimp (Amlot *et al.*, 1987; Liccardi *et al.*, 1996; Helbling *et al.*, 1999; Schäfer *et al.*, 2001).

Sensitisation to some fruits or vegetables may be significantly associated to other foods belonging to the same botanical family as well as with sensitisation to botanically unrelated foods. Clinically this phenomenon has been defined as cluster of hypersensitivity (Eriksson, 1984). The most common recognized clusters in oral allergy syndrome are (Pastorello and Ortolani, 2003): birch-pollen-fruit syndrome; latex-fruit syndrome; and lipid transfer protein sensitisation.

There is often a strong correlation between oral reactions to fresh fruits and vegetables and pollen sensitisation (Wüthrich *et al.*, 1990).

Depending on the severity of clinical symptoms, oral allergy syndrome can be classified into four grades depending on the extent of other organ involvement and systemic reactions. Local oral symptoms are most commonly experienced while the more severe forms are rare (Rodríguez *et al.*, 2000; Ortolani *et al.*, 2000; Ballmer-Weber *et al.*, 2000 and 2001).

Standard diagnostic procedures must include exposure to fresh fruits and raw vegetables because of the labile nature of the allergens most commonly associated with oral allergy syndrome.

3. RESPIRATORY TRACT

3.1 Asthma

Asthma is a reversible obstruction of the small airways. It is associated with constriction of the airways, mucus production and inflammation. Asthma may occur as a manifestation of a food allergic reaction. It may sometimes be the dominating symptom, but is often associated with eczema, urticaria, pollen-related food allergy syndrome (oral allergy syndrome), or gastrointestinal symptoms. Asthmatic symptoms may constitute an important part of a generalized anaphylactic reaction, and it is claimed that death from anaphylactic reactions are more often caused by respiratory problems than hypotension and circulatory failure. Further, it is suggested that asthmatics who are also food allergics are at a peculiar risk of developing the most severe anaphylactic reactions to food.

It is difficult to be sure how many patients with asthma have genuine food-related asthma. Studies in children (Novembre *et al.*, 1988a; Oehling and Cagnani, 1980) and patients of all ages (Onorato *et al.*, 1986) have suggested that 2%-8.5% of asthmatics showed indications of an asthmatic reaction on food challenge.

3.2 Laryngeal oedema

Laryngeal oedema, swelling of the mucosa of the larynx, is often seen as part of an allergic reaction to food, and may lead to airways obstruction and, in worst case, to respiratory arrest.

3.3 Rhinitis

Rhinitis is manifested as an inflammation of the nasal mucosa, which gets swollen and itchy. The condition is often accompanied by clear watery nasal secretion, and by nasal obstruction. Less frequent than asthmatic symptoms, rhinitis has been reported in connection with the intake of specific food items to which the individuals were allergic (Oehling *et al.*, 1992; Wraith *et al.*, 1979). Symptoms suggestive of rhinitis were reported in 13% of 206 infants with cows' milk allergy (Clein, 1954). Symptoms of rhinitis have been reported to occur in response to food challenges (Pelikan and Pelikan-Filipek, 1987).

4. EYES

The main form of allergic reaction in the eyes is conjunctivitis. In conjunctivitis, the surface of the eyes and the inner side of the eyelids get red, swollen and itchy. Conjunctivitis and rhinitis often, but not always, accompany each other, and conjunctivitis tends to occur less frequently than rhinitis. Conjunctivitis has been reported in connection with the intake of specific food items, although less frequently than asthmatic symptoms (Oehling *et al.*, 1992; Wraith *et al.*, 1979).

5. GENERALISED SYMPTOMS – ANAPHYLAXIS

Anaphylaxis is an acute, potentially life-threatening and sometimes fatal condition, involving the cardiovascular system, the respiratory tract, the mouth and the pharynx and the skin, singly or in combination (Sampson and James, 1992; Bock and Atkins, 1990; Yunginger *et al.*, 1988). The initial symptoms often involve the skin or the oropharynx. Symptoms from the mouth region include oedema, tingling and pruritus of the lips, oral mucosa and pharynx. Skin symptoms may be urticaria or more diffuse erythema, angio-edema and pruritus. Respiratory symptoms include bronchospasm, cough, stridor, dyspnoea and wheezing, and may be mistaken as worsening of pre-existing asthma. Oedema of the larynx produces cough, and difficulties with talking, breathing and swallowing. Respiratory function may be severely compromised. Anaphylactic shock may consist of cardiovascular collapse and severe fall of the blood pressure, cardiac arrythmia and in worst case cardiac arrest. In some cases the initial manifestation of an anaphylactic reaction may be loss of consciousness. The symptoms, their sequence and their severity may vary from one epidose to the other and from one individual to another. In fatal food-induced anaphylaxis, initial symptoms commonly developed within 3 to 30 minutes and severe respiratory symptoms within 20 to 150 minutes of exposure (Sampson and James, 1992). Some reactions may, however, show a bi-phasic course and be deceptively mild at their start (Stewart and Ewan, 1996). Also, some reactions are triggered by a combination of food and exercise, and these exercise-triggered food-induced anaphylactic reactions may occur several hours after food intake.

The register of all fatal anaphylactic reactions in England and Wales since 1992 suggests that there are about 20 deaths annually and that about one third of these reactions are due to foods (Pumphrey, 2000).

Anaphylactic reactions to food are associated with IgE-mediated allergy. Peanut is the most common foodstuff reported to cause the condition in Europe (Hourihane *et al.*, 1996), but a number of other food allergens may cause anaphylactic reactions.

6. DIAGNOSIS OF FOOD ALLERGY

The diagnosis of food allergy and food intolerance depends on clinical insight, suspicion, and acumen in interpreting the history and examination of the patient. It is often difficult because of the variable and subjective nature of the symptoms and the lack of objective clinical signs.

The key to clinical diagnosis of immunological and non-immunological adverse reactions to food and food ingredients is the patient's history, particularly the temporal relationships between exposure and reaction.

It is important to be aware that although allergic reactions to food are common in children, such reactions can occur also in adults.

The family history is useful in all types of adverse reaction to food. A family history of atopy will increase the index of suspicion of immune-mediated adverse reaction. If one parent is atopic there is a 20-40% chance of a child developing this type of condition and, if both parents are atopic, a 50-80% chance. A family history of the condition may be present in coeliac disease and in adverse reactions to food of non-immunological origin, such as disaccharidase deficiencies. Ethnic origin may be important in these conditions, symptomatic lactase deficiency being classically more common amongst Chinese and Africans than in Caucasians.

An important clue to the role of a foodstuff in causing a patient's problem can be derived from the resolution of these problems when an offending foodstuff is eliminated from the diet.

6.1 Specific diagnostic tests

Diagnostic procedures for allergic disease of the gastrointestinal tract in childhood have been detailed by several professional bodies (AAAAI, 1998; ESPACI and ESPGHAN, 1999; EAACI, 1999; AAP, 2000; AGA, 2001; Bindslev-Jensen and Poulsen, 1998; Daniel, 1990; Leinhas *et al.*, 1987; Kalach *et al.*, 2001; Wershil *et al.*, 2002; Bachert and Cauwenberge, 2003). The following tests are commonly used for the diagnosis of food allergies and coeliac disease.

6.1.1 Skin prick test

In cases of suspected IgE-mediated immunological reactions to food a skin prick test may be performed. In it a small amount of an allergen in solution is placed on the skin and then introduced into the epidermis by gently pricking the skin surface. A positive reaction is manifested as the development of a wheal, the diameter of which can be measured to grade the reaction. The diagnostic accuracy of a skin prick test in suspected food allergies varies

according to the possible offending food. Negative reactions have a 95% accuracy of there not being an IgE reaction; however, positive tests have only a 50-60% predictive accuracy.

There is a need for standardisation of allergens and of derived test materials, as well as protocols to facilitate epidemiological and other multicentre studies of adverse reactions to foods, particularly allergic reactions. Care must be taken during these investigations as severe reactions can be precipitated, and the tests should be done under the supervision of health care professionals.

6.1.2 Measurement of specific serum IgE antibody levels

The radioallergosorbent test (RAST) and derived immunochemical tests are used in determination of food-specific serum IgE antibodies. These tests correlate variably with the diagnosis since their clinical sensitivity and specificity vary according to the conditions used. However, depending on the incriminated food, sufficient levels of specific IgE are a good indication as to prevent oral provocation test in highly sensitised patients (Sampson and Ho, 1997; Rancé *et al.*, 2002b; Bernard *et al.*, 2003).

In order to confirm the specificity of the binding of serum IgE to the test food allergen, radioallergosorbent tests are sometimes complemented by inhibition studies where the IgE-binding capacity is inhibited by various competitors that are related with the incriminated food.

The lack of standardisation of tests for the determination of antibodies to dietary antigens, and the lack of discrimination between high and low affinity antibodies have made quantitative evaluation of different studies difficult. There is a need for their optimisation to facilitate both the interpretation of published studies and patient management.

6.1.3 Food challenge

The diagnosis of IgE-mediated and other immunologically-mediated adverse reactions to food can only be confirmed by exclusion of the suspected food substance with amelioration of symptoms and their subsequent recurrence on re-introduction of the offending food. This can be done on an open basis or by single-blind challenge when the offending food is given to the subjects who are kept unaware of what they are being given. Open challenge is less convincing than a single-blind challenge. Ideally, the condition should be evidenced by the DBPCFC, which is considered the standard because all objective and subjective bias is removed. However, DBPCFCs are costly, time-consuming and not easy to do. A DBPCFC is sometimes impractical as a diagnostic tool, as there is a risk of severe reactions.

6.1.4 Diagnostic tests for coeliac disease

The standard for diagnosing coeliac disease is small intestinal biopsy. Serological test (IgA transglutaminase antibodies) help in diagnosis and screening.

6.1.5 Atopy patch test

The atopy patch test identifies allergens which may be involved in causing atopic dermatitis. It involves application of an allergen under an occlusive dressing for 48 hours onto non-affected part of the patients' skin. The test area is then examined for reddening and consistent

with a delayed hypersensitivity reaction. Confirmation of the result is needed by a food elimination and challenge test (Perackis *et al.*, 2003).

6.1.6 Tests of respiratory function

Tests of respiratory function will be useful where respiratory signs and symptoms are present in immunologically-mediated adverse reactions to food. Such tests may include those for assessing narrowing of the airways and/or inflammation (bronchopulmonary provocation).

6.1.7 Other tests in immune-mediated adverse reactions to food

Indicators, such as flow cytometric studies of peripheral blood mononuclear cells and IgE in faecal extracts (Abernathy Carver *et al.*, 1995; Jaffe *et al.*, 1994; Kolmannskog *et al.*, 1984; O'Mahony *et al.*, 1990) may identify groups of food allergic patients but their usefulness in the diagnosis of food intolerance in the individual remains to be demonstrated. A more detailed analysis on a cellular level (Beyer *et al.*, 1997) may increase the diagnostic power of *in vitro* tests.

III. EPIDEMIOLOGY OF FOOD ALLERGY

Though food allergy and/or food intolerance are considered to be a relatively frequent medical problem, there are no systematic data that would enable the accurate calculation of the incidence and prevalence of adverse reactions to food and food ingredients in the European countries. Difficulties in the estimation of the frequency of food allergies in the general population stem from the following facts:

- The data available usually refer to hospital populations of sensitive individuals among whom food allergies are more common than in the general population. Population-based studies are required to assess the true incidence and prevalence of food allergies.

- The standard for the diagnosis of food allergy, i.e. the DBPCFC can be implemented only under strict conditions and is often avoided in highly sensitised subjects.

- Other diagnostic criteria are used, ranging from the questionnaire reported self-perception of food allergy, to positive skin prick, or specific IgE tests. These criteria often lead to overestimation of the prevalence of clinically relevant food allergy. On the other hand, absence of an IgE-mediated reaction does not exclude the possibility of food allergy.

- In many studies, allergy (and presumably non-immunologic intolerance) is not differentiated.

- There are serious discrepancies in the way the International Classification of Diseases (ICD) coding is used to characterise and classify food allergic reactions across Europe (WHO, 1975 and 1993). The variety of ICD-9 and ICD-10 codes that have reportedly been used in 11 EU countries to identify food allergies is presented in Table 2. It should also be noted that in the 9th version (ICD-9), there is no specific reference to food allergy, apart from dermatitis due to ingested food.

In its report on adverse reactions to foods and food ingredients, the SCF estimated that among the general population approximately 1-3% is allergic to foods (SCF, 1995). A few years later, in the context of the EU Scientific Cooperation Task 7.2 available data on food allergies from 11 EU countries were evaluated (EU SCOOP Task, 1998). Notwithstanding the paucity of data, the report showed that there is a wide difference between the number of adults who perceive that they are intolerant to food (between 3.8% and 25%) and the number confirmed with food challenge (0.8-2.4%). National prevalence data on food allergy and intolerance in children were scant in all countries. Perceived levels of intolerance ranged from 3.8-27%, but challenge testing limited the prevalence to 1.7% at age 0-6 months in the Netherlands, and 6% at age 18 months in Denmark.

The overall occurrence of food allergy changes with age, and so do the specific allergies (Kagan, 2003). Egg and milk allergy are quite common among infants, but are most often (but not always) rapidly outgrown. Shellfish allergy is an example of a specific allergy that is more common among adults than children, while peanut allergy is common among children as well as adults. The age dependency of food allergy is part of an only partly understood pathophysiological phenomenon, the so-called allergic march. The allergic march is a name for the observed common age-dependent sequence of allergic manifestations: food allergy, atopic dermatitis, asthma, and allergic rhino-conjunctivitis. The change with age of some

specific allergies may partly be explained by exposure factors. Milk consumption is high for small children, while shellfish is consumed more commonly by schoolchildren and adults. However, part of the change in specific allergies with age might be due to immunological and physiological maturation processes.

Table 2. ICD codes presumed to have been used for the classification of food allergic reactions in 11 European Union countries

Code	Diagnostic description
ICD-9	
693.1	Dermatitis due to ingested food
995.0	Anaphylactic shock
995.1	Angioneurotic oedema
995.3	Allergy, unspecified
E865	Accidental poisoning from foodstuffs and poisonous plants
490	Bronchitis
491	Chronic bronchitis
492	Emphysema
493	Asthma
493.0	Asthma, extrinsic
493.1	Asthma, intrinsic
493.9	Asthma, unspecified
495.9	Unspecified allergic alveolitis
496	Chronic airways obstruction, not elsewhere classified
995.2	Unspecified adverse effect of drug, medicament and biological substance
ICD-10	
L27.2	Dermatitis due to ingested food
T78.0	Anaphylactic shock due to adverse food reaction
T78.1	Other adverse food reactions, not elsewhere classified
T78.2	Anaphylactic shock, unspecified
T78.3	Angioneurotic oedema
T78.4	Allergy, unspecified

Source: EU SCOOP Report of experts participating in Task 7.2, 1998

There is little information on gender differences in food allergy in children. In adults, a clinical impression is that food allergy is somewhat more common in women (Schäfer *et al.*, 2001). Of the first 250 cases reported to the Norwegian National Reporting System and Register of Severe Allergic Reactions to Food, the female to male ratio was about 3:2 (Løvik and Namork, 2004). The putative gender difference could be due to physiological differences or to differences in health-seeking behaviour.

The prevalence of food allergy is highly dependent on geographical area. For example allergy to hazelnut is more common in areas where birch pollen is abundant, because of the birch pollen-hazelnut cross-reactivity. The dietary patterns in a given country are also of importance (for instance, fish allergy appears to be more common in areas where much fish is consumed, like Norway, Portugal and Japan).

Several studies indicate that 75% of allergic reactions among children are due to a limited number of foods, namely egg, peanut, cows' milk, fish and various nuts. Among adults, the main foods which are responsible for 50% of allergic reactions are fruits of the latex group

(kiwi, banana, etc.), fruits of the *Rosaceae* family (apples, pears, prunes), vegetables of the *Apiaceae* family (carrot, celery, etc.), and various nuts and peanuts (Kanny *et al.*, 2001; Dubuisson *et al.*, 2002). It should be noted that manifestation of food allergy is by definition interactive. It requires the susceptibility of the host and the exposure to the allergen. Little is known about the variability of genetic susceptibility among populations, or the factors that may modify allergic response (see Chapter IV). Differences in potential of exposure, however, may limit the generalisability in the European population of data from any single European country on the most common allergenic foods.

There is clear evidence that the prevalence of atopy has increased over the last decades (Linneberg *et al.*, 2000; Strannegard and Strannegard, 2001; Kosunen *et al.*, 2002). Although for food allergy this evidence is lacking, it is assumed that this increased prevalence extends to it as well (Moneret-Vautrin and Kanny, 1995a; COT, 2000; Dubuisson *et al.*, 2002). A published study reporting data from hospital admissions in the UK provides support towards an increasing frequency of food allergies (Gupta *et al.*, 2003).

The prevalence of food allergy in the general population has been roughly estimated to be around 1-3% in adults and 4-6% in children. Thus, it appears that food allergy can affect the lives of a considerable number of people in Europe with conditions ranging from very mild to potentially fatal. Given the apparent public health significance of the problem and the paucity of the existing relevant epidemiological data, population-based studies and standard diagnostic criteria are required to assess the true incidence and prevalence of food-allergic diseases.

IV. INFLUENCE OF GENETIC, GEOGRAPHICAL AND OTHER FACTORS IN THE DISTRIBUTION OF FOOD ALLERGIES

Allergies are among the most common environmentally determined diseases. The occurrence of allergies follows the general rules in environmental medicine: the outcome -health or disease- is determined by complex interactions of exposure factors and personal susceptibility determinants of the exposed individual. This rule also applies to food allergy. It should be mentioned already here, however, that unknown factors governing the prevalence of allergy in general also appear to be very important for the prevalence of food allergy. Food allergy in general will, therefore, to a large degree show geographical variations corresponding to the variations of allergies in general. This general variation is then modified by regional variations in prevalence of allergies to specific foods which are caused by local factors, like pollen exposure and differences in food habits.

1. EXPOSURE FACTORS

Some foods are more allergenic than others. That means they have a greater intrinsic capacity to induce allergic sensitisation and elicit allergic reactions than less allergenic foods. Examples of highly allergenic foods are peanuts, sesame seeds and shrimps. Other foods, for example potatoes, more rarely induce allergy in spite of high levels of consumption.

1.1 Allergen exposure

The amount consumed is considered an important determinant for allergy. This factor has two levels: the eating habits and the protein allergen level of a particular food.

1.1.1 Eating habits

In an area where a certain food is commonly consumed, the risk of allergy to that food will be larger than in areas where that particular food is more rarely eaten. For example, fish allergy appears to be most common in areas where much fish is consumed, like Norway, Portugal and Japan. Shrimp allergy is common in the southern states of USA where shrimp consumption is high. Peach allergy appears to be most common in countries where peach is a common part of the diet, like Spain, Portugal and Italy, and is rare in countries where the consumption of peaches is low, like the Nordic countries. Mustard allergy appears to be particularly common in France and Spain, while problems with celery allergy are most often experienced in Germany, Switzerland and France.

If a food is commonly eaten, not only will the induction of allergy to that food be more likely, but allergic reactions will also more frequently be triggered than if the food is rarely eaten, and allergy to that particular food will also for that reason appear to be more common.

The dose-response relationship for the consumption of a specific food and the development of allergy (sensitisation) to that food is unknown. A certain amount of consumption of the particular food is necessary for an allergy to develop, but it is not known if the dose-response curve (or parts of it) is linear or (for example) exponential, or whether it levels out at some level of exposure. The dose-response relationship is very dependent on genetic and other individual factors.

1.1.2 Protein allergen level

On the protein allergen level, common proteins that are present in large quantities in a food will statistically have a greater chance of showing up as allergens than proteins that are present in small quantities. Storage proteins of many nuts and seeds are an example. These proteins may sometimes make up half the weight of the seed or nut. The amount of some allergens will depend on plant variety and growing conditions -this may contribute to geographical variation of some food allergies, and to variation in allergenicity of a given plant product (Codina *et al.*, 2003; van Amerongen *et al.*, 2002).

1.2 Food processing and preparation

Processing and preparing food may increase or decrease allergenicity. The same raw product may be processed and prepared in different ways, according to local traditions and the socio-economic setting -in the broad sense depending on local cultural factors. An example is peanuts- the way of preparing peanuts by dry roasting has been documented to produce a more allergenic food than for example the Chinese way of preparing peanuts by frying or cooking (Maleki *et al.*, 2000; Beyer *et al.*, 2001).

1.3 Co-factors for the development of allergy and triggering of allergic reactions

Infections and other conditions increasing "leakiness" over the intestinal mucosa may also increase allergen uptake, and has traditionally been believed to influence the development of allergy. The importance of the mentioned factors is, however, still uncertain. Food allergic reactions are known to be facilitated by physical exercise, alcohol, non-steroid anti-inflammatory drugs (NSAIDs) and cold drinks. The fat content of the challenge vehicle has been found to have a profound influence on the clinical reaction experienced after allergen ingestion in double-blind placebo-controlled food challenge (Grimshaw *et al.*, 2003). This is an example of the so-called "matrix effect", which means that the material surrounding the food allergen when the food is eaten can have an effect on whether or not an allergic response will develop, how fast it will develop and how strong it will be. Therefore, the dish eaten and food habits may influence to some extent that an allergen in the food will trigger a reaction.

1.4 Cross-reacting allergens

An important co-factor for food allergy is the exposure to cross-reactive allergens. The best example of this is pollen exposure (e.g. birch pollen, mugwort) which will often induce respiratory allergy to the pollen. Because of cross-reactive allergens, pollen allergy will also influence the occurrence of food allergy, e.g. allergy to apples, peach, and carrots. In particular, this allergy will manifest itself as the so-called oral allergy syndrome, also called the pollen-food allergy syndrome. Most often, the condition manifests itself with relatively harmless manifestations from the mouth when the food is eaten, but sometimes the reaction may be more severe. In an area where sensitivity to birch and mugwort pollen is prevalent, 30 to 50% of these patients will experience symptoms when ingesting fruits and vegetables. Therefore, in this area, the prevalence of food allergy in adults will be higher than in a similar area without the pollen exposure.

An example of the pollen-food allergy syndrome is the relationship between birch pollen (respiratory) allergy and hazelnut (food) allergy. Because of birch pollen-hazelnut cross-reactivity, food allergy to hazelnut will be more common in areas where birch pollen is

abundant and the finer allergen specificities and possibly also the clinical character of hazelnut allergy will differ between the areas. In Northern Europe, where birch pollen is common, most cases of hazelnut allergy develop secondarily to birch pollen allergy because of an immunological cross-reactivity between birch and hazelnut. In contrast, in Southern Europe, where birch pollen allergy is rare, hazelnut allergy more often is a primary allergy.

2. INDIVIDUAL SUSCEPTIBILITY DETERMINANTS FOR FOOD ALLERGY

2.1 Genetic factors - atopic predisposition

Atopy predisposes to food allergy. Atopy appears to be determined by a rather large number of genes, with no dominating gene having been identified so far. Rather, the prevalent view is that different combinations of genetic and environmental factors are important for allergy development at different locations and in different situations.

2.2 Genetic factors - specific immune-response genes

A limited number of reports describe association of specific food allergies with specific HLA types. Examples are an association of HLA-DQ7 with cows' milk protein allergy in Italian children (Camponeschi *et al.*, 1997), HLA-DRB1, DQB1, and DPB1 with peanut allergy (Howell *et al.*, 1998), and HLA-DRB1*08 for peanut allergy (six-fold increased risk) and HLA-DRB1*12 (13-fold increased risk) for carrot allergy (Boehncke *et al.*, 1998). Sénéchal *et al.* (1999) described involvement of HLA-DR7 in the allergic response to apple and pollen, but concluded that the increased susceptibility conferred by HLA-DR7 was more related to atopy than to allergen-specific responses. Hypersensitivity to the fish parasite *Anisakis simplex* has been found to be associated with the HLA-DRB1*1502-DQB1*0601 haplotype (Sánchez-Velasco *et al.*, 2000). Some HLA-types show distinct geographical variation. This variation could, in principle, also contribute to the geographical variation of food allergy.

2.3 Ethnicity

Differences of food allergy due to ethnicity could be caused both by genetic differences (e.g., in the HLA system), by different food habits, and by a low prevalence of allergy in the country of origin in the first years of life if immigration has taken place. Food habits clearly are important in some cases, e.g. birds' nest allergy is predominantly a problem in ethnic Chinese people. Information on food allergy in general in different ethnic groups is scarce. It appears that immigrants to Europe from less developed countries generally have fewer atopic diseases when coming to Europe, but over a decade gradually will adapt to the new environment and become more similar to people who have grown up in the country in question (Kalyoncu and Stalenheim, 1992). In the European Community Respiratory Health Survey (Tobias *et al.*, 2001) it was found that immigrants as a group had similar levels of atopy as non-migrant Europeans. Some studies, however, have found that certain groups of children of immigrants are at relatively greater risk for developing allergies than the population in general, as has been reported for Somali immigrants in Italy (Asseyr and Businco, 1994). There also are reports that food as a trigger of asthma is more common in Asian children than in Caucasian children (Wilson, 1985).

V. GENERAL CONSIDERATIONS ON THE STRUCTURE OF FOOD ALLERGENS

There is no definite relationship between the structure and biological function of a protein and its allergenicity. Most of the known food allergens have a protective or storage function. Some may have a metabolic and structural role. They belong to protein families which have conserved structural features in relation with this biological activity. Storage proteins are the cause of most allergenic reaction to legumes and cereals. Among those storage proteins globulins are very abundant. The globulin fractions of seed storage proteins can be extracted with saline solutions. According to their sedimentation coefficient determined by ultra centrifugation, they are divided in a smaller fraction, i.e. 7S globulins also known as vicillins and a bigger one i.e. 11S globulins or legumins. 7S globulins (e.g. peanut Ara h 1) are in general trimers with subunits with a molecular weight around 50-60 kDa. Post-translational modifications such as glycosylation often occur. 11S globulins are formed by associations of 6 subunits with a molecular weight around 60 kDa, each of them being formed by a non-covalent association of two polypeptide chains. They are rarely glycosylated. Both 7S and 11S fractions share conserved sequence motifs comprising identical or chemically similar amino acid residues and a common 3D conformation. This tertiary structure called β barrel structure is made of series of anti parallel β sheets associated with α helices which form a cavity (a cup) comprising a binding site for hydrophobic ligand (Breiteneder and Ebner, 2000).

Interestingly, it is noteworthy that many allergens of animal origin also share this β barrel structure which is a characteristic of the lipocalin family involved in the transport of hydrophobic ligands (e.g. including milk β-lactoglobulin).

Several allergens of plant origin also belong to the 2S albumin family (Mills *et al.*, 2004). It includes several groups of low molecular weight proteins (e.g. conglutinins as Ara h 2). They are characterized by a high content in sulphur-containing amino acid residues and are often constituted of 2 polypeptide chains organized in a structure rich in alpha helices stabilized by disulphide bonds involving 8 well-conserved cysteine residues. The major role of 2S albumins is to provide proteins to the developing seed. They also have a defensive role against pathogenic fungi (see below). Storage proteins also include the prolamin family which contains the major storage proteins of gluten-containing cereal grains. They are characterized by a high content in proline and glutamine. Because of the conserved similar 3D structure rich in alpha helices, 2S albumins are also classified within the prolamin super family as well as lipid transfer protein (LTP) (Pastorello *et al.*, 2002b).

The lipid transfer protein family is made of low molecular weight monomeric proteins (around 7-9 kDa). They are supposed to be involved in the synthesis of cutin and have therefore a protective role in the plant, and particularly in the fruit. Lipid transfer proteins have common structural features also shared by other members of the prolamin super family. They have high amino acid sequence homologies, conserved motifs of cysteine residues involved in disulphide bonds. They have a very compact and stable tertiary structure made of association of alpha helices and loops stabilized by 8 disulphide bonds. This defines a central cavity which contains a lipid binding site. Binding with hydrophobic ligand also contributes to the stabilization of the molecule.

Lipid transfer proteins are frequent and potentially severe allergens; they are also listed as one of the numerous defence protein families also called pathogenesis-related proteins that are responsible for most of the allergic reactions to fruits from the *Rosaceae* family.

A compact 3D structure, ligand binding, and glycosylation are factors which contribute to protein stability which is sometimes related to allergenicity. However it should be pointed out that labile proteins with loose structure, sensitive to protease degradation may also be potent allergens (e.g. milk caseins).

VI. ANALYTICAL METHODS FOR ALLERGEN DETECTION

1. FOOD ALLERGENS: INTRODUCTION AND NOMENCLATURE

Many allergenic foods from plant and animal sources have been reported to cause allergic reactions after contact, inhalation or ingestion in humans. Combination of food science and medical science today allows defining the relevant food allergens contained in these food sources chemically. Food allergens are proteins and most are glycoproteins. Several food allergens have been isolated, purified and characterised in detail. International allergen nomenclature by International Union of Immunological Societies (IUIS allergen nomenclature [http://www.allergen.org, March 02, 2003]) indicates allergen source and species (e.g., *Arachis hypogaea* for peanut), the systematic allergen name (e.g., Ara h 2), the biochemical name (e.g., conglutinin) and the molecular weight (e.g., 17 kDa) for known food allergens.

Food allergens are natural substances with different biological properties. Some are seed storage proteins, some are transport proteins or regulatory proteins and some are enzymatically active (proteases, carbohydrases, enzyme inhibitors). Epitopes are the active part of an allergenic molecule inducing immune response and causing an allergic reaction. Sequential and conformational epitopes are present in food allergens. Sequential epitopes are mostly recognised by T cells whereas conformational epitopes are generally affecting B cells, leading, for example, to the production of specific IgE. IgE-binding analyses using human sera is an important tool in identifying allergens and their relevant epitopes.

2. FOOD ALLERGENS: ANALYTICAL METHODS AND DETECTION LIMITS

Reliable detection and quantification methods for food allergens are necessary in order to ensure compliance with food labelling and to improve consumer protection (Besler, 2001; Poms *et al.*, 2004). Physicochemical methods have been described as well as immunological methods (Table 3). The food sample extraction procedure (using solvents and buffers) is a decisive initial step in food allergen analysis with a strong impact on recovery and performance of the subsequent detection system.

Table 3. Methods in food allergen analysis

Physicochemical methods	**Immunological methods**
Kjeldahl nitrogen	Immunodiffusion
Nephelometry, high performance liquid chromatography (HPLC)	Counterelectrophoresis
	Radioimmunoassay
Mass spectrometry	Enzyme-linked immunosorbent assay (ELISA)
Capillary electrophoresis	Radioallergosorbent inhibition
PCR for allergen-specific DNA	Immunoblotting

PCR = polymerase chain reaction

Any given analytical method should be based on a defined reference material and a reliable standard curve. The usual criteria of sensitivity, specificity, reproducibility, precision and accuracy have to be fulfilled. Still, there remain problems of cross-reactivity, of matrix effects and of food processing (Poms, *et al.*, 2004; Stern, *et al.*, 2001). The most frequently used technique is ELISA. However, it has to be kept in mind that immunogenicity and pathogenicity of the given food allergen are not necessarily identical and that any given

analytical method should be backed up by sufficient clinical information, e.g., taking into account matrix effects and thresholds levels (Grimshaw *et al.*, 2003). Usually immunological results should be confirmed by physicochemical methods. Radioallergosorbent inhibition and immunoblotting using human sera can be used to confirm allergenicity. Recently, PCR and ELISA have been combined and biosensor technology has been applied to detect food allergens with high sensitivity. Physicochemical methods are not necessarily related to the biological activity of the allergen in question. Biological activity may remain when the protein is denatured. Assays that have been developed for native proteins may but do not always work with processed food allergens (Negroni *et al.*, 1998).

3. DETECTION OF RELEVANT FOOD ALLERGENS

3.1 Cereals

Cereals with regard to gluten causing coeliac disease are a highly heterogeneous group. Daily intake of 100 mg of gliadin (the alcohol soluble fraction of gluten) has been found to induce clinical symptoms. However, for lack of conclusive clinical data the current Codex Alimentarius limit for gluten-free foods of 200 mg gluten/kg food may require reconsideration in the future (Stern, *et al.*, 2001). For gluten analysis in food, a European Gliadin Reference is now available and currently under certification by the EC Institute for Reference Materials and Measurements (IRMM-480) (van Eckert, 2002). An ELISA assay has been described with increased sensitivity and specificity by Valdés *et al.* (2003). This system is clearly superior to older methods (Denery-Papini *et al.*, 1999; Skerritt and Hill, 1991). It is based on a defined standard, has a detection limit relevant to known threshold data and may be solving problems of standardisation and accuracy particularly in the low gluten concentration range.

With regard to IgE-mediated food allergy, cross-reaction of cereals with grass pollen is an important issue. All the major cereals have been shown to elicit food allergies (to be differentiated from coeliac disease). Only some cereal allergens have been directly identified (Palosuo *et al.*, 1999; Pastorello *et al.*, 2000). There is no known threshold and no immunochemical method has been reported to analyze foods for non-gluten cereal allergen components.

3.2 Crustaceans

Crustacean food allergens include shrimp, prawn, crab and lobster. Tropomyosin is the major shrimp allergen. Several additional allergens have been identified and there is an important cross-reactivity between crustaceans and house dust mite (Kutting and Brehler, 2001). A shrimp tropomyosin ELISA for food products (Ben Rejeb *et al.*, 2002) and a shrimp allergen ELISA for clinical extracts (Jeoung, *et al.*, 1997) have been described. Currently there is one ELISA kit for tropomyosin commercially available (Poms, *et al.*, 2004).

3.3 Fish

Fish allergens are among the first isolated, crystallised and identified on a molecular level (Gad c 1, parvalbumin containing at least five IgE-binding epitopes) (Aas, 1976). Fish parvalbumins are abundant and stable. A radioimmunoassay has been described to identify

fish allergens (Taylor *et al.*, 2002). However, no ELISA assay is available for the detection of fish allergens in food.

3.4 Eggs

Hen's eggs contain several major and minor allergens. In egg white and yolk, ovomucoid (Gal d 1) and ovalbumin (Gal d 2) are of prime importance. Several immunodiffusion and ELISA methods have been described for detection of egg proteins. Detection limits were between 0.02 mg/kg to 300 mg/kg (Baumgartner, *et al.*, 2002; Hefle *et al.,* 2001; Malmheden Yman, *et al.*, 1994; Yeung, *et al.*, 2000; Sato *et al.,* 2001; Leduc, *et al.,* 1999). Additionally, there are several egg ELISA test kits commercially available which target either total egg protein, ovomucoid or ovomucoid and ovalbumin together with detection limits between 0.3 and 2.5 mg/kg.

3.5 Peanuts

Several allergenic molecules have been identified and ELISA methods have been described to determine peanut allergens including Ara h 1 and Ara h 2 (Hefle *et al.*, 1994; Yeung and Collins, 1996; Koppelman *et al.*, 1996; Holzhauser and Vieths, 1999; Mills *et al*., 1997; Newsome and Abbott, 1999; Stephan *et al.*, 2002; Pomés *et al.,* 2003). The detection limit is around 1 mg/kg, however, detectability is affected by the food matrix and the processing history of the food (Poms *et al.*, 2003). PCR methods are also available (Poms *et al.,* 2004) There are also systems for multiple nut allergen detection (Ben Rejeb *et al.,* 2003; Blais *et al.*, 2003; Pomés *et al.*, 2003).

3.6 Soy

A number of soy allergens have been identified. Physicochemical methods (López-Tapia *et al.,* 1999) and immunochemical methods (e.g., Hitchcock *et al.,* 1981; Griffiths *et al.*, 1984; Rittenburg *et al.*, 1987, Yasumoto *et al.,* 1990; Macedo-Silva *et al.*, 2001; Heppell *et al.*, 1987; Brandon *et al.,* 1991; Tsuji, *et al.*, 1995; Bando, *et al.,* 1998) have been described. However, so far, the methods are not sensitive enough. PCR methods for soy detection with lower detection limits and commercial ELISA tests are available (Poms *et al.*, 2004).

3.7 Milk

Cows' milk allergy is a common food allergy in infants and young children. Numerous epitopes have been identified in different cows' milk proteins. Due to high sequence homology there are frequent cross-reactions between milk proteins from different species. All major milk allergens can be detected with immunochemical methods based on immunoelectrophoresis, immunoblotting and ELISA. The detection limit range is as low as 2.5 mg/kg (Malmheden Yman *et al.*, 1994; Mäkinen-Kiljunen and Palosuo, 1992; Plebani, *et al.*, 1997) or even less (ng/kg) for β-lactoglobulin (Negroni, *et al.*, 1998). Numerous ELISA kits for the detection of milk proteins or specific milk allergens are commercially available (Poms *et al.*, 2004)

3.8 Nuts

Nuts are important food allergens with potentially severe reactions. Several allergens have been described and some have been cloned. For most nuts ELISA methods have been

described with high sensitivity and detection levels reaching as low as 0.2 mg/kg (Almond: Hlywka *et al.*, 2000; Roux *et al.*, 2001; Brazil nut: Clemente *et al.*, 2004; Cashew nut: Wei *et al.*, 2003; Hazelnut: Scheibe *et al.,* 2001; Koppelman *et al.*, 1999; Holzhauser and Vieths, 1999; Blais and Phillippe, 2001; Ben Rejeb *et al.,* 2003; Stephan *et al.,* 2002; Walnut: Niemann and Hefle, 2003). For specific nut allergen detection various ELISA test kits and DNA-based PCR and PCR-ELISA methods are commercially available (Poms *et al.*, 2004).

3.9 Celery

Different celery allergens have been identified (Api g 1, Api g 4, Api g 5 and the panallergen profilin and cross-reactive carbohydrate determinants). There is a protein homology between Api g 1 and a birch allergen. Celery is a frequent ingredient in processed foods (Ebner *et al.*, 1995; Breiteneder, *et al.*, 1995). There is no common method available for the quantification of celery allergens in foods.

3.10 Mustard

Only recently mustard has become known to be a common food allergen. Two components Sin a 1 and Bra j 1 have been identified as individual allergens with similar amino acid composition. No specific detection method has been described.

3.11 Sesame

Of 3 identified sesame seed allergens, Ses i 1 and Ses i 2 are 2S albumins with partial homology to allergens from sun flower seeds, castor bean and Brazil nut. For sesame allergens immunochemical assays with a detection level below 1 mg/kg of food have been reported (Brett *et al.*, 1998) and detection kits are commercially available (Poms *et al.*, 2004).

4. DETECTION LIMITS FOR INTENTIONALLY-ADDED INGREDIENTS IN FOOD

Residual amounts of food allergens after processing, even traces, can cause clinical reactions. For detection of derived products there are generally no known tolerable threshold doses. Analytical methods developed for native proteins do not necessarily measure derivatives. Non-intentionally-added ingredients and contaminants (Miller, 1978) are beyond the scope of this Opinion.

5. CONCLUSION

Many test systems are in use and commercially available for food allergen analysis, most of which rely on immunochemical methods. Major problems remain with matrix effects, insufficient extraction, detection limits above the range of clinical reactivity, insufficient specificity due to cross-reaction and insufficient reproducibility of results. Therefore a systematic approach of method validation should be followed implying reference materials and robust reproducible test systems.

VII. POSSIBLE EFFECTS OF PROCESSING ON ALLERGENICITY OF FOODS

It has been suggested that food processing could reduce the allergenicity of some foods and it has been subsequently proposed that this might serve as a tool for a reduction of the allergenic potential of processed foods.

The rationale behind this concept is that allergenic constituents of a food are proteins. Heat and other treatments alter the structure of proteins and, as a consequence, alter their allergenic potential and therefore the allergenicity of the whole food. This view relies on several tacit assumptions. It assumes that there is a definite relationship, or at least a high correlation, between the structure and physicochemical properties of a protein and its allergenicity and that decreasing the allergenicity of one allergenic protein component of a food will reduce the allergenicity of the whole food, e.g. all proteins will react similarly to the processing. It thus does not take into account the variability of the allergen repertoire of a whole food and the multiplicity of the allergenic structures within the whole food. Moreover, it assumes that this reduction of allergenicity will be sufficient to prevent any allergic reaction to the modified food in a large population of allergic patients and ignores the genetic/geographic variability of the immune response in atopic humans. None of these assumptions is well founded, as will be discussed in this chapter.

1. ALLERGENIC FOODS *vs* ALLERGENS IN FOODS

The term "food allergen" refers both to the complex whole food and to the chemically defined compounds that are responsible for allergenicity, i.e. the proteins. Allergenicity of a given complex food is never due to a single protein component but to numerous different proteins which constitute the allergen repertoire of the food. Due to the diversity and variability of the human IgE response all of them are not always recognised by all patients allergic to this food. Those that are recognised by more than 50% of a population of allergic patients to the food are termed major allergens. This concept relates only to the frequency of recognition of IgE antibodies but it is not related to the severity of the clinical manifestations of an allergic reaction. Clinical reactions may be similar whether they are triggered by major or minor allergens. The allergenicity of each single protein is due to numerous molecular immunoreactive structures, i.e. the IgE-binding epitopes that are widespread within the protein molecule. The characterisation of the different epitopes on an allergen molecule is called the epitope mapping. Similar to allergens, not all epitopes are recognised by all the patients allergic to the protein, some are immunodominant while others are only recognised by a few patients.

Depending on their structure, two kinds of epitopes are described. Some are conformational because they are associated to the secondary and tertiary structure of the protein. Once the protein is denatured, conformational epitopes are modified or destroyed. Other epitopes are linear because they are formed by a sequence of amino acid residues on the peptide chain of the protein. It has recently been shown that some epitopes may have a particular clinical significance depending on their structure and location within the molecule. Short linear IgE-binding epitopes which may be located in hydrophobic parts of allergenic proteins could be used as markers of a persistent food allergy, i.e. to milk and to peanut (Chatchatee *et al.*, 2001). Such characterisation of epitopes, and particularly IgE-binding epitopes, may in the future provide information on persistence and severity of clinical reactions.

There are therefore 3 levels of structures (whole food, protein and epitopes) involved in the interaction with IgE antibodies and responsible for allergenicity of a given whole food. The situation becomes even more complex due to the possible allergic cross-reactions which exist between foods or between foods and pollen. As an example frequent cross-reactions are observed between birch pollen and hazelnut, apple and more generally fruits of the *Rosaceae* family. Cross-reactions are also observed between pollen of compositae (mugwort) and celery. Also important are foods that cross-react with latex, e. g. chestnut, walnut, kiwi, banana, avocado.

Those cross-reactions are due to the presence of proteins that share similar sequence homology and/or structural features or common epitopes. They involve common highly conserved structures which generally correspond to important biological activities and functions that are vital for the plant. Foods belonging to the latex group all possess defence proteins (chitinase) that all share a common "hevein" domain which is also present in the latex prohevein and that is responsible for most of the cross-reactivities.

Although there is no clear relationship between the function of a protein and an allergenic potential, almost all known food allergens have a metabolic, protective (defence) or storage function. They are also generally considered proteins with a globular compact structure stabilised by hydrogen and disulphide bonds, often glycosylated, stable to processing, and resistant to proteolysis by digestive enzymes.

2. EFFECTS OF HEAT TREATMENTS AND PROCESSINGS ON PROTEIN STRUCTURE AND ON ALLERGENICITY

Significant alterations in protein structure do occur during heat treatments, the nature and extent of such changes being dependent on the temperature and duration of the thermal processing as well as on the intrinsic characteristics of the protein and the physicochemical conditions of its environment (e.g. pH). Typically loss of tertiary structure is followed by (reversible) unfolding, loss of secondary structure (55-70°C), cleavage of disulphide bonds (70-80°C), formation of new intra-/inter-molecular interactions, rearrangements of disulphide bonds (80-90°C) and the formation of aggregates (90-100°C) (Davis and Williams, 1998). These modifications reflect a progressive passage to a disorganised structure with denaturation of the proteins that adopt an unfolded, random-coil conformation. Besides those physical transformations, chemical modifications of the protein may also occur at high temperatures (100-125°C and higher). These may involve formation of covalent bonds between the lysine residues of a protein and other constituents of the food matrix leading to various adducts.

It appears that there are no general rules regarding the consequences of thermal treatment on allergenicity. Some allergens or, more properly, some allergenic foods, are described as heat stable (e.g. milk, egg, fish, peanuts and products thereof), while others are considered partially stable (e.g. soybean, cereals, celery, tree nuts and their products) or labile (fruits of the *Rosaceae* family and carrots) (Besler *et al.*, 2001). In addition, thermal processing can create new allergenic epitopes as well as destroy existing epitopes. As an example Maillard reaction contributes to the formation of new immunologically reactive structures (neo allergens) (Davis and Williams, 1998; Berrens, 1996). Whether and how heat treatments may significantly alter the allergenicity of a food is thus a complex question.

In a comprehensive and informative study on a population of patients allergic to birch pollen and to raw hazelnuts, Hansen *et al.* (2003) found that roasting (i.e. 140°C for 40 min) significantly reduced (approximately 100-fold) the allergenicity of Cor a 1.04 (a major hazelnut allergen) and of hazelnuts. This is in contrast to what has been previously observed with Ara h 1 (a major peanut allergen) where Ig-binding was increased approximately 90-fold in roasted *vs* raw peanuts (Maleki *et al.*, 2000). Interestingly, thermal treatment of peanuts at lower temperatures such as boiling (100°C) or frying (120°C), where heat-induced conformational changes take place but where the Maillard reaction is unlikely to occur, did not affect the allergenic properties of Ara h 1. This may explain why the prevalence of peanut allergy may depend on the dietary habits and type of cooking traditionally used in various populations of different countries, i.e. peanut allergy is rare in China where peanuts are eaten boiled or fried whereas it is frequent and more severe in western countries where peanuts are eaten roasted (Beyer *et al.*, 2001).

In addition the fact that allergenicity of a protein is not directly associated to the integrity of its native structure may hamper the detectability of food allergens and lead to inappropriate and dangerous interpretations since a potential health risk remains. As an example the major milk protein β-lactoglobulin is a globular compact protein, thermo-labile, resistant to degradation by digestive proteases. Indeed it is denatured by heat treatments and loses its 3D and 2D structure. As a consequence it is no longer detected by the classical analytical methods used for its determination in foods. Those assays generally used antibodies raised against the native form of the protein that recognise conformational epitopes but do not recognise linear epitopes which are the only ones to remain in the denatured form. However those linear epitopes are well recognised by IgE of allergic patients and the denatured β-lactoglobulin keeps all the allergenic potential. After heat treatment, the protein has denatured but the allergenicity remains although it is no longer detected unless an appropriate additional assay adapted to the modified protein is used (Negroni *et al.*, 1998).

Similar considerations apply when other processes are used, e. g. hydrolysis. It is generally recognised that hydrolysis reduces the antigenicity of a protein and protein hydrolysates are currently used in the so-called hypoallergenic formulae. However, breakdown products as short as 14-15 amino acid residue peptides have been shown to still be allergenic. In addition, if proteolysis can destroy some epitopes, particularly conformational epitopes, it can also unmask linear epitopes, that were buried into the three-dimensional native structure and/or located in hydrophobic domains of the protein. They thus become available for IgE-binding and manifestation of their allergenicity (Haddad *et al.*, 1979; Wal, 2002)

The genetic modification of food crop plants has been presented as a technology that has the potential to reduce the allergenicity of some foods. Two approaches are currently being explored. One is to down-regulate the expression of the structural genes that encode allergenic proteins. This has led to the so-called hypoallergenic rice and, more recently, soybean. The second approach is to modify the amino acid sequence of the immunodominant epitope(s) in the major allergen, by mutation of the appropriate DNA sequence, in order to reduce its IgE-binding activity. This is currently being applied to soybean and peanut. It would be necessary to ensure that resulting cultivars did not amplify alternative proteins or express novel proteins that might also be allergenic (Tada *et al.*, 1996; Yang *et al.*, 2003).

This issue of the significance of the residual allergenicity which remains after processing of the food is a general issue whatever the process and is now outlined below.

3. SIGNIFICANCE OF THE REDUCTION OF ALLERGENICITY THAT CAN BE OBTAINED VIA PROCESSING

Hansen *et al.* (2003) stressed that trace amounts of the residual protein that remain in the roasted hazelnuts can still bind IgE and elicit symptoms in highly sensitive patients and that a significant number of patients in the study population (ca. 30%) proved still to be clinically reactive.

This raises the important question of the clinical relevance of such a "limited" reduction of allergenicity and of its significance and applicability in terms of risk management and protection of allergic consumers. This is linked to the more general question of threshold doses. Bindslev-Jensen *et al.* (2002) have proposed a statistical approach to determine threshold levels from published data in the literature and then to derive an "acceptable intake" for a given proportion of an allergic population (e.g. 90%), using a paradigm similar to that used in classical toxicology. Up to now, the recorded/calculated data show that a substantial part of the population reacts to very low amounts of allergens, particularly those of the peanut, which appears even more important because patients with severe reactions react to lower threshold doses than patients with mild symptoms (Wensing *et al.*, 2002a).

Hansen's paper (2003) also gives an illustration of other issues to be answered to provide reassurance to allergic consumers. 1) Will reducing the allergenicity of one or two major allergens mechanically reduce the allergenicity of the whole food? 2) Will the heterogeneity and variability in the genetic background of individuals and in environmental conditions induce differences in susceptibility and therefore change the level of protection of sub groups of allergic patient populations within the EU? It is known that individuals in Northern and Central Europe who are allergic to hazelnuts are almost exclusively sensitised to Cor a 1.04 which is related to and immunologically cross-reacts with the major birch pollen allergen Bet v 1, whereas allergic populations of Mediterranean countries are essentially sensitised to another major allergen of hazelnut, i.e. Cor a 8, which is a lipid transfer protein (Pastorello *et al.*, 2002a). Both types of protein are present in the same food, i.e. the hazelnut, but lipid transfer proteins and Bet v 1 pollen-related allergens have different structural and physicochemical features. Non-specific lipid transfer proteins belong to a widespread family of plant proteins that share similar characteristics: they are heat-stable and resistant to proteolytic degradation by digestive enzymes, whereas Bet v 1-related allergens are altered during thermal treatments and quite rapidly and extensively hydrolyzed by proteases. This makes a considerable difference to the every day life of allergic patients and in the management of their allergy. "True" food allergies to lipid transfer proteins and to foods containing lipid transfer proteins elicit more severe clinical manifestations than allergic reactions to the same foods due to pollen cross-reactive allergens and they require the compliance with a very strict diet which should avoid any product from a particular food even after processing and heat treatment. The same processing of the same allergenic food may thus differently affect two distinct populations who are not sensitive to the same allergenic constituents of the food.

4. CONCLUSION

It is difficult to anticipate the effects on allergenicity of protein/food modifications due to thermal treatments as well as to any (bio) technological process used in the food industry for

production, storage and processing (Poms and Anklam, 2004). Moreover, the impact may differ completely from one allergenic food constituent to another. As a consequence, depending on which allergenic constituent of the food is responsible for the sensitisation, the process may be clinically relevant, i.e. have a beneficial impact, for some allergic individuals but not for others. It is therefore difficult to decide how extensive and how uniform or specific the product information, with respect to labelling of food allergens and products thereof, should be to provide a suitable warning and an appropriate means of prevention to the various sub groups of the allergic patient population.

VIII. THRESHOLD DOSES

The notion of determining threshold levels for allergenic foods below which sensitised consumers are not at risk of developing allergic reactions has attracted much attention from regulatory bodies, consumer associations and industry throughout Europe.

This concept derives from well-established risk assessment procedures, mostly relating to toxic substances, during which acceptable daily intakes are derived from experimental studies determining the NOAEL (Pelekis *et al.*, 2003; Calabrese and Baldwin, 1994). Uncertainty factors (often between 100-1000) are then applied to account for the inter and intra individual variation of humans and for extrapolation from animal studies to humans.

1. DETERMINING THRESHOLDS

Most of the clinical studies of patients suffering from food allergic diseases are performed in a way that would not show the lowest provoking dose under controlled conditions (Hourihane *et al.*, 1997; Wensing *et al.*, 2002b). In risk assessment terms these studies would establish the lowest observed adverse effect level (LOAEL) which however does not provide a scientific basis for the recommendation of acceptable levels of allergen intake and the determination of a NOAEL (Morisset *et al.*, 2003a).

In addition to the issues mentioned above, the broad and diverse spectrum of food allergic diseases adds another level of complexity when considering thresholds.

2. FOOD CHALLENGE STUDIES

The standardised DBPCFC is widely considered to be the standard for the analysis of threshold levels in food allergic patients (Bahna, 2003; Sicherer *et al.*, 2000a). Even this standard is interpreted differently and varies from investigator to investigator. Variability is particularly related to the scoring of the patient's subjective and objective symptoms and their severity. In addition, a number of clinicians exclude the most severely allergic patients from their challenge studies. These patients are likely to react to the smallest amount of allergens and would have the most severe reactions (Taylor *et al.*, 2002).

Major variables affecting the determination of threshold doses are related to factors detailed below (Table 4).

The distribution of individual threshold doses which provoke an allergic reaction in different cohorts of allergic individuals is highly variable (Hansen *et al.*, 2003; Wensing *et al.*, 2002b; Norgaard and Bindslev-Jensen, 1992; Hourihane *et al.*, 1997) and is provided in the section of this Opinion which deals with individual allergens in detail.

Allergen doses reported to trigger adverse reactions in controlled studies range from micrograms to milligrams, and sometimes grams, of administered allergenic food. It is not always stated whether these doses relate to the administered allergenic protein equivalent or allergen containing whole food items. In some studies the allergenic food is not given in the form it is usually eaten or it is freeze dried or modified in other ways or introduced as flour. (Hourihane *et al.*, 1997). Although such food preparations are necessary to fulfil strict

DBPCFC criteria, they may affect the threshold levels and the outcome of the study (Grimshaw *et al.*, 2003).

Threshold doses in allergic individuals in the normal population, who are either unaware of their allergy or may not wish to seek medical advice, are entirely unknown.

Table 4. Variables affecting the setting of a threshold dose

Severity of the allergic condition
Symptoms used as the clinical read-out system (subjective *vs* objective symptoms and their associated severity)
Different administration protocols, challenge conditions and food preparations
Allergen content and matrix of challenge foods
Total amount of administered dose and time frame
Reproducibility (false positives and negatives)
Co-factors (for example exercise, alcohol, medication)
Patient population (different geographical distribution of underlying sensitisation rates for cross-reacting allergens)
Individual's ethnicity

3. PREDICTION OF INDIVIDUAL'S SENSITIVITY AND ALLERGEN THRESHOLD

It could be clinically important if laboratory parameters could predict a threshold level and severity and allergic reactions in allergic patients. This approach, although theoretically interesting and appealing, has yielded conflicting results in clinical studies of different populations (Osterballe and Bindslev-Jensen, 2003; Sampson, 2001; Boyano Martínez *et al.*, 2001; Eigenmann and Sampson, 1998; Morisset *et al.*, 2003a; Niggemann *et al.*, 2001). Since the specific IgE levels and skin test are only loosely related to the likelihood and severity of an allergic reaction and currently only applicable to a small number of allergens (for example peanut, egg and milk), knowing the specific immunological sensitisation level will not be helpful for the consumer or allow the industry to safely target products to consumers with different degrees of sensitisation.

4. CONCLUSION

Current clinical, epidemiological and experimental data do not allow us to determine safe allergen threshold levels which would not trigger an adverse reaction in a sensitised consumer. Even if this could be achieved and sensitive and reliable analytical methods were available for most common allergens, the feasibility of this approach would need to be studied prospectively in different populations.

IX. COELIAC DISEASE AND ALLERGY TO CEREALS

COELIAC DISEASE

SUMMARY

Coeliac disease (synonymous gluten-sensitive enteropathy, sprue) is an autoimmune disease triggered by the cereal protein gluten that occurs in wheat and rye. Unlike atopic food allergy it is not based on an IgE-mediated reaction. The causal relation between gluten and its "toxicity" in individuals genetically predisposed to develop coeliac disease is firmly established and forms the basis for inclusion of gluten and coeliac disease in food regulations and declarations in order to prevent harmful effects of gluten-containing food or food components in coeliac patients. A network of genetic predisposition, of biochemical processes modifying the triggering protein and of a complex T cell-mediated immune reaction at the small intestinal level characterises the disease, which shows many clinical forms. The classical form has become rare today, the more frequent oligosymptomatic forms occur in all age groups. Extraintestinal manifestations have to be considered.

Prevalence of coeliac disease including classical, oligosymptomatic, and silent forms in children and adults is now known to be as high as 1:200 in Europe. Therapy consists of a gluten-free diet. Gluten is a well-defined wheat storage protein rich in glutamine and proline. Gluten and its alcohol-soluble component gliadin contain repetitive peptide units known to elicit the immunological and clinical effects at the molecular level. Peptide sequences like QPQPFPPQQPYP and PQQPFPQ are potent T cell epitopes in coeliac disease. They trigger the autoimmune small intestinal inflammatory response leading to malabsorption. Complete acid hydrolysis destroys coeliac-eliciting properties of gluten. However, partial hydrolysis and enzymatic degradation and heat treatment during food processing do not destroy coeliac-triggering peptide units. Gluten-free foods intended for dietary use can be analyzed for residual gluten content by immunochemical methods. However, there are no conclusive clinical data on the threshold of gluten sensitivity of coeliac patients. The current Codex Alimentarius limit for gluten-free foods of 200 mg gluten/kg food therefore requires reconsideration.

1. DEFINITION OF COELIAC DISEASE, PREVALENCE

Coeliac disease is an autoimmune disorder triggered by gluten. It is a life-long disease with permanent gluten intolerance and is characterised by a severe small intestinal mucosal lesion (villus atrophy) typically exhibiting a flat mucosa (Marsh, 1992; Collin *et al.*, 1994; Fasano and Catassi, 2001; Stern *et al.*, 2001). The small intestinal lesion is responsive to elimination of the trigger by a gluten-free diet except for a very few severe cases of non-responsive adult coeliac disease. World-wide prevalence of coeliac disease based on the clinical diagnosis and on classical gastrointestinal symptoms is about 1:3,000 (Fasano and Catassi, 2001). However, the classical picture with abdominal distension, steatorrhea and deficient growth has become rare. Oligosymptomatic forms (patients with anaemia, retarded puberty, dental anomalies, oral ulcers, infertility, abdominal pain, arthritis, neurological and psychiatric complaints) have become more predominant (Collin *et al.*, 1994 and 1999). The term "silent coeliac disease" is used for these oligosymptomatic patients who have the full small intestinal lesion, however. Considering the classical picture and silent coeliac disease together, the overall prevalence is as high as 1:200 in Europe and the Western World. Very similar prevalence data have been

brought about by screening studies in Europe and in North and South America (Fasano and Catassi, 2001). Coeliac disease is associated with other autoimmune diseases, with type 1 diabetes mellitus and with secretory IgA deficiency. First degree relatives of index patients have a ten percent risk to develop coeliac disease themselves (Marsh, 1992; Fasano and Catassi, 2001; Sollid, 2002). Underestimation and unawareness of the diagnosis coeliac disease is still common, particularly in the adult population (Iceberg hypothesis). For all these reasons, coeliac disease is a major source of morbidity and an important economical factor.

2. CHARACTERISATION OF GLUTEN/GLIADIN

Gluten is defined as the rubbery dough forming protein that remains when wheat flour is washed to remove starch (Stern *et al.*, 2001; Wieser, 1996). Gluten is characterised by a unique high content of glutamine and proline. It is a seed storage protein and consists of glutenin and gliadin. Glutenin is a high-molecular protein fraction insoluble in alcohol. Its toxicity potential in coeliac disease has not been well characterised. The alcohol-soluble gliadin contains mainly monomeric low-molecular proteins. The ratio of total gluten to gliadin can vary from 1.3 to 1.6 in flours from different wheat cultivars. Both fractions glutenin and gliadin consist of numerous partially close-related protein components. Gliadins (molecular weight 28,000-39,000) contain repetitive peptide units such as QPQPFPPQQPYP (one-letter code for amino acids) and PQQPFPQ. These repetitive peptide units are contained in α, γ, ω subtypes of gliadin which have all been shown to elicit the disease equally. Recently, a 33-mer peptide was identified as a primary initiator of the inflammatory response in coeliac disease (LQLQPSTQPQLPYPQPQLPYPQPQLPYPQPQPF, Shan *et al.*, 2002).

Wheat, rye and barley contain these unique proteins (group name prolamins). Maize, rice, millet and sorghum do not show any of those. Oats contains low amounts of the prolamin type. Wheat, rye and barley have been established to trigger coeliac disease (Wieser, 1996) whereas maize, rice and buckwheat were found not to be harmful. There is some disagreement about the coeliac toxicity of oats (Janatuinen *et al.*, 2002).

Food processing does affect coeliac "toxicity" of gluten, e.g., complete acid hydrolysis abolishes toxicity. However, partial hydrolysis and enzymatic peptic-tryptic degradation of gluten does not affect coeliac-triggering properties since the important peptide units are left unaffected. Heat treatment (baked products) does not change coeliac "toxicity". All food technology processes, however, affect extractability and detectability of gluten which are important in any attempts to measure gluten quantitatively in food (Stern *et al.*, 2001).

3. GENETICS AND PATHOPHYSIOLOGY

Coeliac disease is strongly associated with HLA-DQ2 and DQ8 (Marsh, 1992; Fasano and Catassi, 2001; Sollid, 2002; Schuppan, 2000). The primary HLA association in coeliac disease is to the HLA-DQA1*0501, DQB1*0201 gene found in over 90% of patients. Gluten peptides are presented by DQ2- and DQ8- positive antigen-presenting cells to immunocompetent cells of small intestinal lamina propria. Tissue transglutaminase, which has also been identified as the important endomysial autoantigen in coeliac disease, is released and it may potentiate antigen presentation by deamidating or cross-linking gluten peptides (Schuppan, 2000). As a result, T cell activation, cytokine production, mucosal inflammation and destruction evolve. As a secondary event, production of humoral antibodies to the

autoantigen transglutaminase and to gliadin occurs. Immune pathophysiology of coeliac disease involves mechanisms different from classical IgE-mediated food allergy. As a consequence, time course and clinical manifestations of the reactions are different in coeliac disease and food allergy. However, the causal relation between gluten and its coeliac "toxicity" in genetically predisposed persons is firmly established and forms the basis for inclusion of gluten and coeliac disease in food regulations and declarations in order to prevent harmful effects of food or food components in coeliac patients (*cf.* Stern *et al.*, 2001).

By small intestinal organ culture and by T cell stimulation experiments, different gluten peptides have been shown to elicit coeliac-specific "toxic" effects (Wieser, 1996; Van de Wal *et al.*, 1998; Anderson *et al.*, 2000; Ellis and Ciclitira, 2001; Arentz-Hansen *et al.*, 2002; Shan *et al.*, 2002; Vader *et al.*, 2002). Deamidation was found to enhance stimulatory capacity of gluten peptides for specific T cell clones. However, coeliac disease-eliciting properties and immunogenicity of gluten peptides are not identical. At the level of T cell reactivity, stimulatory gluten peptides have been identified up to the molecular level. There is a diverse repertoire of gluten peptides eliciting a coeliac response including immuno-dominant T cell stimulatory peptides rich in proline residues (Arentz-Hansen *et al.*, 2002; Vader *et al.*, 2002).

4. CLINICAL FEATURES AND DIAGNOSIS

The classical picture of coeliac disease has become rare today forming just the tip of a diagnostic "iceberg" (Marsh, 1992; Collin *et al.*, 1994 and 1999; Fasano and Catassi, 2001). Epidemiologic studies have shown a shift from infant to adolescent and adult age at diagnosis and from the classical picture towards patients with a wide range of less clear-cut and milder symptoms like deficiency syndromes, abdominal pain, liver disease, osteoporosis, arthritis, neuropsychiatric and dermatologic disease (dermatitis herpetiformis Duhring). Silent and latent forms of coeliac disease are hidden below the diagnostic "waterline" of the iceberg. Extraintestinal manifestations have to be considered (Fasano and Catassi, 2001). Longstanding untreated coeliac disease can lead to gastrointestinal malignancies, particularly T cell lymphoma (Fasano and Catassi, 2001).

According to current diagnostic criteria established by the European Society for Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) in 1990, diagnosis of coeliac disease relies on small intestinal biopsy showing a severe mucosal lesion and subsequently restoration on a gluten-free diet with disappearance of symptoms (Fasano and Catassi, 2001). Serologic methods like determination of gliadin antibodies and of autoantibodies against endomysium and tissue transglutaminase are a useful adjunct to the diagnostic procedure particularly for screening.

5. GLUTEN-FREE DIET AND THRESHOLD FOR GLUTEN SENSITIVITY

A gluten-free diet excluding wheat, rye, barley and traditionally also oats is the conventional cornerstone treatment of coeliac disease. Nevertheless in coeliac patients the relationship between the quantity of gluten ingested and the severity of clinical symptoms and histological abnormalities is still undefined (Wieser, 1996; Ciclitira *et al.*, 1984; Selby *et al.*, 1999; Kaukinen *et al.*, 2000). Individual variation and clinical heterogeneity of coeliac patients pose difficult problems for an attempt to find an acceptable threshold value for trace amounts of gluten to be allowed in gluten-free foods (Stern *et al.*, 2001).

Since the normal daily intake of gluten is 15-20 grams in the adult European population, any effort has to be undertaken to exclude gluten from the diet of coeliac patients. Challenge studies and dietary survey studies (Ejderhamn *et al.*, 1988; Catassi *et al.*, 1993; Kaukinen *et al.*, 1999; Jansson *et al.*, 2001; Laurin *et al.*, 2002; Peräaho *et al.*, 2003) have shown that a daily intake of 100 mg of gliadin was sufficient to elicit typical coeliac changes whereas a daily intake of gliadin between 4-14 mg did not cause any small intestinal mucosal damage in coeliac patients (see Table 5). In two studies from the same group (Kaukinen *et al.*, 1999; Peräaho *et al.*, 2003) it was concluded that wheat starch-containing gluten-free flour products are acceptable in the gluten-free diet. Further it was concluded that general dietary compliance is much more important than the intake of trace amounts of gluten present in wheat starch-based gluten-free foods.

Much criticism applies to "threshold studies" in coeliac disease (Table 5): some dietary survey studies have been very small. None of those studies did apply direct gluten analysis. Thus, calculated figures based on assumptions were given. Interference of clinical evaluation by confounding variables (non-coeliac unspecific untoward effects; Kaukinen *et al.*, 2000, Selby *et al.*, 1999) is limiting the value of retrospective long-term studies. To minimise further confounding factors, only studies controlled by biopsy have been included in Table 5. *In vivo* challenge studies have been limited by small numbers and short duration. The base line of a gluten-free diet was not always well-defined and even at the beginning of some of those studies small intestinal mucosa showed some degree of damage. Besides, it was very difficult to guarantee a gliadin intake of less than 100 mg/day while consuming an ordinary gluten-free diet. Contamination of dietary constituents and inadvertent dietary transgressions are not rare in the setting of a clinical study. Challenge studies did not include negative controls. Therefore, clinical data are insufficient today with regard to establishing valid threshold values. Further well-controlled clinical studies using small dose challenge and dietary records including gluten analysis are necessary until a more meaningful discussion on standards for gluten-free foods can be started.

Table 5. Effects of gliadin/gluten in coeliac disease: Dose-response studies (controlled by biopsy)

Dietary survey studies							
Name	**Year**	**Children (c) Adult (a)**	**Number**	**Quantity**	**Gliadin/ Gluten**	**Time**	**Toxicity**
Ejderhamn	1988	c	11	4-14 mg/day*	gliadin	10 years	non-toxic
Kaukinen	1999	a	52	34 mg/day*	gliadin	8 years	non-toxic
Selby	1999	a	89	not specified	wheat starch	8 years	non-toxic
Peräaho	2003	a	57	not specified	wheat starch	1 year	non-toxic
***In vivo* challenge studies**							
Name	**Year**	**Children (c) Adult (a)**	**Number**	**Quantity**	**Gliadin/ Gluten**	**Time**	**Toxicity**
Ciclitira	1984	a	7	10 mg/day	gliadin	8 hrs	non-toxic
Catassi	1993	c	20	100 mg/day	gliadin	4 weeks	toxic
Jansson	2001	c	27	200 mg/kg/day	gluten	4 weeks	toxic
Jansson	2001	c	27	500 mg/kg/day	gluten	4 weeks	toxic
Laurin	2002	c	24	100 mg/kg/day	gluten	13 weeks	toxic

* = calculated

Unlike most cases of food allergy (Bousquet *et al.*, 1998) there appears to be a low level threshold of trace amounts of gluten which might be included without a hazard for the majority of coeliac patients. The Codex Alimentarius Committee on Nutrition and Food for Special Dietary Uses adopted a first Codex Standard on gluten-free food in 1981 (CCNFSDU, 1994, CODEX-STAN 118-1981). It defined the cereals that are toxic to coeliac patients (wheat, rye, barley, oats and crossbred varieties) and set limits to the amount of gluten allowed in raw materials to produce gluten-free food. Since at that time no method for measuring gluten was available, the threshold value was set at 0.05 g nitrogen per 100 g dry matter determined by the Kjeldahl method (referring to wheat starch). A revision of this standard is now underway (Codex Alimentarius Commission, 2000). The most striking differences between the old standard and the proposed new standard is that while the old standard is restricted to ingredients the proposed new standard applies to all foods labelled as gluten-free. A three-fold definition of gluten-free is presented: (1) those gluten-free foods consisting of ingredients that do not contain any prolamin from wheat or *Triticum* species such as spelt, kamut or durum wheat, rye, barley, oats or their crossbred varieties with a gluten level not exceeding [20] mg/kg; (2) those consisting of the above ingredients which have been rendered "gluten-free" with a gluten level not exceeding [200] mg/kg; (3) a mixture of the two ingredients listed above with a gluten level not exceeding [200] mg/kg. (The square brackets indicate that there was insufficient information to make a final decision on the figure.) Methods for sample extraction and chemical analysis of foods for gluten/gliadin content were also included in the revision of the standard for gluten-free foods. However, in the light of recent findings (see below) this part of the draft is outdated.

At present, clinical data are not sufficient to back up the [200] mg/kg threshold suggested. Obviously there is a lack of good clinical studies including appropriate controls carried out, long-term and using objective gluten measurement. The current figure of [200] mg gluten/kg food is arbitrary and does not include any safety factor. This applies also to the remark included in the draft revised standard "The total daily intake of prolamin for coeliac patients should not exceed 10 mg/day" (CX/NFSDU 00/4). From the clinical point of view, a threshold value in terms of a daily allowable intake would be preferable to the product-centred mg/kg figure which does not take into account the relative consumption of different dietary and non-dietary foods in coeliac patients.

6. METHODOLOGY OF GLUTEN ANALYSIS

Considerable progress has been made in gluten analysis of food. For this purpose immunochemical assays and non-immune methods have been introduced (Skerritt and Hill, 1991; Sorell *et al.*, 1998; Denery-Papini *et al.*, 1999). Sensitivity, specificity and reproducibility of most earlier methods for gluten analysis have been unsatisfactory (Stern *et al.*, 2001).

A European gliadin reference is now available and currently in the process of certification by the EC Institute for Reference Materials and Measurements. This material serves as a reference for the detection of gliadin/gluten in food samples. A large collaborative study of a new R5 ELISA assay accompanied by non-immunological cross-testing has been finished recently. Data on extractability, sensitivity and limit of detection (1.5 mg/kg), on robustness and reproducibility of this test system are promising. The assay is based on a monoclonal antibody reacting with QQPFP residues (Sorell *et al.*, 1998; Valdés *et al.*, 2003). This system is solving problems of standardisation and accuracy in gluten analysis particularly in the

relevant low level range. Further analytical problems arising from heated or hydrolyzed products and problems concerning disturbing matrix effects are, however, still far from being solved by current methods. Unexpected food contamination by gluten can occur in solid food and beverages, i.e., safety of gluten-based clarification of wine has not been studied for coeliac patients (Cattaneo *et al.*, 2003).

7. CONCLUSION

Any effort should be made to keep the diet of coeliac patients as gluten-free as possible. Gluten analysis is helpful in the control of gluten-free products but some analytical problems remain, and, more important, clinical data are insufficient today to back up a threshold value of gluten to be allowed for all coeliac patients.

ALLERGY TO CEREALS

SUMMARY

Cereals are a staple food worldwide and provide three-quarters of the protein in the human diet. The great majority of cereals belong to the grass family (*Gramineae*). Cereals can cause several different immuno-mediated disorders, including coeliac disease, baker's asthma, and food allergy. IgE-mediated allergic reactions are immediate or delayed after ingestion, their severity varies from mild to very severe, and the clinical picture includes oral allergy syndrome, urticaria, flare-up of atopic dermatitis, respiratory and gastrointestinal symptoms, and even anaphylaxis (usually exercise-induced anaphylaxis). Allergy to cereals in the general population is not very frequent, as few cases are reported in relation to the great consumption; in children, however, wheat is a major cause of food allergy.

The diagnosis of cereal allergy is hampered by a very high degree of IgE cross-reactivity between seed and pollen allergens, so that patients with grass pollen allergy often show a positive skin prick test or RAST to cereals, even in the absence of food allergy symptoms. Conversely, some cereal-induced disorders such as atopic dermatitis and irritable bowel disease may not have detectable specific IgE. For all these reasons DBPCFC is always the standard for the diagnosis of cereal allergy.

The allergens identified up to now are water/salt-soluble proteins (albumins and globulins) and alcohol-soluble proteins (prolamins). The former include alpha-amylase inhibitors, which are responsible for allergy by both inhalation (baker's asthma) and ingestion (cereal food allergy); the latter include gliadin, which is responsible for food-dependent exercise-induced anaphylaxis, atopic dermatitis and immediate-onset food allergy. Gliadin is a heat-stable allergen, which shows enhanced resistance to enzymatic digestion after cooking, and therefore has the characteristics of a perfect food allergen.

1. BACKGROUND

The term "cereal" indicates any kind of plant producing grains which are milled in order to obtain edible flour. It follows that "cereals" do not belong to a single botanical family, though

the great majority of them are from the grass family, named *Poaceae* or *Gramineae* (wheat, rice, maize, oat, barley, rye, spelt); some belong to the family of *Polygonaceae* (buckwheat), others to the family of *Fabaceae* (soybean).

Cereals are a major source of food in all parts of the world, and account for 72% of the protein in the human diet. World production of all cereal grains is about 1,600 billion tonnes annually. Wheat is the leading cereal grain, representing about one-third of world cereal production, followed by rice and maize. Nearly two-thirds of the wheat produced is used for food; the remaining is used for feed, seed and non-food applications. Wheat is consumed as food in several different forms, all of which involve some degree of processing: products such as breakfast cereals are obtained from the whole kernel, but the majority of wheat for food is first milled into flour to be used for baked goods. About 6% of wheat undergoes industrial processing into starch and gluten, which are used in food as protein enrichment and binding or strengthening agents.

Due to their global distribution and wide consumption, the potential of cereals to cause disease is a matter of concern. A well-known cereal-related disorder is coeliac disease, which is caused by an immunological reaction to a gluten protein fraction named gliadin in wheat, secalin in rye, and hordein in barley, which is absent in maize and rice.

IgE-mediated allergic reactions to cereals were first described as occupational diseases, caused by the inhalation of cereal flour by bakers or millers, called “baker’s asthma”. More recently, cereals have also been demonstrated to be involved in food allergy, as they can provoke a number of reactions after ingestion, such as oral allergy syndrome, urticaria, flare-up of atopic dermatitis, respiratory and gastrointestinal symptoms, and even anaphylaxis (usually exercise-induced anaphylaxis). Symptoms can be immediate or delayed after ingestion, and their severity varies from mild to very severe reactions. The cereals described as causing type I hypersensitivity reactions are wheat, rice, maize, barley, oat, rye and buckwheat.

2. FREQUENCY

2.1 Prevalence

Cereals are not a frequent cause of food allergic reactions in adults, since few cases of cereal allergy were reported in the literature, despite the enormous quantity of cereals that are consumed every day all over the world. Cereal allergy is, however, quite frequent in children, as confirmed by many DBPCFC studies performed on children affected by atopic dermatitis. In these series, wheat was one of the six most common offending foods, together with egg, milk, soy, peanut and fish (Burks *et al.*, 1988; James and Sampson, 1992). In a review of DBPCFC tests performed over a 13-year period in children with atopic dermatitis, Sicherer *et al.* (2000a) demonstrated that 40 out of 196 children with a positive challenge test had wheat allergy (20%). Among cereals, wheat is the most frequent cause of allergy: Jones *et al.* (1995) studied 31 children with cereal allergy confirmed by DBPCFC, and found that 26 (84%) were allergic to wheat, 5 to oat, 5 to maize, 4 to barley, 4 to rye, and 1 to rice.

3. CLINICAL FEATURES

3.1 Wheat and other gluten-containing cereals

The main problem in evaluating food allergic reactions to cereals is the presence of a very high degree of cross-reactivity between IgE antibodies towards seeds and pollen tissues. In fact, even though the term "cereal" is applied to any edible flour source, the majority of cereals belong to the grass family. For this reason, a low positive predictive value has recently been shown for any level of IgE antibodies to wheat below 100 kU/L (Sampson, 2001). Thus, it is necessary to apply strict clinical parameters to make a diagnosis of cereal allergy. In particular, DBPCFC is the standard test for this food, which only rarely causes severe reactions.

Jones *et al.* (1995) studied a population of 145 patients suffering from atopic dermatitis with positive skin prick test to one or more cereals (wheat, rye, barley, oat, rice, corn). All were submitted to DBPCFC: a total of 228 challenges were performed administering up to 10 g of cereals. Only 45 positive reactions occurred in 31 out of 145 patients (21%); all the reactions were immediate, occurring within two hours of challenge. Twenty-five out of 31 had a positive challenge to only one grain (19/25 to wheat, 3/25 to corn, 1/25 each to barley, rice and rye); three patients (10%) had positive challenge to two grains; three to four grains. In conclusion, 67% of patients with positive skin prick test to cereals had no clinical evidence of hypersensitivity after the specific challenge, thus demonstrating once again the high frequency of cross-reactivity between cereals and grasses extracts and the low prevalence of food allergic reactions to these food items in patients sensitised to grasses.

James *et al.* (1997) diagnosed an IgE-mediated allergic reaction to wheat in seven children presenting with symptoms like urticaria, anaphylaxis and vomiting, and with positive skin prick test and RAST results for wheat. Six of them underwent a DBPCFC confirming their clinical reactivity to wheat; one had a convincing clinical history of severe anaphylaxis after ingestion of wheat and was not subjected to the food challenge.

More recently, Armentia *et al.* (2002) from Spain recruited 18 adults with a suspected food allergy related to cereals, from the reviewed data of a database of 16,281 patients studied in their allergy unit during the previous ten years. They were submitted to DBPCFC on the basis of history and skin prick test results: 11 of them had a positive result with barley, 6 with wheat and 3 with rye.

All these DBPCFC studies thus demonstrate that typically, even if rarely, cereals elicit the typical immediate symptoms of allergic diseases such as atopic dermatitis, urticaria, angio-edema and anaphylaxis.

In other studies, however, based on open or double blind food challenges, it was found that wheat can also cause other kinds of hypersensitive reactions. In particular, delayed hypersensitivity symptoms with onset 24 hours after ingestion have been reported by Scandinavian authors. Varjonen *et al.* (2000) described the cases of 18 children with atopic dermatitis: all of them were submitted to open oral challenges with up to 10 g of wheat given twice daily. Eight patients developed immediate symptoms and 5 delayed symptoms (urticaria, eczema, erythema and pruritus, wheezing). A good correlation was found between positive skin prick test to gliadin or elevated gluten RAST and positive wheat open challenge.

Delayed onset symptoms were also described in the study by Majamaa *et al.* (1999) in children with negative skin prick tests and specific IgE results for wheat.

The finding of a positive oral challenge to wheat in patients with no evidence of sensitisation is another peculiarity of wheat allergy, as pointed out by Simonato *et al.* (2001a) and Niggemann *et al.* (2001). Simonato *et al.* (2001a) selected twenty patients affected by irritable bowel syndrome in whom hypersensitivity to wheat was suspected on the basis of history and results of elimination diet and open oral challenge. Nine of them had negative CAP/RAST results, despite positive open challenge. Niggemann *et al.* (2001) provided evidence that non-IgE mediated mechanisms are responsible for over 20% of positive challenge reactions to wheat in children with atopic dermatitis. More recently, the Finnish group already mentioned (Palosuo *et al.*, 2001a) showed immediate (48%) and delayed (20%) hypersensitivity symptoms in 27 out of 40 children submitted to open or double blind oral wheat challenge, recruited on the basis of a history suggestive of wheat allergy. The interesting result was that IgE antibodies to the gluten fraction of wheat were not detected in the 8 children with delayed symptoms, while they were detected in the majority (84%) of the children with immediate symptoms.

Another interesting aspect of allergic reactions to cereal products is the observation of a quite unusually high frequency of food-dependent exercise-induced anaphylaxis. Cereal-dependent exercise-induced anaphylaxis was described in 18 patients with positive skin prick test to wheat flour by the same Finnish group (Palosuo *et al.,* 1999). Similar results were found in 11 patients in Japan (Dohi *et al.*, 1991).

4. IDENTIFIED ALLERGENS

4.1 Wheat

Wheat grain proteins are divided into four classes on the basis of their solubility: water-soluble albumins (15% of the total), salt-soluble globulins (5%), 70% ethanol-soluble prolamins, which include gliadins (40%) and acid- or alkali-soluble glutenins (40%). A more recent classification of wheat storage proteins is based on molecular characteristics rather than on solubility: high molecular weight prolamins, corresponding to high molecular weight-glutenin subunits (about 100 kDa), sulphur-poor prolamins, corresponding to omega-gliadins (45-60 kDa) and sulphur-rich prolamins, comprising low molecular weight-glutenin subunits, alpha-, beta- and gamma-gliadins (31-45 kDa).

The gluten fraction of cereals has been extensively studied because of its causal role in coeliac disease.

The allergenic potential of cereal proteins was first demonstrated in wheat flour, which causes occupational respiratory disease: the fraction involved is that of water/salt soluble albumins and globulins, which are easily extractable from the primary allergenic source. To this group belong the alpha-amylase inhibitors, proteins of 15-17 kDa molecular weight, whose role in baker's asthma has been widely assessed.

Few cereal allergens causing food allergic reactions have been identified to date. Some of them have been recognised by sera from patients selected on the basis of history and positive skin prick test or RAST (IgE-dependent hypersensitivity); others by sera from patients

submitted to oral open or double-blind challenges, whether positive or negative to skin prick test and RAST (IgE-dependent or IgE-independent hypersensitivity).

Jones *et al.* (1995) identified a 20 kDa and a 47 kDa protein as potential specific food allergens of wheat; in fact, these proteins were recognised by sera from patients with oral sensitisation to wheat confirmed by DBPCFC, and no evidence of grass pollen allergy according to history or skin prick test results. Grass-sensitised patients, on the contrary, did not have IgE antibodies that bound to these fractions; however, these allergens were not purified and characterised at a molecular level.

James *et al.* (1997) showed that wheat 15 kDa alpha-amylase inhibitor, the major allergen in baker's asthma, was able to sensitise not only by inhalation, but also via the gastrointestinal route: in fact it was a significant wheat allergen identified in a population of atopic children with positive DBPCFC for wheat.

Armentia *et al.* (2002) confirmed this finding by showing that patterns of IgE-binding components in barley, rye and wheat were very similar, using sera from individuals sensitised by the inhalation or the intestinal route, irrespective of age. Protein bands of around 11-16 kDa, probably allergenic members of the alpha-amylase inhibitor family, were immuno-detected in wheat, barley and rye. In both these studies however the water-insoluble allergens were not investigated.

Simonato *et al.* (2001a) identified an allergen of the same molecular weight as wheat alpha-amylase inhibitor (16 kDa) recognised by the sera of atopic patients positive for wheat CAP/RAST and open challenge. This allergen was slightly bound by sera from non-atopic patients who, despite positive open challenge, had negative CAP/RAST for wheat; these patients recognised, however, some proteins of the gluten fraction, such as the 42 kDa protein, which is a major allergen.

Gliadin, the antigenic protein of wheat responsible for coeliac disease, was recently identified also as the major allergen involved in wheat-dependent, exercise-induced anaphylaxis of adult subjects and in immediate allergy to ingested wheat in children (Varjonen *et al.*, 2000; Palosuo *et al.*, 1999 and 2001a). This observation arose from the study of the already mentioned population of 18 adult patients with a history of systemic anaphylactic reactions after ingestion of cereals followed by exercise. They recognised two different kinds of gliadins as major allergens: the 65 kDa omega-gliadin (firstly mentioned as gamma-gliadin) and the 40 kDa alpha-gliadin. Similarly, Morita *et al.* (2001), using a RAST inhibition test to gluten by using purified gamma-gliadin, showed that this was the most important allergen in four adult patients with wheat-dependent, exercise-induced anaphylaxis. The omega-gliadin allergen, previously believed specific only for wheat-dependent, exercise-induced anaphylaxis, was subsequently found as the causative allergen also in a group of 18 children with atopic dermatitis (Varjonen *et al.*, 2000) and in a group of 19 children with immediate hypersensitivity symptoms evoked by blinded or open wheat oral challenge (Palosuo *et al.*, 2001a).

Recently, the same group of authors demonstrated that tissue transglutaminase, an intestinal enzyme locally activated during exercise, is able to cross-link omega 5-gliadin-derived peptides, causing a marked increase in IgE-binding. This finding can give a rational basis to the previous observations about the role of this gliadin in wheat-dependent exercise-induced anaphylaxis (Palosuo *et al.*, 2003).

The high frequency of IgE positivity towards omega 5-gliadin in wheat allergic patients induced the previously mentioned researchers to suggest the use of this allergen to perform skin prick tests to identify patients allergic to wheat without carrying out dangerous food challenges.

Different results came from a Japanese group, which found some heterogeneity of the allergens involved in anaphylactic symptoms in 7 young children; they did not identify a correlation between the allergenic pattern and the clinical features of anaphylaxis. Taken together, these results indicate a difficulty in making hypoallergenic wheat flour, lacking specific protein components to prevent anaphylaxis (Takizawa *et al.*, 2001).

Table 6. Wheat allergens

Allergen	Family	Molecular weight	Population studied	Authors
Not assigned	Unknown	20 kDa and 47 kDa	5 patients with positive DBPCFC to wheat and absence of sensitisation to grass	Jones *et al.*, 1995
Not assigned	Alpha amylase inhibitor	15-16 kDa	6 children with positive DBPCFC and 1 with severe anaphylaxis to wheat	James *et al.*, 1997
			10 atopic patients with positive CAP/RAST and open challenge to wheat	Simonato *et al.*, 2001a
Tri a 19	Gliadin	65 kDa	19 children with immediate hypersensitivity symptoms during blinded or open wheat oral challenge	Palosuo *et al.*, 2001a

5. CROSS-REACTIVITIES

5.1 Clinical cross-reactivity

Since almost all cereals belong to the *Gramineae* family, it is not surprising that a very high degree of IgE cross-reactivity should exist between allergens from cereal seeds and allergens from grass pollen tissue, as described by many authors (Baldo *et al.*, 1980; Sutton *et al.*, 1982; Prichard *et al.,* 1985; Walsh *et al.*, 1987; Sander *et al.*, 1997). This cross-reactivity, demonstrated *in vitro*, has little clinical significance, since few grass pollen-allergic patients also have food allergy to cereals. The different route of sensitisation may explain the different clinical reactivity to the same allergens: this is true for baker's asthma, a disease in which cereal allergen causes symptoms only when inhaled, and not when ingested.

Food allergy to cereals is often due to monosensitisation to a single cereal; in fact, as reported by Jones *et al.* (1995), only 20% of patients with cereal allergy demonstrated by DBPCFC are clinically reactive to more than one cereal grain. This percentage is quite small, but it is higher than that found in other food families like legumes.

5.2 Cross-reacting allergens

Many cereal allergens are cross-reactive with grass pollen allergens, as demonstrated by Jones *et al.* (1995), who found only two wheat-specific allergens (i.e., not cross-reacting with homologous grass pollen allergens) of 20 and 47 kDa, which were not characterised. However, this is only an *in vitro* cross-reactivity; up to now there are no studies on clinically significant cross-reactive allergens of cereals and grasses. As for the cross-reactivity among different cereal grains, Palosuo *et al.* (2001b) demonstrated that the 65 kDa gliadin wheat-dependent, exercise-induced anaphylaxis cross-reacted with homologous proteins from other cereals, the gamma-70 secalin of rye and the gamma-3 hordein of barley.

6. POSSIBLE EFFECTS OF FOOD PROCESSING ON ALLERGENICITY AND DERIVED PRODUCTS

6.1 Wheat

Since wheat is always consumed cooked, it is obvious that its allergenicity survives thermal treatment; however, few studies focused on the effect that different processing methods may have on wheat allergenicity. Simonato *et al.* (2001b) demonstrated that some wheat allergens, like the alpha-amylase inhibitor, are destroyed by heating, and others, like the prolamins, are thermostable. They also found that bread baking can enhance the resistance of allergens to digestion in the gastrointestinal tract, as it induces the formation of protein aggregates stabilised by heat-induced interactions. In fact, the IgE-binding capacity of unheated bread dough was decreased by enzymatic digestion with pepsin and pancreatin, while that of breadcrumb and crust which underwent thermal treatment was not.

7. THRESHOLD DOSES

Sicherer *et al.* (2000a) studied a population of 196 children suffering from atopic dermatitis with suspected food allergy, over a 13-year period: 40 patients had a positive DBPCFC test for wheat, with doses ranging from 400-500 mg to 8-10 g of food. The authors demonstrated that low amounts of wheat were able to provoke an allergic reaction, as 25% of patients had symptoms at the first dose (500 mg or less), while 88% of patients reacted to doses of less than 8-10 g. Similar results were reported by Niggemann *et al.* (1999), who performed DBPCFC in 107 children with atopic dermatitis, with positive results to wheat in 15 patients at doses of 10 g of wheat protein or less.

8. CONCLUSION

IgE-mediated allergy to cereals is caused both by inhalation of cereal flour (baker's asthma) and by ingestion of cereal-based products (food allergy). Cereal-induced food allergy is a well-documented disease: many DBPCFC studies have been performed lately, which confirm that cereals are also able to determine anaphylactic reactions (type I hypersensitivity), and not only coeliac disease, another immuno-mediated disorder caused by gluten-containing cereals. Some of the culprit allergens have been identified, mainly albumins/globulins and prolamins. However, little is known about the modifications of cereal allergenicity after physical and chemical treatments and about the heat resistance of the single allergens.

X. ALLERGY TO CRUSTACEANS

SUMMARY

Crustaceans are a common food in Europe. Allergy to crustaceans appears to be among the most common food allergies, and can cause life-threatening reactions. Allergy to crustaceans has been proven by DBPCFC studies. The major allergens have been well characterised. In addition, crustaceans have a number of allergens causing reactions in a smaller number of crustacean allergic individuals. The major allergen of crustaceans is the abundant muscle protein tropomyosin, which has been characterised at the molecular level. Tropomyosins in different crustaceans show even greater similarity between them than parvalbumins do in finfish. This is the reason for extensive clinical cross-reactivity and cross-sensitisation between crustaceans. There is some cross-reactivity between crustaceans and molluscs, in particular squid, which may be of clinical importance. Also, there is cross-reactivity between crustaceans and snails, mites and cockroaches, apparently caused by tropomyosin, which is a allergen found in many species. Crustaceans have a number of minor allergens that may cause more or less species-specific allergy. Tropomyosins are heat-resistant, and crustacean allergenicity is not much influenced by food processing. There is little information on the lowest dose of crustaceans that can cause a clinical reaction. Reactions to 14-16 grams of shrimp or equivalent amounts of shrimp extract (less than 32 mg protein) have been observed in DBPCFC studies.

1. BACKGROUND

Crustaceans are commonly eaten in all European countries. Few accurate consumption data are available, but seafood intake is considered to vary considerably between different regions, depending on local traditions and supplies. The highest consumption appears to be found in Iceland, where the 1993-1995 estimate of gross per capita fish and crustaceans intake was approximately 91 kg live weight (cited by USDA-ERS, 1999). According to the same source, in Portugal the intake was 59 kg, Norway 47 kg, Spain 43 kg, Sweden 29 kg, France 27 kg, Italy 22 kg, United Kingdom 19 kg, Germany 13 kg, and Hungary 4 kg. Consumption is known to vary greatly between individuals.

It should be noted that, similar to fish, for crustaceans the route of exposure appears to determine whether food allergy or respiratory allergy develops. In some crustacean-processing workplaces, respiratory allergy to crustaceans has been a considerable problem because of inhaled allergen (Goetz and Whisman, 2000; Desjardins *et al.*, 1995; Jeebhay *et al.*, 2000; Lemière *et al.*, 1996; Lopata and Jeebhay, 2001; Patel and Cockroft, 1992).

2. FREQUENCY

For the discussion below and interpretation of data given, it must be kept in mind that test positivity is something different from clinical food allergy. Test positivity will show a higher prevalence than clinical allergy.

Fish and crustaceans are generally considered to be among the four foods most commonly provoking severe food anaphylaxis (Sampson, 2000).

2.1 Prevalence of crustacean allergy – general population

In a questionnaire-based survey of the general population in France (33,110 persons under 60 years, corresponding to a total population of 40,000 individuals, i.e. the French population 1:1000), Kanny *et al.* (2001) found an estimated prevalence of food allergy of 3.24%, based on self-perception data (not DBPCFC studies). Among the most frequent allergens were crustaceans. Specific data for the prevalence in the general population of allergy to crustaceans are not available.

2.2 Prevalence of crustacean allergy – food allergic adults and children

Rancé *et al.* (1999a) report a patient series of 703 patients with food allergies, confirmed by labial or single-blind placebo-controlled food challenge. In these patients shrimp and crab accounted for about 1.7% of food allergic reactions. In a later paper on 163 asthmatic children with food allergy studied by DBPCFC, giving 250/385 positive tests, shrimp accounted for 4.5% of positive reactions (Rancé and Dutau, 2002). In a multicentre study of cases with anaphylactic reactions to food (Moneret-Vautrin and Kanny, 1995b), crustaceans accounted for 9/81 cases. In a study of 500 children with atopic dermatitis, 24% were judged to be allergic to crustaceans (without DBPCFC) (Guillet and Guillet, 2000). In a study by André *et al.* (1994) of 580 patients with pathological reactions to food, 60 presented with severe, near-fatal reactions. Of these, on the basis of clinical history, skin prick testing and serum specific IgE, crustaceans were incriminated in 17% of cases. DBPCFCs were not performed. Of the 580 patients, 34% were found to have specific IgE (RAST) for crab.

Bock and Atkins (1990) studied 480 children with a history of adverse reactions to food by DBPCFC, and found that in the 185 children with positive reactions (n=245), there were 2 reactions to shrimp (both in children >3 years).

Crespo *et al.* (1995c) studied 355 children with food allergy in Spain. Based on clinical history, skin prick tests and specific IgE, crustaceans caused reaction in 6.8% of the patients (3.8% of 608 reactions).

In a study from South Africa consisting of individuals perceiving adverse reactions to seafood (n=105), Lopata and Jeebhay (2001) found that the two most common seafood species were prawns (47%) and rock lobster (44%). Specific IgE to 3 seafood groups (crustaceans, finfish and molluscs) as determined by testing of 12 seafood species by RAST gave 131 positive reactions on the group level in 80 individuals. Fifty per cent of the reactions were against crustaceans.

In a study of adult food allergic patients in Gran Canaria, Spain, Castillo *et al.* (1996) found that 50/107 patients were sensitised (specific IgE) to crustaceans. Of these 48 were sensitised to shrimp. However, 92/107 were also sensitised to house dust mites. There was a positive association between crustacean sensitivity and cockroach sensitivity; this was very marked for reaction against lobster extract, and to a lesser degree also for shrimp.

In a study of 14-year old Danish schoolchildren (Mortz *et al.*, 2003), the prevalence of skin prick test positivity to shrimp was 8.1% (n=74) in children with atopic dermatitis, 11.6% (n=190) in children with inhalant allergy (without atopic dermatitis), and 2.0% (n=205) in normal controls.

3. CLINICAL FEATURES

3.1 General clinical manifestations

Generally, the pattern of allergic symptoms after ingestion of crustaceans appears similar to the symptoms due to other foods (Lopata and Jeebhay, 2001). Bock and Atkins (1990) in a DBPCFC study of 480 subjects up to age 19 years found that cutaneous reactions were the most frequent symptoms for all foods tested, followed by gastrointestinal symptoms. Respiratory manifestations were recorded less frequently; however, asthma (wheezing) as the sole symptom was noted in four (of 245) positive reactions to DBPCFC.

Food allergic reactions to crustaceans may be systemic, but may also be local in the form of an oral allergy syndrome.

Shrimp has been implicated in food-dependent exercise-induced anaphylaxis (Mathelier-Fusade *et al.*, 2002).

3.2 Anaphylaxis in highly crustacean allergic individuals

A number of reports of severe allergic reactions triggered by crustaceans can be found in the literature. Yunginger *et al.* (USA) (1988) reported seven cases of fatal food-induced anaphylaxis. One of these was judged to be triggered by crabmeat. Kemp *et al.* (1995) report on 89 cases of anaphylaxis caused by food. Crustaceans (defined as shrimp, scallops and crab) were the most common incriminating food in this study (26 patients). Pumphrey (2000) reported 37 fatal food anaphylaxis cases from UK. Three of these were judged to be caused by "seafood". The absence of crustaceans as an offending food in the study reported by MacDougall *et al.* (2002) from UK and Ireland (8 fatal cases, 55 severe or near fatal), may possibly be due to the fact that this was a paediatric study.

3.3 Natural history of crustacean sensitisation

In contrast to clinical allergy to milk, egg, wheat and soy which typically is outgrown (in spite of persistent positive skin tests), clinical allergy to crustaceans often is life-long (Bock, 1982; Eigenmann *et al.*, 1998; Sampson and Scanlon, 1989; Hill *et al.*, 1989).

4. IDENTIFIED ALLERGENS

Identified crustacean allergens are shown in Table 7.

The only major allergen identified in shrimp is the 34-36 kDa muscle protein tropomyosin (Shanti *et al.*, 1993; Subba Rao *et al.*, 1998). At least 80% of shrimp-allergic subjects react to tropomyosin and it binds approximately 85% of the shrimp-specific IgE from shrimp allergic subjects; all other shrimp allergens bind IgE from less than 25% of the shrimp allergic subjects (Ayuso *et al.*, 2002a; Leung and Chu, 1998; Leung *et al.*, 1994; Shanti *et al.*, 1993; Daul *et al.*, 1994; Subba Rao *et al.*, 1988). Tropomyosin is an important allergen not only in shrimp (Pen a 1) (Hoffman *et al.*, 1981; Naqpal *et al.*, 1989; Daul *et al.*, 1991), but also in other crustaceans, such as the lobsters *Panulirus stimpsoni* (Pan s 1) and *Homarus americanus* (Hom a 1) (Mykles *et al.*, 1998; Leung *et al.*, 1998a), and crab *Charybdis feriatus* (Cha f 1) (Leung *et al.*, 1998b).

Table 7. Identified crustacean allergens

Allergen	Source of allergen	Family	Molecular weight	Population/source of antibodies	Authors
Crustaceans					
Pan s 1	Lobster *P. stimpsoni*	Tropomyosin	34 kDa	Sera from 10 patients with crustacean allergy	Leung *et al.*, 1998a
Hom a 1	Lobster *H. americanus*	Tropomyosin	34 kDa		
Cha f 1	Crab *Charybdis feriatus*	Tropomyosin	34 kDa	Sera from 10 patients with hypersensitivity and specific IgE to crab	Leung *et al.*, 1998b
Met e 1	Shrimp *Metapenaeus ensis*	Tropomyosin	34 kDa	Sera from 8 patients with a history of anaphylaxis to shrimp	Leung *et al.*, 1994
Pen i 1	Shrimp *Penaeus indicus*	Tropomyosin	34 kDa	Sera from 2 patients with allergy to cooked shrimp	Naqpal *et al.*, 1989 Shanti *et al.*, 1993
Par f 1	Shrimp *Parapenaeus fissurus*	Tropomyosin	39 kDa	Sera from shrimp allergic patients	Lin *et al.*, 1993
Pen a 1	Shrimp *Penaeus aztecus*	Tropomyosin	36 kDa	Sera from 34 shrimp allergic individuals	Daul *et al.*, 1994 Reese *et al.*, 1997 Ayuso *et al.*, 2002a
Pen m 2	Tiger shrimp *Penaeus monodon*	Arginine kinase	40 kDa	Sera from 18 shrimp allergic patients	Yu *et al.*, 2003
Related mollusc allergens					
Tod p 1	Squid *Todarodes pacificus*	Tropomyosin	38 kDa	Sera from 4 patients with squid allergy, and 10 patients with shrimp allergy	Miyazawa *et al.*,1996
Cra g 1	Oyster *Crassostrea gigas*	Tropomyosin	35 kDa	Serum from 4 mollusc- and crustacean allergic patient(s)	Ishikawa *et al.*, 1997, 1998a
Cra g 2	Oyster *Crassostrea gigas*	Tropomyosin	35 kDa	Serum from 4 mollusc- and crustacean allergic patient(s)	Ishikawa *et al.*, 1997, 1998a
Tur c 1	*Turbo cornutus*	Tropomyosin	35 kDa	Serum from 4 mollusc- and crustacean allergic patient(s)	Ishikawa *et al.*, 1998b

Recently, a second class of shrimp allergen, Pen m 2 from the black tiger shrimp *Penaeus monodon*, was described by proteomics and immunological analysis (Yu *et al.*, 2003). The allergenicity was proven by skin testing of shrimp-allergic individuals. Interestingly, the allergen was found to be an arginine kinase, and thus represents a new class of cross-reactive *Crustacea* allergen. Arginine kinase was previously described as a cross-reactive invertebrate allergen by Binder *et al.* (2001).

Lehrer *et al.* (1985) demonstrated the presence of at least seven allergens in shrimp, and reactivity limited to minor allergens can confer a degree of species-specific allergy to species of shrimp and other crustaceans.

5. CROSS-REACTIVITIES

5.1 Crustacean cross-reactivities

5.1.1 Cross-reactivity and cross-allergy between different crustaceans

Tropomyosin is the major allergen of shrimp and other crustaceans and concomitant clinical and *in vitro* immunological hypersensitivity between different crustaceans is commonly observed and suggests strong cross-reactivity between shrimp, prawn, lobster, crayfish and crab. Torres Borrego *et al.* (2003) estimates that a person with allergy to one type of crustacean presents a risk of 75% of reacting against another crustacean species.

Tropomyosin has been found to be a "pan-allergen" (Leung and Chu, 1998; Subba Rao *et al.*, 1988) with extensive sequence identity between crustaceans (Leung and Chu, 1998), causing the extensive serological and clinical cross-reactivity between shrimp, prawn, lobster, crab (Lehrer, 1986), and crayfish (Daul *et al.*, 1993; Carillo *et al.*, 1992).

5.1.2 Cross-reactivty and cross-allergy between crustaceans and molluscs

Crustacean allergic subjects often react to cuttlefish, abalone, limpet, squid, oyster, mussel, scallop and clam. IgE reactivity of sera from 9 crustacean allergic individuals was found with a 38 kDa protein, shown to be tropomyosin, in the muscle extract of all 10 molluscs tested (Leung and Chu, 1998). In addition, varying reactivity of the sera with a number of other mollusc allergens was observed (Lehrer and McCants, 1987; Shibasaki *et al.*, 1989; Morikawa *et al.*, 1990). Serological and clinical cross-reactivity with oyster e.g. *Crassostrea gigas* is sometimes observed (the allergen Cra g 1 is a tropomyosin) (Ishikawa *et al.*, 1998a; Leung *et al.*, 1996; Lehrer and McCants, 1987). The cross-reactive allergen was tropomyosin (Goetz and Whisman, 2000).

Serological and clinical cross-reactivity appears to be observed particularly often between crustaceans and the mollusc squid e.g. *Todarodes pacificus*. The squid allergen Tod p 1 is a tropomyosin (Miyazawa *et al.*, 1996). Carrillo *et al.* (1992) found serologic cross-reactivity between squid (*Loligo vulgaris*, common in the Mediterranean and Atlantic oceans) and shrimp, lobster and crab.

Of 10 children sensitised to molluscs, 9 showed equally strong skin prick test and specific IgE reactivity against crustaceans, which suggests that crustacean allergy may be the primary allergy (Crespo *et al.*, 1995c).

However, all the mentioned clinical cross-reactivities between crustaceans and molluscs are based on case histories, sometimes including elimination diets, and not on DBPCFC.

5.1.3 Cross-sensitisation and cross-allergy between crustaceans, snails, house dust mite and helminths

The snail (*Turbo cornutus*) allergen Tur c 1 is a tropomyosin (Ishikawa *et al.*, 1998b), and also the house dust mite allergen Der p 10 (Aki *et al.*, 1995; Asturias *et al.*, 1998b) and cockroach (*Periplaneta americana* and *Blatella germanica Bla*) allergens Per a 7 and Bla g 1 (Asturias *et al.*, 1999; Santos *et al.*, 1999; Pomes *et al.*, 1998). A number of examples of clinical and *in vitro* concomitant hypersensitivity between crustaceans, molluscs, insects, arachnids and nematodes suggest that tropomyosin is an important cross-reactive allergen causing clinical cross-allergy between invertebrates (Ayuso *et al.*, 2002b; Banzet *et al.*, 1992; Carrillo *et al.*, 1994; Crespo *et al.*, 1995c; Martínez *et al.*, 1997; Pascual *et al.*, 1997; Petrus *et al.*, 1997; Reese *et al.*, 1999b; van Ree *et al.*, 1996; O'Neil *et al.*, 1985; Witteman *et al.*, 1994; Koshte *et al.*, 1989). Ayuso *et al.* (2002 a and b) supplemented with data from Reese *et al.* (1999a) found that the IgE-binding regions of Pen a 1 partly or completely overlap with those in other allergenic tropomyosins such as Pen i 1 from the shrimp *P. indicus*, Tur c 1 from the snail *T. cornutus* and Cra g 1 from the oyster *C. gigas*. Of the five major IgE-binding regions of Pen a 1, all showed 100% homology with the corresponding regions of the lobster fast muscle tropomyosin Hom a 1, explaining the high degree of cross-reactivity among crustaceans. Regions 2 and 5 of Pen a 1 may be of particular importance, as the homologous sequences in oyster (Cra g 1) and snail (Tur c 1) bind IgE antibodies of mollusc allergic subjects, supporting the notion that tropomyosin is the cause of clinically relevant cross-sensitisation between crustaceans and molluscs (Lehrer and McCants 1987; Reese *et al.*,1997).

Clinically relevant cross-reactivity between crustacean and house dust mite allergens has been described (Witteman *et al.*, 1994), and the term "mite-crustaceans-mollusc-syndrome" is sometimes used (Kutting and Brehler, 2001). The primary sensitisation is believed most often to be "respiratory" allergy to house dust mites, which then sometimes causes food allergic reactions to crustaceans or molluscs. However, there are also observations on allergy to mites or cockroaches subsequent to sensitisation to crustaceans (de Blay, cited by Ayuso *et al.*, 2002a). There is evidence that the cross-reacting allergen is tropomyosin, and the Pen a 1 IgE-binding regions show high sequence homology with corresponding regions of Per a 7 and Der p 10 (60-100%), suggesting that there are similar IgE-binding epitopes in arthropods (Ayuso *et al.*, 2002 a and b).

Hyposensitisation with mite allergen may increase the risk for anaphylactic reactions to crustaceans and snails (Pajno *et al.*, 2002; Banzet *et al.*, 1992; van Ree *et al.*, 1996).

Of particular interest is possibility of cross-reactivity between crustaceans and helminth tropomyosin. There are clinical observations suggesting cross-reactivity between crustaceans (shrimp), molluscs (oysters in the case cited) and the fish parasite *Anisakis* (Pascual *et al.*, 1997; Gonzáez Galán *et al.*, 2002); possible clinical cross-reactivity between crustaceans and *Anisakis* is supported by the findings of *in vitro* immunological cross-reactivity between *Anisakis*, *Blatella germanica* and chironomids (Pascual *et al.*, 1997) and the observation by Ayuso *et al.* (2002a) of a sequence homology of 94% between IgE-binding regions of the shrimp allergen Pen a 1 and corresponding regions of tropomyosin from the helminth *Onchocerca volvulus*.

5.1.4 Absence of cross-reactivity of tropomyosins between crustaceans and vertebrates

In vertebrates, the muscle protein tropomyosin is not an allergen of importance. With 57 sera from meat allergic individuals, weak IgE reactivity to any of four mammalian tropomyosins was detected only in 2 out of 57 sera (Ayuso *et al.*, 1999), and vertebrate tropomyosin is considered to be non-allergenic (Restani *et al.*, 1997; Ayuso *et al.*, 2000; Leung *et al.*, 1996). Extracts of salmon, tuna, trout, pollack and mackerel all failed to significantly inhibit a shrimp RAST (O'Neil *et al.*, 1993), indicating that unique IgE epitopes are present among crustacean tropomyosins, which explains the absence of cross-reactivity between fish and crustaceans.

6. POSSIBLE EFFECTS OF FOOD PROCESSING ON ALLERGENICITY AND DERIVED PRODUCTS

It is a common experience that boiled crustaceans elicit clinical allergic reactions. It was early observed that shrimp has a major heat-stable allergen (Hoffman *et al.*, 1981; Subba Rao *et al.*, 1988; Naqpal *et al.*, 1989, Crespo *et al.*, 1995b). Tropomysin, the major crustacean allergen, is heat-resistant (Leung *et al.*, 1994).

7. THRESHOLD DOSES

There is little information in the literature on the lowest doses of any crustacean causing a clinical allergic reaction. In the case of inhaled food sensitive asthma, the doses are likely to be small. For ingested allergen, clinical experience also indicates that the dose may be low. Bernstein *et al.* (1982) report that a patient in a DBPCFC reacted to 14.0 grams of shrimp. Daul *et al.* (1988) report that during DBPCFC, four reactions occurred to a dose of four "shrimp equivalents", that is to 32 mg of shrimp extract (corresponding to four medium-sized 4 gram shrimps [16 grams]). The dose of protein that provoked the reactions must have been lower than 32 mg.

8. CONCLUSION

The allergenicity of crustaceans is evidenced by a large number of clinical studies, and confirmed by DBPCFC. Crustaceans appear to be among the food allergens that most commonly cause food-allergic reactions. No shellfish has been found safe. The reactions sometimes are very severe. The major allergen, tropomyosin, is well characterised. It is heat-resistant. Few studies address the question of the lowest dose of crustacean allergen that can elicit a reaction. Two studies report reactions to doses of 14 and 16 grams of shrimp or equivalent amounts of shrimp extract (less than 32 mg protein).

XI. ALLERGY TO FISH

SUMMARY

Fish is an important food in Europe. Allergy to fish appears to be among the most common food allergies, and can cause life-threatening reactions. Allergy to fish has been proven by DBPCFC studies. The major allergens have been characterised. In addition, fish has a number of allergens causing reactions only in a small number of fish allergic individuals.

The major allergen in fish is the abundant muscle protein parvalbumin, which has been characterised at the molecular level. Parvalbumins in different species of fish show great similarity, and probably are responsible for the extensive cross-sensitisation and clinical cross-reactivity between fishes. Minor allergens may cause more or less species-specific fish allergy. Except for parvalbumin fish collagen may play a similar role, but this has been less well documented. The major fish allergens are heat-resistant and are not much influenced by food processing. The lowest dose of fish reported to cause a clinical reaction is 5 mg.

1. BACKGROUND

Fish is a common food in all European countries. Traditionally consumption has been highest in coastal areas, but this pattern may have become less pronounced. Few accurate consumption data are available, but fish intake is considered to vary considerably between different regions, depending on local traditions and supplies. The highest consumption appears to be found in Iceland, where the 1993-1995 estimate of gross per capita fish and crustaceans intake was approximately 91 kg live weight (cited by USDA-ERS, 1999). According to the same source, in Portugal the intake was 59 kg, Norway 47 kg, Spain 43 kg, Sweden 29 kg, France 27 kg, Italy 22 kg, United Kingdom 19 kg, Germany 13 kg, and Hungary 4 kg. According to a different source (Yearbook of Nordic Statistics, 1991) the consumption of fish in Iceland was 80 kg/person per year, in Sweden 31 kg/person per year.

It is commonly thought that differences in consumption of a food are reflected in more or less corresponding differences in the occurrence of food allergy to the same food. However, this picture appears to be modified by other, unknown factors that at least to some extent influence also the occurrence of respiratory allergies. In the context of the European Community Respiratory Health Survey 502 individuals in Reykjavik, Iceland and 434 adult individuals in Uppsala, Sweden were studied (Gislason *et al.*, 1999). In Reykjavik, 20 individuals (4%) were positive to one or more allergens in a food panel (fx5) compared to 27 (6%) in Uppsala. With regard to the single food allergens in the panel, 16 positive reactions (in 9 individuals) were found in Reykjavik compared to 47 (in 20 individuals) in Uppsala ($p<0.05$), mirroring the situation with regard to sensitisation to respiratory allergens. Sensitisation to fish was 0.2% both in Uppsala and Reykjavik, a number too low to allow any conclusion with regard to differences in prevalence of fish allergy, but the study illustrates the tendency to co-variation of respiratory and food allergen sensitisation. Keeping in mind that sensitisation only inaccurately reflects the (lower) occurrence of clinical allergy, we must assume that other factors than the amount of fish and crustaceans eaten may have an equally strong influence on the development of allergy to these foods. However, once allergy has developed, fish and crustacean allergy is likely to present a greater problem for the allergic individual in areas where these foods are more often consumed.

It should be noted that the route of exposure appears to determine whether food allergy or respiratory allergy to fish develops. In some fish-processing workplaces, respiratory allergy to fish has been a considerable problem because of inhaled allergen (Douglas *et al.*, 1995; Jeebhay *et al.*, 2000; Lopata and Jeebhay, 2001; Rodríguez *et al.*, 1997). In food allergy, presumably induced by fish allergen in the food, fish allergen in the food will cause food allergy symptoms. However, also if fish allergen is inhaled, e.g. in the vapour from boiling fish or in the air at a fish market, symptoms of asthma and even generalised anaphylactic symptoms may develop (Crespo *et al.*, 1995a; Taylor *et al.*, 2000).

2. FREQUENCY

2.1 Prevalence of fish allergy – general population

The prevalence of "food allergy" as perceived by the general population is several times larger than the prevalence that can be verified by standard diagnostic procedures (Björnsson *et al.*, 1996; Jansen *et al.*, 1994). Most (but not all) cases of food allergy are presumed to be positive for specific IgE or skin prick test positive to the food in question. A negative specific IgE test to a food has been considered to have an almost 100% negative predictive value for the outcome of food challenge (Ortolani *et al.*, 1989).

For the discussion below and interpretation of data given, it must be kept in mind that test positivity is different from clinical food allergy, and the prevalence of test positivity will be higher than the prevalence of clinical allergy.

Fish allergy was demonstrated in a classical study early in the history of allergology (Prausnitz and Küstner, 1921), and fish and crustaceans are generally considered to be among the four foods most commonly provoking severe food anaphylaxis (Sampson, 2000).

Some data on fish sensitisation in the general population are given in Table 8.

2.2 Prevalence of fish allergy – food allergic adults and children

Fish was one of six foods found in DBPCFC to be the most common allergens (Bock and Atkins, 1990; Sampson and Albergo, 1984). In a study with labial challenge and single-blind placebo-controlled food challenge in France, Rancé *et al.* (1999b) found codfish to account for 7.1% of confirmed food hypersensitivity in a study of 378 children with food hypersensitivity. Similar results were found by some of the same authors in an earlier study, possibly including some of the same patients (Rancé and Dutau, 1997). In another paper from 1999a, Rancé *et al.* report a patient series of 703 patients with food allergies, confirmed by labial or single-blind placebo-controlled food challenge. In this study, cod and salmon accounted for 4.8% of food hypersensitivity reactions. In a later paper on 163 asthmatic children with food allergy studied by DBPCFC, giving 250/385 positive tests, codfish accounted for 6% of positive reactions (Rancé and Dutau, 2002). In a multicentre study of cases with anaphylactic reactions to food (Moneret-Vautrin and Kanny, 1995b), fish accounted for 8/81 cases. In a study by André *et al.* (1994) of 580 patients with pathological reactions to food, 60 presented with severe, near-fatal reactions. Of these, on the basis of clinical history, skin prick testing and serum specific IgE, fish accounted for 13%. DBPCFCs were not performed.

Table 8. Fish sensitisation in the general population*

Population	Age (years)	Number	Male (M)/ Female (F)	% test positive	Method	Authors
General Iceland, Sweden	33.0±6.9 32.6±7.4	502 434	48.3/51.7 48.7/51.3	0.2 0.2	IgE	Gislason *et al.*, 1999
General Denmark	14.1 mean	469 397 866	F M M+F	0.2 0.0 0.1	IgE	Mortz *et al.*, 2003
General Sweden	20-40	1397	~50/50	0.3	IgE	Björnsson *et al.*, 1996

* No data on clinical allergy

In Sweden, 39% of all paediatric patients with food allergy in one study had fish allergy (Dannaeus and Inganãs, 1981). In two studies in Spain, 18% and 30% of all children with clinical food allergy had symptoms related to the ingestion of fish (Boyano Martínez *et al.*, 1987; Crespo *et al.*, 1992).

Bock and Atkins (1990) studied 480 children with a history of adverse reactions to food by DBPCFC, and found that in the 185 children with positive reactions (n= 245), there were 8 reactions to fish (3 in children <3 years, 5 in children >3 years).

In a DBPCFC study collected over 13 years and consisting of 196 children (median age 5 years and 9 months; 45% male) with atopic dermatitis (Sicherer *et al.*, 2000a), 513 challenges to six common allergenic foods were positive. Of these 12 were fish challenges (egg 267, milk 117, soy 53, wheat 40, peanut 24).

Novembre *et al.* (1998b) report on 95 episodes of anaphylaxis occurring in 76 (50 boys, 26 girls) children (mean age ± SD = 6.1 ± 4.6 years). Fifty-four (54%) of the episodes were attributed to food allergy; 16/54 (30%) were judged to have been triggered by fish, which was the most common triggering food in this patient series.

Crespo *et al.* (1995c) studied 355 children with food allergy in Spain. Based on clinical history, skin prick tests and specific IgE, fish was found to be among the three most commonly involved foods, causing reactions in 30.4% of the patients (17.8% of 608 reactions).

In a study from South Africa consisting of individuals perceiving adverse reactions to seafood (n=105), Lopata and Jeebhay (2001) found that the two most common seafood species were prawns (47%) and rock lobster (44%). Specific IgE to 3 seafood groups (crustaceans, finfish and molluscs) was determined by testing of 12 seafood species by RAST. This gave 131 positive reactions on the group level in 80 individuals. Twenty per cent of the reactions were against finfish.

In a study of 14-year old Danish schoolchildren (Mortz *et al.*, 2003), the prevalence of skin prick test positivity to codfish was 5.4% (n=74) in children with atopic dermatitis, 2.6% (n=190) in children with inhalant allergy (without atopic dermatitis), and 0.5% (n=205) in normal controls.

3. CLINICAL FEATURES

3.1 General clinical manifestations

Generally, the pattern of allergic symptoms after ingestion of fish appears similar to the symptoms due to other foods (Lopata and Jeebhay, 2001). Bock and Atkins (1990) in a DBPCFC study of 480 subjects up to age 19 years found that cutaneous reactions were the most frequent symptoms for all foods tested, followed by gastrointestinal symptoms. Respiratory manifestations were recorded less frequently; however, asthma (wheezing) as the sole symptom was noted in four (of 245) positive reactions to DBPCFC. Clinical manifestations caused by fish in a DBPCFC study in 196 children (median age 5 years and 9 months; 45% male) with atopic dermatitis (Sicherer *et al.*, 2000a) which gave 12 positive challenges, were cutaneous (100%), gastrointestinal (42%) and respiratory (33%). Between 15% and 20% of the reactions were judged to be severe. Helbling *et al.* (1999) in a DBPCFC study noted oral allergy syndrome, urticaria, generalised pruritus, nausea and emesis, abdominal cramps, nasal congestion, chest tightness, and wheezing.

In a clinical study of 20 fish allergic children (de Martino *et al.*, 1990), prominent symptoms were urticaria/angio-edema, vomiting, asthma, and worsening of atopic dermatitis.

3.2 Anaphylaxis in highly fish allergic individuals

A considerable number of reports of severe allergic reactions triggered by fish can be found in the literature. Yunginger *et al.* (1988) from USA reported seven cases of fatal food-induced anaphylaxis. One of these was judged to be triggered by fish. Sampson *et al.* (1992) from USA reported 6 fatal and 7 near-fatal cases. One of the near-fatal cases had reacted to fish. Kemp *et al.* (1995) report on 89 cases of anaphylaxis caused by food. Tuna fish was the cause in one patient. Bock *et al.* (2001) from USA reported 32 cases of fatal food-induced anaphylaxis, of these one was judged to be triggered by fish eaten in a school lunch. Pumphrey (2000) reported 37 fatal food anaphylaxis cases from UK. Three of these were judged to be caused by "seafood". Eigenmann and Calza (2000) report on 14 patients with an established history of an anaphylactic reaction, four of these reactions were caused by fish intake. The absence of fish and crustaceans as an offending food in the studies reported by Macdougall *et al.* (2002) from UK and Ireland (8 fatal cases, 55 severe or near fatal), may possibly be due to the fact that this was a paediatric study.

3.3 Natural history of fish sensitisation

Fish allergy often is manifest in small children. In one published study of 79 patients with clinical food allergy to fish (Pascual *et al.*, 1992), the age of onset was as follows: 0-6 months 24%, 7-12 months 51%, 13-18 months 8%, 19-24 months 6%, >24 months 11%. Age distribution in a patient study can be biased for several reasons, but the data are in conformity with the perception that fish allergy tend to develop in the first year of life, but somewhat later than allergy to cows' milk and hens' egg.

In contrast to clinical allergy to milk, egg, wheat and soy which typically is outgrown (in spite of persistent positive skin tests), clinical allergy to fish often is "life-long" (Bock, 1982; Eigenmann *et al*, 1998; Sampson and Scanlon, 1989; Hill *et al.*, 1989).

4. IDENTIFIED ALLERGENS

Identified fish allergens are shown in Table 9.

4.1 Finfish allergens

Both fish muscle, fish skin and fish roe contain food allergens.

4.1.1 Fish muscle

Codfish allergens were the first food allergens to be purified and characterised (Aas, 1967; Aas and Jebsen, 1967; Coffee and Bradshaw, 1973; Elsayed and Bennich, 1975). Codfish contains one major allergen contained in the sarcoplasmic proteins of fish muscle, Gad c I. Gad c I is a parvalbumin (Aas, 1976; Elsayed and Apold, 1983). The allergenic determinants appear to be sequential, a fact that possibly may explain the low tendency for remission of fish allergy. Gad c 1 contains at least 5 IgE-binding epitopes distributed along its polypeptide chain.

Recently, a new parvalbumin allergen in Atlantic cod, Gad m 1, encoded by a gene distinct from that of Gad c 1, has been identified (Das Dores *et al.*, 2002a).

Parvalbumins from fish represent extremely abundant and stable allergens. They are considered by some authors to be the major and sole fish allergens for 95% of patients suffering from IgE-mediated fish allergy (Swoboda *et al.*, 2002). Parvalbumins are small (12 kDa; 108-109 amino acid residues) calcium-binding muscle proteins, and are present in high amounts in the white muscles of lower vertebrates and in lower amounts in fast twitch muscles of higher vertebrates, and have a function in calcium buffering and possibly in muscle relaxation. Allergenicity is greatly reduced by calcium depletion (Bugajska-Schretter *et al.*, 2000).

Parvalbumin has been found to be a major allergen in various other fish species. Cod has been mentioned above. Hamada *et al.* (2003) demonstrate that parvalbumin is a major allergen in three species of mackerels (*Scomber japonicus*, *S. australasicus and S. scombrus*) said to be the fish most frequently involved in IgE-mediated food allergy in Japan. Based on amino acid sequence data, the parvalbumin protein family can be subdivided into two evolutionary distinct lineages: the α group and the β group. The mackerel parvalbumin had 108 residues, being a member of the β-type parvalbumins. Sera from four of five patients who had experienced clinical hypersensitivity to fish, contained IgE-binding the parvalbumins from all three species, demonstrating the cross-reactivity of the parvalbumins.

Bugajska-Schretter *et al.* (2000) described carp parvalbumin, Lindström *et al.* (1996) salmon parvalbumin.

Collagen has recently been proposed to be an important fish allergen (Hamada *et al.*, 2001), although further verification is needed. A high-molecular weight allergen from bigeye tuna muscle was found to bind specific IgE from 5 out of 8 allergic patient sera, but none of the normal control sera. The authors concluded that the allergen was collagen, probably type 1 collagen which is the representative collagen in fish muscle.

Table 9. Identified fish allergens

Allergen	Source of allergen	Family	Molecular weight	Population/source of antibodies	Authors
Fish					
Gad c 1	Baltic cod *Gadus callarias*	Parvalbumin	12 kDa	Sera from fish allergic donors	Elsayed and Aas, 1971; Elsayed and Bennich, 1975
Gad m 1	Atlantic cod *Gadus morhua*	Parvalbumin	12 kDa	Sera from patients with adverse reactions to Atlantic cod	Das Dores *et al.*, 2002b
Cyp c 1	Carp *Cyprinus carpio*	Parvalbumin	13 kDa	Sera from 30 patients allergic to fish	Bugajska-Schretter *et al.*, 2000
Sco j 1	Pacific mackerel *Scomber japonicus*	Parvalbumin	11 kDa	Sera from 5 fish allergic patients	Hamada *et al.*, 2003
Sco a 1	Spotted mackerel *Scomber australasicus*	Parvalbumin	11 kDa		
Sco s 1	Atlantic mackerel *Scomber scombrus*	Parvalbumin	11 kDa		
Sal s 1	Atlantic salmon *Salmo salar*	Parvalbumin	12 and 14 kDa (two isoforms)	Sera from 11 patients with a history of fish allergy	Lindström *et al.*, 1996
Tra j 1	Horse mackerel *Trachurus japonicus*	Parvalbumin	10.5 kDa	Sera from 2 fish hypersensitive patients	Shiomi *et al.*, 1998
Not assigned	Codfish, hake, whiting, dab, salmon, tuna	Aldehyde phosphate dehydro-genase (ADPH)	36 kDa, 41 kDa	Sera from 13 codfish allergic patients	Galland *et al.*, 1998; Das Dores *et al.*, 2002a
Fish parasite Anisakis					
Ani s 1	*Anisakis simplex*	Enzyme (?)	24 kDa	Sera from patients with clinical *Anisakis simplex* hyper-sensitivity and specific IgE against the parasite	Moneo *et al.*, 2000; Gómez-Aguando *et al.*, 2003

Collagen is ubiquitously found as an extracellular matrix protein in animals. Native collagen is composed of three homo or hetero α-chains twisted together to form a triple helix, and is insoluble in water at low temperature. If collagen is denatured, each α-chain is released from the triple helix, and the denatured form of collagen, i.e. gelatine, is water-soluble. Allergenicity appears to be heat-stable. Similar to parvalbumin, the heat-stable collagen may cause extensive cross-allergenicity among fishes.

If the importance of collagen as a major allergen in fish is confirmed, this will somewhat reduce the relative importance of parvalbumin, and explain several inconsistencies in earlier observations. For example, in two species of tuna fish (albacore and yellowfin), parvalbumin was recognised by only one of eight sera from tuna fish-sensitive patients (Yamada *et al.*, 1999). The relative importance of parvalbumin *versus* collagen perhaps may be found to differ both between patients and between fish species. One patient in the study described by Hamada *et al.* (2001) reacted (IgE-binding) to both tuna allergens, while others reacted predominantly to one or the other of the allergens.

Baltic codfish has been found to contain, in addition to the one major allergen Gad c I, a number of other intermediate and minor allergens (Aukrust *et al.*, 1978a; Aukrust *et al.*, 1978b; Dory *et al.*, 1998). Hansen *et al.* (1996) by immunoblotting demonstrated 18 IgE-binding bands in freshly prepared codfish extract. Das Dores *et al.* (2002b) characterised a new minor allergen of raw codfish (4 of 13 codfish allergic patients reacted against the allergen) of 41 kDa (Galland *et al. cf.* 1998).

Species-specific (more or less) allergens have been reported in swordfish, tuna, trout, pollock and codfish (Kelso *et al.*, 1996; Mata *et al.*, 1994; Galland *et al.*, 1998; Yamada *et al.*, 1999). The latter author found an allergen that was specific for Yellowfin tuna, as compared to Albacore tuna. There is evidence that the allergenicity of different fish species may to some extent differ, with hake and cod being among the more allergenic, and albacore tuna being among the least allergenic (Pascual *et al.*, 1992).

4.1.2 Fish skin

Muscle allergens on fish skin may be released during cooking, and fish skin is also often eaten (e.g. grilled fish). Fish skin is used for the preparation of gelatine, and as mentioned above, it has been proposed that fish collagen is an allergen of importance (see below).

4.1.3 Fish roe

A report on repeated anaphylactic reactions after intake of Russian Beluga caviar has recently been published (Untersmayr *et al.*, 2002). The patient had no clinical allergy to fish, and was skin test and specific IgE negative to fish (test fish species not specified). Serum contained specific IgE to several proteins in Beluga and Sevruga caviar (derived from sturgeon) and also to a lesser degree to proteins in "false" caviar (lumpsucker, salmon and trout), but there was skin test positivity only to Beluga and Sevruga caviars. Considering the near absence of other reports on allergy to fish roe, this allergy appears to be rare.

4.2 Other fish-related allergens

Since 1990, fish parasites of the genus *Anisakis* have been recognised to contribute to fish-related anaphylaxis (Kasuya *et al.*, 1990). The ingestion of seafood contaminated with third-stage *Anisakis* larvae can induce a specific IgE response and elicit a reaction, usually urticaria but sometimes anaphylaxis, even when the fish is cooked. A major parasite allergen, Ani s 1, has been isolated and characterised (Moneo *et al.*, 2000). The allergen shows no homology to previously identified proteins. The allergen is located in secretory granules of the excretory gland (Gómez-Aguado *et al.*, 2003). An association between *Anisakis* sensitisation and *Askaris*, *Daphnia*, chironomids, Atlantic shrimp, and German cockroach has been described (Kennedy *et al.*, 1988; Pascual *et al.*, 1997), and tropomyosin has been suspected to be the

putative cross-reacting allergen (Asturias *et al.*, 2000; Ayuso *et al.*, 2002a), but this has so far not been proven.

5. CROSS-REACTIVITIES

5.1 Finfish cross-reactivities

There is broad but somewhat unpredictable cross-reactivity immunologically and clinically among fishes. This cross-sensitisation and clinical cross-reactivity are caused by the major allergen parvalbumin, and by other shared allergens. Torres Borrego *et al.* (2003) state that 50% of individuals allergic to one type of fish will react to a second species of fish, and that up to 40% of patients sensitised to one or more species of fish do not present symptoms on consuming other species. The best tolerated fish appears to belong to the *Scombroideae* family, which includes tuna.

5.1.1 Serological and skin prick test cross-reactivity

Bernhisel-Broadbent *et al.* (1992a) studied skin prick test cross-reactivity to 10 fish species in 11 patients. Skin prick tests were positive to all 10 fish in 8/11 patients, and the remaining three patients had skin prick test reactivity to at least two fish.

In another study, a majority of sera reactive with one of six fish species, reacted with at least five of six species tested. Fewer sera reacted against Albacore tuna (Pascual *et al.*, 1992). In another study, all out of 30 sera from patients allergic to fish displayed reactivity to parvalbumin. Four of five sera from fish allergic patients reacted to all parvalbumins isolated from three species of mackerel (Hamada *et al.*, 2003).

Among fish allergic patients (n=9) 56% reacted serologically against all fish tested (hake, mackerel, tuna, salmon). In an immunoblot study of IgE reactivity to individual antigens in 7 different fish species, the same authors found both cross-reactive but also species-specific allergens.

5.1.2 Clinical cross-reactivity

The absence of certain allergens from certain species, e.g. an allergen present in Yellowfin tuna but not in Albacore tuna (Yamada *et al.*, 1999), allow some patients to react clinically to some species of fish but not to others. Also, not all of the five epitopes demonstrated for the cod allergen Gad c 1 (Elsayed and Apold, 1983) may be of equal importance, and patients may show differential reactivity to individual epitopes as well as to individual allergenic proteins.

Hansen *et al.* (1997) report cross-reactivity to cod, mackerel, herring and plaice of sera from 8 clinically codfish allergic patients (verified by DBPCFC). De Martino *et al.* (1990) demonstrated extensive cross-reactivity between 17 fish species as determined with skin prick test, RAST, and clinical history. The authors conclude that cod allergy appears to be, on the whole, a reliable index of fish allergy.

Bernhisel-Broadbent *et al.* (1992a) studied clinical cross-reactivity to 10 fish species in 11 patients. Positive oral challenges occurred to only one fish in 7/11 patients, two fish in one

patient, and three fish in two patients (each patients was challenged with four to six fish species). The authors conclude that most fish allergic patients are able to eat one or more fish species without symptoms.

Helbling *et al.* (1999) performed skin prick tests, serological studies and DBPCFC in 9 subjects with clinical symptoms attributed to fish allergy. The skin prick tests and serological studies confirmed the broad skin prick test and serological cross-reactivity among fishes, but also that individuals with high IgE values to some species of fish can have low or undetectable levels of specific IgE to other fishes. In DBPCFC, three of four individuals tested with three species of fish reacted to all three species of fish, one of two subjects challenged with two fish species reacted to both. The data support the notion that broad clinical cross-reactivity between various species of fish is not uncommon. The authors emphasise that fish allergic subjects should be advised to avoid all fish species until a fish species can be proven safe to eat by food challenge.

6. POSSIBLE EFFECTS OF FOOD PROCESSING ON ALLERGENICITY AND DERIVED PRODUCTS

6.1 Finfish

The major fish allergen parvalbumin corresponding to the cod allergen Gad c 1 is resistant to boiling and other high temperature processing. Gad c I is a "sequential" allergen, remaining allergenic after heating at 100^{o}C for 10 min and after digestion with proteolytic enzymes or denaturation with chemicals (Elsayed and Aas, 1971; Aas, 1976; Elsayed and Apold, 1983). The allergenicity is decreased by polymerisation or by acetylation of the Tyr-30 residue, but these chemical modifications cannot be exploited commercially to reduce the allergenicity of codfish (Apold and Elsayed, 1980).

Bigeye tuna collagen was studied by Hamada *et al.* (2001) and was found to be very thermostable as to its allergenicity. Even when denatured to gelatine by heating in a boiling water bath for 120 minutes, the bigeye tuna collagen retained 90% of its original binding ability for IgE.

A number of other fish allergens are temperature-sensitive. In the study of 8 tuna fish allergic patient by Yamada *et al.* (1999), only one of eight patients appeared to have IgE-binding to parvalbumin, and a number of other IgE-binding proteins were observed. However, although IgE-binding proteins were observed in cooked or canned tuna, the biologic function was absent when tested in the histamine release assay. This may explain how cooked or canned fish, e.g. salmon and tuna, may be tolerated by individuals who react to undercooked or raw fish (Bernhisel-Broadbent *et al.*, 1992b).

Surimi, in contrast, appears to retain much of fish allergenicity. This difference in allergenicity compared to canned fish may partly be due to the fact that canned tuna and salmon are cooked for up to 14 hours, whereas surimi is cooked only briefly and at low temperature (Musmand *et al.*, 1996).

It should be noted that some patients appear to react to cooked fish but to not raw fish. Already in the classic paper by Prausnitz and Küstner (1921), it was noted that Küstner was sensitive to cooked codfish but not to raw codfish.

In conclusion, heat treatment during food preparation or processing may in some cases reduce allergenicity, but it is not a reliable method to render fish less allergenic.

6.2 Allergenicity of highly processed and novel fish-based products

The complexity of this field is high. These foods may contain potentially allergenic proteins from several sources, e.g. egg white, or enzyme inhibitors from legumes or chicken. At the same time, products may be made that are highly purified or hydrolyzed and perhaps have reduced fish allergenicity (Mireles DeWitt and Morrissey, 2002; Bindslev-Jensen *et al.*, 2003).

6.2.1 Surimi

Surimi is a product made of minced and thoroughly washed fish meat. It can be made from one species of fish (e.g. cod, Alaskan pollack or mackerel surimi) or it can be prepared from a mixture of many species of fish and retains most of its allergenicity after processing (Mata *et al.*, 1994; Musmand *et al.*, 1996; Helbling, 1992). Small fish and fish species not used for other purposes may be used for this industrial fish product (Venugopal and Shahidi, 1995). Surimi is reported to lack most of soluble sarcoplasmic muscle proteins, and is composed mainly of myofibrillar protein (Lee, 1986). Surimi may have added starch, egg white, meat protein, seafood-derived flavourings, spices, seasonings and other ingredients, forming an elastic gel after cooking. Surimi has numerous applications in food industry, the most well-known is probably crab meat imitation ("crabsticks") and other imitation crab, shrimp, scallops, and seafood snacks. Surimi-meat blend products, such as "meatless" hot dogs, hybrid ham and bologna, sausages, pepperoni sticks, and pizza toppings are being developed and tested-marketed (Musmand *et al.*, 1996).

Mata *et al.* (1994) studied surimi allergenicity. Fresh codfish and surimi (source not specified) were compared. The investigators found an allergenic protein in SDS-PAGE gel filtration eluates of surimi which was also found in codfish eluates, with a molecular mass of 63 kDa. This is different from the major codfish allergen Gad c I which has a molecular weight of 13 kDa, and which may presumably have been washed out during surimi preparation. The authors speculate that this protein may represent an additional allergen present in both codfish and surimi. The 63 kDa protein was the single allergenic protein detected in surimi. Six codfish allergic patients all reacted to surimi in skin prick tests, and the allergenicity was also demonstrated by specific IgE-binding by surimi, by surimi inhibition of specific IgE-binding by codfish and by the leukocyte histamine release test.

Helbling (1992) investigated the allergenic potential of surimi in a group of 30 adults allergic to fish. Fifty per cent of the group had positive skin test reactions to both pollack and surimi extracts, and 30% had positive RAST tests to both surimi and pollack. The authors demonstrated cross-reactivity between a surimi-derived pizza topping and several fish species, including pollack, by RAST inhibition test.

Musmand *et al.* (1996) reported the first case of surimi allergy in a patient verified by DBPCFC, which reacted to 1 g of surimi.

6.2.2 Gelatine

Gelatine is commonly made from other sources, but can also be prepared from fish. For example, the source of gelatine in kosher foods is often fish skins, including skin from commonly allergenic fish, such as cod. Some muscle tissue is likely to be adherent to the skin; however, the presence of fish allergens in fish skins to be used for gelatine production has not been documented (Taylor and Hefle, 2000).

The gelatine-making process must be expected to involve rather dramatic modification of fish proteins (Taylor and Hefle, 2000). However, the allergenicity of fish gelatine has been reported in one study, describing a child with sensitivity to gelatines from codfish and salmon (Sakaguchi *et al.*, 1999). Unfortunately, it was not reported whether this child had allergy to fish. Bovine gelatine, to which the child also was sensitive, did not inhibit IgE reactivity to the fish gelatines. Also other studies have indicated that there is no IgE cross-reactivity between fish and mammal collagens (Hamada *et al.*, 2001). André *et al.* (2003) studied IgE-binding to gelatines extracted from tuna flesh and tuna skin, as well as porcine and bovine gelatines. Only 3 of 100 sera from fish allergic individuals tested gave evidence of reactivity to gelatine extracted from tuna skin. Cross-reactivity between fish gelatines and porcine and bovine gelatines was not observed. The authors conclude that the risk of adverse reactions to tuna skin gelatine seems to be significantly lower than the risk of reaction to fish.

Isinglass has far more than hundred years been widely used to clarify alcoholic beverages (Hickman *et al.*, 2000). It is derived from the swim bladder of certain tropical fish and consists predominantly of collagen. It works by aggregating particles in the beverage, so that these then can be removed by sedimentation or filtration. No information on putative allergenicity of isinglass has been found in medical literature.

6.2.3 Ice-structuring protein

Ice-structuring proteins are naturally occurring proteins that bind to ice and structure ice crystal formation. Their natural function is to help protect organisms in cold habitats from ice crystal damage. Ice-structuring proteins have been reported to have a number of potential commercial applications, including the food industry. Ice-structuring proteins can be isolated from fish living in or near Arctic waters, and have been produced using recombinant baker's yeast. Ice-structuring protein has been examined with regard to allergenicity. The protein preparations did not bind specific IgE to fish, and other evidence for allergenicity was not found (Baderschneider *et al.*, 2002; Bindslev-Jensen *et al.*, 2003).

6.2.4 High-pressure pasteurisation

High pressure with little or no heating above room temperature represent a novel way of "pasteurisation", found effective for the killing of bacteria. This form of "pasteurisation" has certain advantages over traditional pasteurisation by high temperature. No published data on altered allergenicity of fish or crustacean proteins after high-pressure pasteurisation have been found. Because the effect on the food appears to be milder than the effect from traditional pasteurisation, it seems likely that no new allergens are introduced. On the other side, the milder treatment may not significantly reduce allergenicity either.

7. THRESHOLD DOSES

7.1 Finfish

In a double-blind, placebo-controlled oral food challenge study in 196 children (median age 5 years and 9 months; 45% male) with atopic dermatitis (Sicherer *et al.*, 2000a), 17% (2 of 12 children) reacted already to the lowest dose of 400 or 500 mg of fish. No strong correlation between skin prick test wheal size and the dose causing a reaction or the reaction severity was observed (correlation coefficients -0.14 and 0.39, respectively; not statistically significant).

Helbling *et al.* (1999) in a DBPCFC study found that the lowest dose of fish employed elicited subjective symptoms in 2 of 9 individuals and objective signs in 1 individual. In five challenges both subjective and objective symptoms and signs were observed with doses of 1 g or lower.

Hansen and Bindslev-Jensen (1992) in a DBPCFC study found that the minimum amount of codfish needed to elicit a reaction (starting at 5 mg and going up to 5 g) was 6 mg. Taylor *et al.* (2002) site data on lowest provoking dose of fish from various investigators reported at a roundtable conference on threshold doses. Data (lowest observed provoking dose of fish in DBPCFC) are as follows: Rancé 16 mg; Moneret-Vautrin 65 mg (15 mg in single blind study); Bindslev-Jensen and Hansen 5 mg (cod), 500 mg (mackerel), 5 mg (herring), 6000 mg (plaice); Bock 200 mg.

It should be noted that all doses cited above relate to mg fish and not to mg protein, the protein dose will be lower.

8. CONCLUSION

The allergenicity of fish is evidenced by a large number of clinical studies, and confirmed by DBPCFC. Fish appears to be among the food allergens that most commonly cause food allergic reactions. No fish has been found safe. The reactions sometimes are very severe. The major allergens are well characterised. The major allergens are heat-resistant. The lowest reported allergen dose provoking a clinical reaction was 5 mg of fish.

XII. ALLERGY TO EGGS

SUMMARY

Egg allergy represents one of the most frequent allergies in the population at levels of affected individuals similar to milk and peanut allergies. Sensitisation to egg with subsequent allergic symptoms in childhood often occurs without known oral exposure. Egg sensitisation rates at 12 months of age in infants with an atopic history are around 7% and are predictive of asthma in later life. The frequency in childhood is around 2-3% and similar to those reported in adults. Clinical features are comparable to those seen in other food allergic diseases from mild oral and skin reactions to severe anaphylaxis and death. Egg allergens are found in egg white and egg yolk and are partially characterised and identified as Gal d 1 to Gal d 5. The effects of heat and food processing are not likely to abolish allergenicity and most egg allergic individuals react to cooked and raw eggs.

Clinical and serological cross-reactivities exist to some extent to other bird and mammalian eggs. Patients with food allergies to egg yolk may also suffer respiratory symptoms caused by bird exposure.

Egg lysozyme is used in the preparation of medications and also in the food industry as bactericide to prevent the growth of anaerobic bacteria. Egg lecithin is also used as emulsifier (E322) and there is the possibility of residual allergenicity of these preparations in severely allergic patients.

Egg albumin is used as a clarifying or fining agent in the production of red wine. Reports of allergies to the protein content after clarification appear to be rare although well conducted studies are lacking.

Threshold doses or more precise lowest doses at which severely egg allergic patients reacted are in the microgram and low milligram range.

1. BACKGROUND

In several westernised countries, egg allergy is one of the most frequent allergies together with milk and peanut (Bernhisel-Broadbent *et al.*, 1994; Eggesbo *et al.*, 2001a; Eigenmann and Calza, 2000; Hill *et al.*, 1999; Kanny *et al.*, 2001; Schäfer *et al.*, 1999). Egg sensitisation at one year of age is highly predictive of asthma in later life (Kulig *et al.*, 1998).

2. FREQUENCY

Egg allergy prevalence estimates based on parental perception vary between 3% and 7% at the age of two years (Eggesbo *et al.*, 2001a; Kajosaari, 1982). On the basis of positive skin tests the prevalence was estimated to be 3.2% (Hill *et al.*, 1999). In an opinion poll-like questionnaire-based study on 33,110 individuals in France representing a sample of the normal population 3.5% reported symptoms of a food allergy of which 4% thought they were reacting to eggs (Kanny *et al.*, 2001). In a population based study (3289 children born in 1992) using a stepwise diagnostic procedure including a double blind food challenge, a mean

of 2.6% ± 1% of children have been diagnosed with egg allergy at 2.5 years of age (Eggesbo *et al.*, 2001a).

The positive predictive value of parents reporting adverse reactions to eggs increases to 100% in those patients who reacted 3 times to a food and where egg was the most likely causative ingredient (Eggesbo *et al.*, 2001a).

3. CLINICAL FEATURES

The clinical symptoms caused by hens' eggs in egg allergic patients are similar to those reported in general for other food allergies (see Table 1).

4. IDENTIFIED ALLERGENS

Major allergens of the eggs of the species *Gallus domesticus* are known, characterised and classified as Gal d 1-5 by the International Union of Immunological Societies (IUIS) although major allergen sources are still unassigned (Cooke and Sampson, 1997; Eigenmann *et al.*, 1996a; Elsayed and Stavseng, 1994; Holen and Elsayed, 1990; Honma *et al.*, 1996 and 1994; Kahlert *et al.*, 1992; Mine and Zhang, 2002; Szepfalusi *et al.*, 1994).

The egg white (albumen) content is classified as:

Gald 1 (ovomucoid 28 kDa)
Gal d 2 (ovalbumin, 43 kDa)
Gal d 3 (conalbumin, 76 kDa)
Gal d 4 (lysozyme, 14 kDa)
Gal d 5 (serum albumin, 69 kDa)

Major yolk components include:

Lipovitellin (400 kDa)
Phosvitin (~175 kDa)
Low density lipoprotein (unknown)
Low density lipoprotein (3-10 x 10^6)
Livetins (45-150 kDa)
and other not yet well characterised proteins

Ovomucoid, one of the major egg allergens for clinical reactions (Bernhisel-Broadbent *et al.*, 1994) is relatively heat-stable and individuals reacting to this allergen will be reacting to raw and cooked eggs. There are a number of reports investigating the effects of heat and chemical denaturation procedures on major egg allergen *in vitro* (Anet *et al.*, 1985; Bernhisel-Broadbent *et al.*, 1994; Cooke and Sampson, 1997; Kato *et al.*, 2001; Mine and Zhang, 2002; Sakai *et al.*, 1998; Elsayed and Stavseng, 1994; Holen *et al.*, 2001; Kahlert *et al.*, 1992; Shimojo *et al.*, 1994).

5. CROSS-REACTIVITIES

Clinical cross-reactivities are generally restricted to avian eggs to a varying extent. Hens' egg white immunologically cross-reacts with egg white from turkey, duck, goose, seagull (Langeland, 1983). In this study all egg whites contained proteins able to bind human IgE antibody of patients with allergies to hens' egg white. Several cross-reacting proteins in egg white were also detected in egg yolks and to some extent hen and chicken sera and flesh. Occasionally, patients with allergies to chicken and other avian meats are reported who are able to eat eggs without symptoms (Anibarro *et al.*, 2000; Cahen *et al.*, 1998). One report (Anibarro *et al.*, 2000) describes patients allergic to duck and goose eggs without sensitisation to hens' eggs. The probability of cross-reactions is likely to be affected by the interspecies relationships (Kelso *et al.*, 1999).

Individuals with both inhalant allergies to birds and allergic reactions after egg intake exhibit IgE antibodies against α-livetin which is a component of both egg yolk and birds' feathers (Bausela *et al.*, 1997; de Blay *et al.*, 1994; Hoffman and Guenther, 1988; Szepfalusi *et al.*, 1994).

5.1 Other egg-related allergies

5.1.1 Birds' nest allergies

Edible nests of Collocalia species has been used as a Chinese health-improving delicacy (Ou *et al.*, 2001). Anaphylaxis after ingestion has been reported and immunochemical characterisation of a putative 66 kDa allergen found to be homologous to ovoinhibitor, a serine protease inhibitor which is one of the allergens found in egg white (Goh *et al.*, 2000 and 2001).

Birds' nest soups are increasingly being marketed worldwide and could be of clinical relevance to individuals with hens' egg allergies.

5.1.2 Bird-egg syndrome

Patients with food allergies to egg yolk may also suffer respiratory symptoms caused by bird exposure (Quirce *et al.*, 2001; Szepfalusi *et al.*, 1994).

The cross-reacting allergen is partially heat-labile α-livetin (Quirce *et al.*, 2001). Other allergens have been reported (Szepfalusi *et al.*, 1994) and incubation of pooled sera from patients with bird-egg syndrome with bird feather extracts led to complete blocking of IgE-binding to allergens in egg yolk and bird feather extracts. IgE from patients with egg white allergy did not react with allergens in egg yolk or bird feather extract (Szepfalusi *et al.*, 1994).

6. POSSIBLE EFFECTS OF FOOD PROCESSING ON ALLERGENICITY AND DERIVED PRODUCTS

Most egg allergic individuals react to cooked and raw eggs (Langeland, 1982 a and b). Rarely individuals may react only to raw eggs and can tolerate cooked eggs (Eigenmann, 2000). These allergic individuals often exhibit lower egg-specific IgE levels (Boyano Martínez *et al.*, 2001). Heating and freeze drying can reduce the clinical allergenicity in some patients (Urisu

et al., 1997) but does not reliably prevent IgE-binding or clinical reactions. A major reason is likely to be the heat stability of ovomucoid and ovalbumin, both major allergens.

6.1 Egg lysozyme and lecithin

Egg lysozyme is used in the preparation of medications and also in the food industry as bactericide to prevent the growth of anaerobic bacteria (Fremont *et al.*, 1997). Egg lecithin is frequently used in the food industry as emulsifiers (E322). The possibility of residual allergenicity in allergic reactions has been highlighted by Palm *et al.* (1999) who demonstrated a protein content of 11% (Kjeldahl method) in a lecithin preparation which triggered an allergic reaction in a DBPCFC. The threshold was estimated to be between 50-100 mg lecithin.

6.2 Wine clarification

A number of clarifying or fining agents such as bovine gelatine, egg albumin, cereal proteins, and fish-derived icing proteins are commonly used in the production of red wine. Reports of allergies to the protein content after clarification are rare and these procedures have been considered as safe although well conducted studies are missing (Cattaneo *et al.*, 2003; Marchal *et al.*, 2002). In view of the variable use of the fining agent and process, absence of the fining agents in the finished product may not always be guaranteed. Based on this possibility and awaiting the outcome of ongoing research, Australian and New Zealand wine producers declare the source of their fining agents on the label.

7. THRESHOLD DOSES

The determination of threshold levels triggering clinical relevant reactions in allergic individuals is fraught with difficulties for theoretical and practical reasons (Bindslev-Jensen *et al.*, 2002; Taylor *et al.*, 2002).

In very sensitive patients reactions to small amounts of egg (micrograms) have been reported (Wüthrich, 2000; Wüthrich and Ballmer-Weber, 2001). Threshold levels based on food challenge studies are unreliable since a number of patients react already at the first dose. There are also no data on a possible threshold dose of contamination below which no egg allergic patient is likely to experience symptoms (Bindslev-Jensen *et al.*, 2002). Using published data, adjusted for allergen content as good as possible, Bindslev-Jensen developed a statistical model, which would allow extrapolation of allergen doses likely to cause a reaction. Threshold doses for egg allergens were in the same order of magnitude as for milk and peanut. The formula predicts that one in a million of egg sensitive individuals would react to 0.002 mg of hens' egg allergen and 1 in 100 to a dose of 0.15 mg (0.024 lower confidence limit). Extrapolation to raw egg as food consumed would be 0.001 mg (1/1,000,000) and 1.6 mg (1/100) (Bindslev-Jensen *et al.*, 2002). A round-table discussion of paediatric allergy specialists noted clinical reactions in egg allergic patients at the following doses (Table 11).

These observations broadly support the reported threshold doses based on previously reported data (Bindslev-Jensen *et al.*, 2002).

Undeclared egg contamination of food products has been the major reason for food products recall in the United States in 1999. Of a total of 236 recalls for the two most serious classes I

and II, egg contamination accounted for 90 in total of which 66 (73%) fell into the most serious class I (reports of patients experiencing adverse clinical reactions (Vierk *et al.*, 2002).

Table 11. Amounts of protein levels reported to induce adverse reactions in egg allergic individuals [modified after Bindslev-Jensen *et al.*, 2002 and Taylor *et al.*, 2002 as result of a roundtable discussion]

Amount of food	Protein	Reported by
2 mg	0.2 mg	Moneret-Vautrin as reported by Taylor *et al.*, 2002
265 mg	26.5 mg	
20 mg	-	Bock *et al.*, as reported by Taylor *et al.*, 2002
5 mg	0.65 mg	Bindslev-Jensen *et al.*, 2002
400 mg	200 mg	Burcks *et al.*, 2002
0.02 mL	2 mg	Hill as reported by Taylor *et al.*, 2002
1 mg	0.13 mg	Rancé *et al.*, 2002
100 mg	10 mg	Lack as reported by Taylor *et al.*, 2002
200 mg	20 mg	
40 mg	4 mg	Strobel*, 2002
50-100 mg	5-10 mg	Palm *et al.*, 1999

* Strobel, unpublished observation

8. CONCLUSION

Egg proteins are frequent triggers of allergic reactions and represent a major source of food-derived allergens. Both egg white- and egg yolk-derived proteins have been described in clinical reactions to eggs. Most egg allergic individuals exhibit IgE-binding to egg white. A number of egg allergens have been identified and classified as Gal d proteins by the IUIS. There are possible clinical cross-reactivities between other bird and mammalian eggs. Heat denaturation and other food processing treatments do not reliably reduce the allergenicity to a level which would constitute a safe level for severely allergic individuals. Threshold doses reported to trigger reactions in clinical studies range from microgram to low milligram levels of orally administered egg proteins.

XIII. ALLERGY TO PEANUTS

SUMMARY

Peanut, a member of the legume family, is a frequent cause of food allergic reactions. Prevalence of peanut allergy is estimated between 0.5% and 1.1% in the adult population of some American and European countries. This seems however to be a growing phenomenon, whose incidence and prevalence are increasing in the last few years. Peanut allergy usually starts in childhood, at an average age of 5 years, and only a minority of patients outgrows it; tolerance is achieved in about 20% of patients, but cases of re-sensitisation have been described. Severe to life-threatening reactions to peanut are common, more frequently in asthmatic patients; fatalities due to peanut ingestion have been reported, especially in the UK and in the USA. Allergic reactions usually begin a few minutes after ingestion, and frequently involve more than one target organ (e.g. skin, respiratory, gastrointestinal and cardiovascular system).

The standard for the diagnosis of peanut allergy is the DBPCFC, but because of the risk of provoking severe reactions, safer tests are required. Some diagnostic tools are provided by determination of cut-off levels with a high positive predictive value.

At least seven peanut allergens have been identified up to now: the vicillin Ara h 1, the conglutinins Ara h 2, 6 and 7, the glycinins Ara h 3 and 4 and the profilin Ara h 5. Ara h 1 and Ara h 2, which respectively represent 20% and 10% of all peanut proteins, are the most important peanut allergens, recognised by the great majority of peanut allergic patients. These allergens are thermal-resistant, and their allergenicity is enhanced by roasting, possibly because of structural modifications of the molecules or increase in their enzymatic function.

Peanut proteins survive treatments for the extraction of oil from peanut, so that trace amounts of allergenic proteins can be found in peanut oil.

A peculiar characteristic of peanut allergy is the high frequency of reactions following accidental ingestion of peanut-contaminated foods; many studies using oral food challenges highlighted that very low doses of peanut (ranging from 100 μg to 1 g of peanut protein), are able to elicit symptoms in peanut allergic patients.

1. BACKGROUND

Peanut (*Arachis hypogea*) is a member of the legume family, which also includes pea, bean, soybean, lupin and lentil. Peanut consumption has increased during the last decades because of its content in easily digested proteins and its versatility: it can be eaten as a vegetable, crushed or ground as a "butter", roasted or salted as snacks, incorporated into candies and used to produce oil extracted by solvents or pressure. In the United States, one of the world's largest producers of peanuts, one half of the crop is consumed as peanut butter, 20% as table peanuts, 16% is used in candies and baked goods, and the remainder is processed into oil. The wide use of peanuts makes inadvertent exposure extremely frequent: peanut butter, for example, is often used in restaurants to make soft foods more firm or to "glue down" and close egg rolls; deflavoured peanuts (i.e. peanuts that have been pressed, deflavoured and reflavoured) are sold as other nuts such as walnuts and almonds (Loza and Brostoff, 1995).

2. FREQUENCY

Peanut allergy is one of the most common forms of food IgE-mediated reaction. According to epidemiological studies from the UK and USA, its prevalence in adults ranges from 0.48% in the UK (Emmett *et al.*, 1999) to 1.1% (together with tree nut allergy) in the USA (Sicherer *et al.*, 1999). Similar data are reported by French authors (Kanny *et al.*, 2001). The relative epidemic of peanut allergy appears to be a phenomenon of the past two decades (Sampson and Hugh, 2002). A very recent birth cohort study on children aged 0-4 years found an overall prevalence of peanut or tree nut sensitisation of 1.1% (Tariq *et al.*, 1996). The same authors subsequently found an increase in peanut sensitisation from 1.1% to 3.3% and clinical reactions from 0.5% to 1% over a 6-year period (Grundy *et al.*, 2002). These data seem reliable as they came from the results of open oral challenges with peanut and not only from patients' histories as in previous studies.

Peanut is also the most common cause of severe or fatal food-induced anaphylaxis. In 1982 Fries described a number of anaphylactic reactions to peanuts; six years later, Evans *et al.* (1988) described the first fatal reaction in a 24-year old woman after accidental ingestion of peanuts. Since that time, several other episodes of fatal or near-fatal allergic reactions to peanuts have been reported, thus confirming the life-threatening potential of this food. In reviews of fatal, food-induced anaphylaxis in the United States, 7 out of 13 deaths were attributable to peanut (Yunginger *et al.*, 1988; Sampson and Hugh, 1992). In an American registry of fatal food-induced anaphylaxis, 20 of the 32 fatalities were caused by peanut (Bock *et al.*, 2001). In the UK, 10 out of 37 fatalities to food recorded from 1992 to 1998 were caused by peanut (Pumphrey, 2000). In Sweden, two out of six fatal cases were due to peanut and four to soybean (Foucard and Malmheden Yman, 1999). A two-year prospective study in a paediatric population in Great Britain described 55 severe or fatal food allergic reactions, 10 of which caused by peanut (Macdougall *et al.*, 2002).

Nevertheless the incidence and prevalence of peanut allergy are certainly increasing, there are no convincing theories explaining the reasons for this phenomenon.

3. CLINICAL FEATURES

3.1 Natural history

Allergy to peanuts occurs very early in life and is rarely outgrown. According to the data of a voluntary registry (Sicherer *et al.*, 2001), 89% of peanut allergic subjects are younger than 18 years of age (median age 5 years); most children experience their first allergic reaction to peanuts at 14 months of age, and for 74% of them the first reaction occurs on the first known exposure. The high incidence of allergy to peanut in very young children, who do not frequently consume this kind of food, leads to the consideration that occult exposure to peanut may cause sensitisation in these patients: possible routes of occult sensitisation include foetal exposure to allergens ingested by the mother and infant exposure from breast milk (Frank *et al.*, 1999; Vadas *et al.*, 2001), or even application of peanut oil to inflamed skin in children with eczema (Lack *et al.*, 2003).

Peanut allergy seems to be a lifelong problem: Bock and Atkins (1989) were the first to demonstrate that in peanut allergy previously diagnosed by means of DBPCFC, no oral tolerance to peanut developed. Recently, some authors proposed that peanut allergy is

sometimes outgrown (Hourihane *et al.*, 1998; Vander Leek *et al.*, 2000; Spergel *et al.*, 2000). A study by Skolnick *et al.* (2001) suggested that tolerance could be achieved after 4-5 years of allergen avoidance. They found that 21.5% of 223 children with a previous diagnosis of peanut allergy had a negative DBPCFC; however, the oral challenge was performed to confirm peanut allergy only at the end of the study, and not at the start. In a subsequent study, the same authors extended these data by demonstrating that patients with low levels of serum peanut-specific IgE (5 kU_A/L or less) have a greater chance of outgrowing their allergy, as 55% of them tolerated an oral challenge with peanut (Fleischer *et al.*, 2003).

The authors thus recommend that children with low levels of IgE antibodies to peanut (less than 2 kU_A/L) should undergo an oral challenge in order to verify if tolerance was achieved. This raises the problem of how to reintroduce peanut into the diet after a negative peanut oral challenge. Busse *et al.* (2002) reported cases of patients who seemed to have outgrown their allergy and showed negative peanut-IgE levels at the time of reintroduction; these patients subsequently experienced a new allergic reaction to peanut and had an increase in their peanut-IgE levels. The authors speculated that re-sensitisation might have occurred because they ingested only small amounts of peanut intermittently, rather than small amounts frequently or larger amounts intermittently, which might better sustain tolerance. Similar findings were reported by Fleischer *et al.* (2003) who however found a lower recurrence rate (3% *vs* 14%).

3.2 Symptoms

Peanut typically causes severe anaphylaxis, and life-threatening reactions are much more frequent in peanut-sensitised subjects than in patients allergic to other foods (Burks *et al.*, 1999). A voluntary register of individuals with peanut and/or tree nut allergy was established in 1997 to elucidate the most common features of this kind of food allergy through use of a structure questionnaire (Sicherer *et al.*, 2001). A total of 5,149 individuals were registered; registrants were primarily children with an average age of 5 years. Isolated peanut allergy was reported by 3,482 registrants (68%), isolated tree nut allergy by 464 (9%) and allergy to both foods by 1,203 (23%). It was observed that allergic reactions to peanut usually began 3 minutes after exposure, which was mainly by ingestion (91% of cases) and less frequently by skin contact (8%) or inhalation (1%). About half of all patients had manifestations in one target-organ system, 30% in 2, 10-15% in 3, 1% in 4. Skin involvement with urticaria, erythema, angio-edema was the most common clinical feature, occurring in 89% of patients. Other manifestations included respiratory (wheezing, stridor, cough, dyspnoea, throat tightness, nasal congestion), gastrointestinal (vomiting, diarrhoea, abdominal pain) and cardiovascular symptoms (hypotension, arrhythmia, cardiac arrest) in 42%, 26% and 4% of the cases, respectively. Patients with asthma were more likely to have severe reactions than those without asthma (33% *vs* 21%; $p < 0.001$). A peculiar characteristic of peanut allergy was the high frequency of reactions due to accidental ingestion that occurred in 48% of patients being moreover more severe in comparison with initial reactions. The majority of these patients had already had reactions to peanuts that were less severe than the subsequent ones.

3.3 Diagnosis

The diagnosis of a suspected peanut allergy is based on medical history, physical examination, detection of peanut specific IgE through use of skin prick tests or CAP/RAST, and on DBPCFC, which is the standard for diagnosis of every kind of food allergy. Given the risk of severe reactions caused by DBPCFC for peanut it is opinion of the major experts that

new tests are needed to predict the severity of an individual's reaction (Leung and Bock, 2003). Up to now it is internationally accepted that skin prick testing to peanut generally has excellent sensitivity (90-100%) and negative predictive value (75-100%) if good quality extracts or crude peanuts are used, but the specificity (29-58%) and positive predictive value (44-55%) are very poor, leading to possible overestimation of peanut allergy (Sampson and Ho, 1997; Sampson and Albergo, 1984). Compared to skin prick test, the measurement of specific serum IgE antibodies by CAP-FEIA is slightly less sensitive but may have a greater positive predictive value: Sampson found that a value of 15 kU_A/L or greater has a positive and negative predictive value of 100% and 36%, respectively (Sampson and Ho, 1997; Sampson, 2001). The identification of such a cut-off level seems useful to indicate which patients are more likely to have symptoms after peanut ingestion, thus eliminating the need for a DBPCFC. Other authors suggested the combined use of skin prick test and CAP to peanut, in order to reduce the need for prescribing DBPCFC (Rancé *et al.*, 2002). However the suggested IgE concentration, which should be less than 57 kU_A/L, to identify definitely peanut non-allergic patients, is extremely different from that indicated by Sampson's study (Sampson and Ho, 1997).

Nevertheless considering that different symptoms could be related to different IgE specific threshold values it would be important to have other studies combining the diagnostic performances of skin prick test and IgE values in respect to DBPCFC to definitely solve this clinical problem.

Another relevant point is the identification of patients who have a past history of allergic reactions to peanut, but have outgrown their allergy and can thus tolerate peanut, despite a persistently positive specific IgE result. Skolnick *et al.* (2001) proposed that patients with a peanut IgE level of less than 10 kU_A/L be considered eligible for DBPCFC, because of potential resolution of peanut allergy. Beyer *et al.* (2003) proposed that the measurement of serum IgE antibodies specifically directed towards different peptides, representing the immunodominant sequential epitopes on the major peanut allergens Ara h 1, 2 and 3, can help differentiating between patients with symptomatic peanut allergy and those who are sensitised but clinically tolerant (including patients who outgrew their peanut allergy). They demonstrated that, regardless of the peanut-specific IgE levels, three immunodominant peptides on Ara h 2 were recognised by 60-73% of the patients with peanut allergy but only by 6% of the individuals tolerant to peanut. Moreover, two epitopes on Ara h 1 were recognised by 20-40% of allergic and by none of non-allergic subjects. Since 93% of symptomatic, but only 12.5% of tolerant patients, recognise one of these "predictive" epitopes on Ara h 1 or 2, this test could represent a useful tool to avoid DBPCFC in the patients who have peanut-specific IgE levels below the diagnostic decision levels of 15 kU_A/L (Beyer *et al.*, 2003).

4. IDENTIFIED ALLERGENS

Peanut kernels contain about 23-27% proteins, comprising over 50 different types of proteins, 19 of which bind IgE from peanut allergic sera (Clarke *et al.*, 1998). Peanut allergens (Table 12) are heat stable and resistant to treatment: roasting does not reduce peanut allergenicity, but on the contrary enhances it 90-fold (Maleki *et al.*, 2000). One of the first peanut allergens identified was Peanut I, showing two major bands at 20-30 kDa molecular weight (Sachs *et al.*, 1981); subsequently, a 65 kDa concanavalin A-reactive glycoprotein was found to be able to bind over 50% of sera from peanut sensitised patients (Barnett *et al.*, 1985).

Table 12. Peanut allergens

Allergen	Family	Molecular weight	Population studied	Authors
Ara h 1	Vicillin	64.5 kDa	9 children with atopic dermatitis and either positive DBPCFC or severe anaphylactic reactions to peanut	Burks *et al.*, 1991a
Ara h 2	Conglutinin	17.5 kDa		Burks *et al.*, 1992a
Ara h 3	Glycinin	14 kDa	5 children with a history of anaphylactic reactions to peanut	Eigenmann *et al.*, 1996b
Ara h 4	Glycinin	35.9 kDa	For allergen identification: 34 peanut-sensitised patients, with positive history, SPT and RAST + 3 patients with positive RAST For prevalence study: 40 peanut-allergic patients with positive specific IgE	Kleber-Janke *et al.*, 1999
Ara h 5	Profilin	14 kDa		
Ara h 6	Conglutinin	14.5 kDa		
Ara h 7	Conglutinin	15.8 kDa		

More recently, Burks *et al.* (1991a and 1992a) identified three major peanut allergens, named Ara h 1, 2 and 3, abundantly present in peanut kernel, belonging to the vicillin, conglutinin and glycinin families of seed storage proteins, respectively. Ara h 1 represents 20% of all peanut proteins, while Ara h 2 represents 10%. In 9 children with atopic dermatitis and either positive DBPCFC or severe anaphylactic reactions to peanut (laryngeal oedema, severe wheezing, hypotension), Ara h 1 and Ara h 2 were recognised by IgE from more than 95% of patients (Burks *et al.*, 1991a and 1992a). Ara h 3 is a peanut-specific allergen, not cross-reactive to soybean allergens: in a population of five children with a history of anaphylactic reactions to peanuts, the IgE-binding to this 14 kDa allergen was not decreased after pre-incubation of the sera with soy extract. IgE-binding to Ara h 1 and Ara h 2, however, was decreased by 79% and 76%, respectively (Eigenmann *et al.*, 1996b; Burks *et al.*, 1998b). Whether the cross-reactivity between peanut and legumes has a clinical relevance is still a matter of debate.

Other allergens have recently been recognised in peanut through molecular cloning. In a Swiss study, six allergens were identified by screening a cDNA library with a pool of sera from 34 peanut sensitised patients (Kleber-Janke *et al.*, 1999). Additionally, the percentage of reactivity to each recombinant allergen in a population of 40 peanut allergic patients was determined. Two of the six allergens cloned corresponded to the already known major allergens Ara h 1 and Ara h 2. The others were all newly identified allergens, named Ara h 4, a glycinin recognised by 53% of patients; Ara h 5, a profilin recognised by 13%; Ara h 6 and Ara h 7, two conglutinins recognised by 38% and 43% respectively. Interestingly, Ara h 6 and Ara h 7 show a low amino acid sequence identity to each other and to the other peanut conglutinins Ara h 2, even though all three proteins belong to the same 2S albumin family. Moreover, Ara h 6 seems to be responsible for severe allergic reactions (Becker *et al.*, 2001).

5. CROSS-REACTIVITIES

5.1 Clinical cross-reactivity

Besides peanut, the only legume recognised as a relevant allergen is soy; nevertheless, soy allergy is not very common and it is almost exclusive to young children. Even though patients with peanut allergy have extensive serologic cross-reactivity among members of the legume

family (Barnett *et al.*, 1987; Eigenmann *et al.*, 1996b), clinically significant cross-reactivity among legumes appears to be rare. Bernhisel-Broadbent *et al.* (1989) demonstrated that only two out of 41 legume allergic patients, diagnosed by means of DBPCFC, had an IgE-mediated reaction to more than one member of the legume family.

Two studies reported cases of adverse reactions to legumes in peanut allergic patients. The first, by Swedish authors, described four fatalities due to ingestion of foods containing a low concentration of soy; the patients were all asthmatic and severely allergic to peanut, but had no previously known allergy to soy (Foucard and Malmheden Yman, 1999). The second, by a French group, reported seven patients with positive DBPCFC to both peanut and lupine flour (Moneret-Vautrin *et al.*, 1999). The authors reported that symptoms to lupine were caused by cross-reactivity with peanut, as the major lupine flour allergen (molecular weight 43 kDa) was present in peanuts, and IgE cross-reactivity between peanut and lupine allergens was demonstrated by immunoblots inhibition.

A more recent study described three patients with a history of severe allergic reactions after ingestion of pea, who had peanut-related symptoms (confirmed in one case by DBPCFC). These patients reacted to the peanut major allergen Ara h 1 and to the pea allergen vicillin; the immunoblotting inhibition experiments demonstrated that pea was probably the first sensitiser, as IgE-binding to peanut was inhibited by pea but not, or only partially, the other way round (Wensing *et al.*, 2003).

6. POSSIBLE EFFECTS OF FOOD PROCESSING ON ALLERGENICITY AND DERIVED PRODUCTS

6.1 Roasted peanut

The most important characteristic of peanut major allergens is their resistance to heat treatment: after roasting both Ara h 1 and Ara h 2 increase their IgE-binding capacity (Maleki *et al.*, 2000). The mechanisms by which roasting increases IgE-binding to these allergens of peanut are not known. Possible explanations could be structural modifications or functional alterations. It has been demonstrated that after roasting Ara h 1 forms highly stable trimers by intermolecular cross-linking, while Ara h 2 forms intramolecular cross-links without forming higher order structures. Maleki *et al.* (2003) demonstrated that Ara h 2 functions as a weak trypsin inhibitor, and that it shows approximately a 3.5-fold increase in this activity because of roasting. The fact that in the USA peanuts are most commonly eaten after roasting, which affects their IgE-binding properties, could be the reason for the higher frequency of peanut allergy in this population, as suggested by Metzeger (2003).

6.2 Peanut oil

Oil extracted from peanuts is a potentially allergenic source for peanut sensitised individuals, because it contains trace amounts of peanut proteins. Full refining of oils results in the almost complete removal of protein; the protein content of crude oil is in the range of 100-300 μg, while that of refined oil is 100-fold lower. Thus, the determination of the content and immunoreactivity of the residual protein is crucial to assess the allergenic risk of peanut oil (Crevel *et al.*, 2000).

In early studies, some authors suggested that peanut oil did not contain allergenic proteins (Nordlee *et al.*, 1981) and was not able to elicit allergic reactions (Taylor *et al.*, 1981). The latter authors demonstrated by a double-blinded oral provocation challenge performed on ten peanut allergic patients that up to 8 mL of hot-processed peanut oil were well tolerated. Bock and Atkins (1989) safely administered up to 30 mL of purified peanut oil to four subjects with confirmed peanut allergy.

More recently, some case reports of allergic reactions in children and infants fed with milk formulae containing peanut oil were described (Fries, 1982; Moneret-Vautrin *et al.*, 1991; Brown, 1991), and in four atopic dermatitis infants a flare up of dermatitis was induced by oral challenge with peanut oil (Moneret-Vautrin *et al.*, 1994). These reports aroused the suspicion that residual allergenic proteins might be contained in peanut oil.

The processing method used to obtain oil from peanut kernels can influence the allergenicity of the final product: Hoffmann and Collins-Williams (1994) found highly significant amounts of peanut allergen in some brands of cold-pressed peanut oil and no detectable amounts in hot-pressed peanut oils. The protein content of cold-pressed peanut oil was 0.2-3.3 μg/mL; such minute amounts of protein would be able to provoke allergic reactions only in highly sensitive patients.

The protein content and the allergenicity of peanut oil are inversely related to the extent of heat-treatment: Teuber *et al.* (1997) demonstrated that serum IgE from 17 peanut and tree nut allergic patients bound only to unrefined oils treated at 54 to 93°C, while refined oils treated at 230-260 °C were not allergenic.

Hourihane *et al.* (1997) studied 60 patients with peanut allergy proven by open challenge with roasted peanuts. All of them were submitted to DBPCFC with both refined and crude peanut oil: none reacted to the refined oil and only six (10%) to the crude oil. Since commercial peanut oil used for cooking is usually refined, while crude oil is used only rarely, when a peanut flavour is deliberately required, it seems unlikely that peanut oil could cause allergic reactions in peanut sensitised patients.

In contrast with the previous results, a more recent study demonstrated that both crude and refined peanut oil contain allergenic proteins. One of these proteins was recognised by IgE antibodies from a population of 11 peanut allergic patients, 4 of whom also had a positive DBPCFC result with peanut oil; this allergen had a molecular weight (18 kDa) and an isoelectric point (4.5) similar to those of the peanut major allergen Ara h 2 (Olszewski *et al.*, 1998).

Since the information up to know available are contradictory, it is reasonable to conclude that peanut oil should be declared in the label of foods, as it may contain trace amounts of protein.

7. THRESHOLD DOSES

Several studies determined a peanut reactive dose through peanut challenge in order to establish the lowest threshold dose.

Moneret-Vautrin *et al.* (1998) reported 509 cases of children evaluated with oral food challenges and found that 142 had peanut allergy confirmed by either labial or double-blinded

oral provocation tests. This study demonstrated that low doses of peanut are able to induce allergic reactions: over 87% of patients reacted to cumulative doses less than 1000 mg (the weight of one peanut kernel). The same authors found that reactivity to very small amounts was not unusual in peanut allergic patients: in 103 patients with positive single- or double-blind placebo-controlled food challenge to peanut, 18% reacted to 65 mg or less of roasted peanut (3.9% to 15 mg or less); the lowest reactive dose was 5 mg (Morisset *et al.*, 2003a).

Some investigators found even lower reactive doses: Hourihane *et al.* (1997) performed a DBPCFC in 14 adult subjects, demonstrating that as little as 2 mg of ingested peanut protein was able to elicit objective symptoms, and 100 μg subjective symptoms. Wensing *et al.* (2002a) confirmed these results, as they found that in 26 patients subjected to DBPCFC with increasing doses of peanut, the threshold doses eliciting allergic reactions ranged from 100 μg to 1 g of peanut protein. Fifty percent of the study population reacted to 3 mg or less of peanut protein; patients with a history of severe symptoms had lower threshold doses compared with those with mild symptoms. No patient reacted to the lowest dose of 30 μg of peanut protein.

A statistical model elaborated by Bindslev-Jensen *et al.* (2002), using the data from four clinical trials with open or blind food challenges to peanut, demonstrated that the threshold dose giving an allergic reaction of one in a million susceptible patients was 0.7 μg of peanut, while that giving reaction of one in a hundred is 1.2 mg (Bindslev-Jensen *et al.*, 2002).

However, all the challenge-based studies, aimed at definition of a certain threshold dose, show several limitations, as they usually exclude the most sensitive patients presenting history of severe allergic reactions to very minute amounts of the offending food, and they also include patients reacting to the first administered dose of peanut, who could react to even lower doses than the lowest tested.

The case reports of allergic reactions occurred in peanut sensitised patients, after accidental ingestion of foods-containing peanuts, suggest that even minimal traces of peanut proteins can trigger an allergic reaction in these subjects (Table 13). As reported by the US Peanut and Tree Nut Allergy Registry, most of the reactions to peanut occurred in restaurants were due to foods which contained peanut as an ingredient in a sauce, dressing, or egg roll, so that visual identification of the nut by the patient was impossible. The most common source of exposure was desserts (43%), followed by entrees (35%) and appetisers (13%); Asian restaurants and dessert shops were particularly involved (Furlong *et al.*, 2001).

8. CONCLUSION

Peanut is a common cause of allergic reactions, which can be very severe or even fatal and it is not possible to determine a reliable threshold dose. Studies based on oral food challenges with peanut reported that very low doses (from 100 μg to 1 g of peanut protein) are able to elicit symptoms in peanut allergic patients; there are no data on the doses which can cause reactions in highly sensitive patients, who are usually excluded from challenge tests.

Table 13. Case reports of allergic reactions occurred in peanut sensitised patients after accidental ingestion of foods containing peanuts

Foods-containing peanuts	Allergic reaction	Author
Almond icing	Fatal anaphylaxis	Evans *et al.*, 1988
Candy	Vomiting, asthma, urticaria, drowsiness	Foucard and Malmheden Yman, 1999
Hamburger	Asthma, cyanosis, cardiac arrest	
Meatballs	Severe asthma	
Kebab	Asthma, urticaria	
Bun with peanut flakes	Fatal anaphylaxis	
Peanut beverage, home-made		
Dry soup mixture	Systemic reaction	McKenna and Klontz, 1997
Cake	Fatal anaphylaxis	Bock *et al.*, 2001
Egg roll		
Candy bar		
Mexican food		
Candy		
Snack mix		
Cookie		
Peanut sauce		
Chinese food		
Chili containing peanut butter	Fatal anaphylaxis (angio-edema of lips, tongue, glottis, cardiopulmonary arrest)	Yunginger *et al.*, 1988
Cookie	Fatal anaphylaxis (cardiovascular collapse)	
Vietnamese food containing slivered peanuts	Fatal anaphylaxis (glottis edema, cardiovascular collapse)	
Cake	Fatal anaphylaxis (vomiting, asthma, cardiopulmonary arrest)	
Ice cream cone with peanut candy	Not reported	Furlong *et al.*, 2001
Egg rolls closed with peanut butter		
Asian sauce		
Ice cream		

XIV. ALLERGY TO SOY

SUMMARY

Soy is a common dietary protein and soy bean intake among adults in Europe is around 1-2 g/day according to a European multicentre dietary recall study (Keinan-Boker *et al.*, 2002). It is often introduced into the diet from an early age, often as a standard milk formula in healthy children and in children with suspected or proven cows' milk allergy as a substitute, although this practice is now discouraged (SCF, 2003; AAP, 1998; Barrett, 2002).

Prevalence studies are generally lacking. Prevalence rates of 0.3-0.4% in the general population have been quoted. In children with atopic eczema rates of 2-4.4% of reactive children have been described. Around 6% of atopic children with a positive skin test to soy may respond in a DBPCFC. Sensitisation rates are high in children with enterocolitis.

Clinical reactions are similar to those observed cows' milk or egg allergy. Soy allergy may affect the skin, the gastrointestinal tract, the respiratory tract and can cause systemic anaphylaxis.

The natural history normally follows that of milk or egg allergies and many soy allergies have resolved by 3 years of age. Patients with severe peanut allergy can react to soy proteins in 3-6% of cases. In these co-allergies, soy sensitivity is likely to be lost at an earlier stage. On the basis of these reports it seems unwarranted to avoid all legumes in individuals with clinical allergy to one legume only.

Severe and/or fatal anaphylaxis reactions seem to be rare. In reports with a higher soy protein anaphylaxis rate in peanut allergic patients, minute peanut contaminations cannot be entirely ruled out. A number of cross-absorbing peanut and soybean proteins have been described. Major allergenic proteins of soybean are contained in the 2S and 7S fraction (see Tables 14 and 15) and unique soy proteins of 21 kDa and 40 kDa have been described.

Threshold levels of triggering adverse reaction in soy allergic individuals are similar to milk and egg and reported to be in the low milligram range (0.0013 mg-500 mg) although studies that address these questions have not been performed in a satisfactory way. Soy protein-based formula should not be used for cows' milk allergy prophylaxis. The possibly changed allergenicity of genetically modified soy has not been formally addressed and is mainly dependent on the transformation event of the new gene product.

1. BACKGROUND

Consumption of soy is common in children and soy-based formulae were introduced in infant nutrition about 80 years ago. The use of soy-based formulae was extended to the treatment of cows' milk intolerance 75 years ago by Hill and Stuart in 1929.

Since the 1970s, use of soy-based formulae became common, and in 1980s, US consumption was around 25% of that of cows' milk-based formulae. Interest in soybeans and their components has increased mainly because of the potential protective influence of soy on the development of disease, although adverse health effects have also been reported. Soy and soy protein formulae can cause allergies and other intolerance reactions although for many years

after the first adverse description in 1934, soy was considered a weak sensitising protein on the basis of animal studies. Currently there is no doubt that soy is an important food allergen.

A higher prevalence of soy intolerance has been reported in delayed onset enterocolitis and enteropathy syndromes. A significant number of children with cows' milk protein intolerance develop soy protein intolerance when soy milk is used in dietary management.

2. FREQUENCY

The prevalence and incidence of soy allergy in the general population is unknown and is likely to be dependent on local feeding habits and exposure. In many instances soy protein is introduced into the diet at an early age. In the context of this Opinion we will omit discussion of occupational allergies resulting mostly from inhalation by individuals working in soybean processing industries where a skin prick test sensitisation rates of 29% and 36% have been reported (Roodt and Rees, 1995; Zuskin *et al.*, 1992). Rates of over 80% have been described in adult asthma patients who showed symptoms during unloading of soybeans (García-Ortega *et al.*, 1998).

2.1 Studies in food allergic adults and children

True prevalence rates in different populations are not known and reports of children with soy allergy are usually obtained from patients attending specialist medical services related to their atopic state or symptoms related to food intake.

A number of studies report sensitisation rates in children with food allergies and atopic dermatitis or atopic dermatitis alone. Out of 107 children with atopic dermatitis, 16% (45) reacted clinically in a DBPCFC. In order to establish clinically relevant soy-related disease, assessment of sensitisation rates with skin test or RAST are not reliable (Burks *et al.*, 1998a; Eigenmann and Sampson, 1998; Giampietro *et al.*, 1992; Magnolfi *et al.*, 1996). Two Italian studies (Giampietro *et al.*, 1992; Magnolfi *et al.*, 1996) on 1075 food allergic and atopic children report a RAST and skin test positivity, of 22% and 21% of which only 3% and 6% reacted in a DBPCFC or open challenges, representing 1.1% of children referred for atopic disease. Similar DBPCFC positive sensitisation rates of 6%-20% have been reported (Bock and Atkins, 1989; Bruno *et al.*, 1997; Burks *et al.*, 1998a).

2.2 Natural history of soy allergy/sensitisation

The natural history of soy allergy seems to mirror other food allergies of childhood and is transient and most reports state that this condition is lost at about 3 years (Bock, 1987; Host and Halken, 1990; Sampson and Scanlon, 1989). Seventy-six healthy newborns were screened for soybean IgE with RAST in Sweden at 3, 8, 25 and 48 months. Only at 8 months was there a soybean RAST positivity of 6%, at all other time points skin tests were negative. This is in keeping with reports of a transient sensitisation to eggs during the first year of life (Lau *et al.*, 2002). Studies from the USA (Sampson and Scanlon, 1989) report a 26% loss of sensitivity one year after onset of soy, egg, milk and peanut allergies, assessed by DBPCFC. Out of 8 infants with soybean induced enterocolitis, two had lost their reactivity after 25 months (Sicherer *et al.*, 1998). Based on these data it seems -at least for soy-induced enterocolitis- that the resolution is somewhat slower than for cows' milk-induced enterocolitis.

3. CLINICAL FEATURES

The clinical manifestations of soy allergy are similar to those of cows' milk allergy, ranging from severe enterocolitis to atopic eczema and immediate IgE-mediated systemic multisystem reactions. Soy allergy seems not to be responsible for severe life-threatening reactions (Sicherer *et al.*, 2000b) although some doubts have been raised by a Swedish group (Foucard and Malmheden Yman, 1999).

4. IDENTIFIED ALLERGENS

Significant advances have been made in the last decade, particularly in identifying allergenic proteins of peanut and soy (Table 14). Several protein fractions of soy have been identified by ultra-centrifugation and are listed below (Table 15).

Table 14. Soy allergens

Allergen	Proteins/ glycoproteins	Molecular weight	Family	References
Gly m 1.0101, Gly m 1.0102	Soybean hydrophobic seed protein	8 kDa	Lipid transfer protein	González *et al.*, 1995
Gly m 2	Soybean hull protein	8 kDa	Storage protein	Codina *et al.*, 1997; Rihs *et al.*, 1999
Gly m 3	Soybean profilin	14 kDa	Profilin	Rihs *et al.*, 1999
Gly m Bd 30K	Soybean vacuolar (Gly m Bd 30K)	30 kDa	Serine protease	Ogawa *et al.*, 1991; Tsuji *et al.*, 1995
Not assigned	Glycinin	320-360 kDa	Seed storage protein	Djurtoft *et al.*, 1991
Not assigned	Beta-Conglycinin	140-180 kDa	Seed storage protein	Ogawa *et al.*, 1995
Gly m 4	Kunitz-trypsin inhibitor	20 kDa	SAM 22, PR protein	Moroz and Yang, 1980

Several authors report on IgE-binding *in vitro* absorption studies of patients suffering from peanut or soy allergy. Sensitivity to glycinin and Gly m 1 has been reported in over 90% (Djurtoft *et al.*, 1991). Kunitz trypsin inhibitor in 86% (Baur *et al.*, 1996) and profiling (Gly m 3) in 69% (Rihs *et al.*, 1999). A number of reactivities to other not yet completely characterised moieties between 5 and 20 kDa have also been described, mostly in adult patients (Eigenmann *et al.*, 1996b; García-Ortega *et al.*, 1998; Sandiford *et al.*, 1995).

5. CROSS-REACTIVITIES

In decreasing frequencies, the following cross-reactivities against other legumes in soy allergic individuals have been described.

Peanut around 70-90%, green pea ~80%, lima bean ~50%, string bean ~40% (Bernhisel-Broadbent and Sampson, 1989; Bernhisel-Broadbent *et al.*, 1989; Fries, 1971) and wheat flour in soybean sensitised bakers' (Baur *et al.*, 1996). The overall consensus in from all these studies is that *in vitro* cross-reactivities do not correlate with clinical reactivity. Immediate-type allergic reactions in patients with birch pollen after soy protein-containing food can

result from cross-reactivity of bet v 1 specific IgE to the homologous pathogenesis related proteins, especially the PR10 protein SAM 22 (Kleine-Tebbe *et al.*, 2002).

Table 15. Protein fractions of soy

Proteins/Glycoproteins	Amount of total protein
Sedimentation fractions	
2S	20%
7S (incl. Beta-conglycinin 50% and others)	33%
11S	33%
15S	10%
Soluble fractions	
Globulines (salt-soluble)	90%
Albumines (water-soluble)	10%
Water-soluble fraction	
Glycinin (in 11S) and beta-conglycinin (in 7S)	>70%
Trypsin inhibitors (in 2S)	15%
Soybean vacuolar protein (Gly m Bd 30K) (in 7S)	2-3% of seed proteins
60%-ethanol-soluble fraction	
Hydrophobic protein (Gly m 1.0101)	-20 mg/100 g seeds

5.1 Risk of anaphylaxis in highly peanut allergic individuals

During the period of 1984-1992 in France, André *et al.* (1994) retrospectively analysed 580 patients with severe, near fatal reactions and state that in 30% soy has been the trigger. No challenge tests were performed. In a survey of 402 food allergic patients a Swiss report describes a sensitisation rate of 1% (Wüthrich, 1993).

During a period of 4 years (1993-1996) a Swedish group (Foucard and Malmheden Yman, 1999), in 1999 reported 61 cases of severe anaphylactic reactions from a national register. Peanut, tree nuts and soy are deemed to have caused 45/61 reactions. All 4 children who died from soy anaphylaxis were suffering from asthma and severe peanut allergy. Severe reactions occurred after initially mild symptoms and an almost symptom-free interval of about one hour. Among the severe reactions the foods responsible for reactions in the soy allergic patients were ice cream (2), hamburger (2), kebab (1) and soy sauce (1). Peanut allergics who reacted to kebab and hamburger were highly sensitised to peanut and demonstrated also soy-specific IgE. However, in view of the nature of the study, questions as to the true trigger for these fatal reactions remain and hidden peanut exposure as trigger cannot be entirely ruled out.

5.2 Clinical co-existing allergies of peanuts and soy

Although there is a high rate of detection of soy protein binding IgE in peanut allergic patients (Barnett *et al.*, 1987) clinical reports of co-reactivity are rare (Sicherer *et al.*, 2000b). Reported clinical co-reactivity rates in peanut allergics range from 1%-6.5% in placebo-controlled studies (Burks *et al.*, 1998b).

5.3 Cows' milk allergy and sensitivity to soy

Several authors report co-existing clinical soy allergies in patients with cows' milk allergies. These range from 5% (Host and Halken, 1990) in a prospective study to around 50% in a selected group of patients with cows' milk enterocolitis (Burks *et al.*, 1994). It is unclear whether soy allergy in these children represents a *de novo* sensitisation or a cross-reaction of a soy protein component with caseins from milk (Rozenfeld *et al.*, 2002).

In a randomised, prospective study in 170 infants with cows' milk allergy from Finland (Klemola *et al.*, 2002) adverse reaction to a randomly assigned soy formula was confirmed by challenge in eight patients [10% (4.4-18.8%)] and in two patients assigned to an extensively hydrolysed milk formula [2.2% (0.3%-7.8%)]. Adverse reactions to soy were similar in IgE- or non-IgE-mediated milk allergy. Overall, adverse reactions were more common in the under 6 months old infants. IgE to soy was detected in only two infants with an adverse reaction.

Bardare *et al.* (1988) report that out of a group of 50 atopic children with cows'milk allergy and 21 non-atopics, 4% showed a positive RAST test without any difference between those which were fed soy or not. Open soymilk challenge was positive in 10/58 children (6 atopic, 4 non-atopic). Four out of 21 atopic patients with a cows' milk intolerance had a positive soymilk challenge (~20%).

Cantani and Lucenti (1997) report own experiences and a meta-analysis of 17 published papers and conclude that soy protein formula allergies, based on a positive RAST/skin test and open or DBPCFC, have a 3-4% incidence in cows' milk allergic children given soy protein formulae.

6. POSSIBLE EFFECTS OF FOOD PROCESSING ON ALLERGENICITY AND DERIVED PRODUCTS

Storage, fresh or heated, heat denaturation and glycerination and enzymic digestion, all affect the IgE-binding activity of sera obtained from peanut and soy allergic patients. Whereas most exposures to heat between 80-120ºC for 60 minutes leads to a reduction in IgE-binding (Burks *et al.*, 1991b and 1992b; Vieths *et al.*, 1995b). Glycerination as used for skin testing can increase skin-test-positivities after 30 days of storage and new binding epitopes can be revealed (Codina *et al.*, 1998; Müller *et al.*, 1998; Morell *et al.*, 1999).

6.1 Importance of soy preparation for challenge procedures

Burcks and colleagues in 1994 reported that the extent of hydrolysis of soybean formulae (powder *vs* liquid) may affect the outcome in 43 children with enterocolitis. Fourteen (33%) children reacted to a powdered soy formula, 13 reacted to a liquid formula. They also report that out of 10 children with a positive milk challenge 6 also had a positive soy challenge. The authors also observed a challenge order effect, which needs to be kept in mind when designing and reporting food challenge studies.

Table 16. Allergen sources of reported adverse reactions to soy

Reported adverse reactions	References
Food/food additives	
Fatal anaphylaxis after ingestion of sausage pizza fortified with soy protein	Yunginger *et al.*, 1991
Symptoms after ingestion of tofu, soybean salad, soybean sprouts, and spring rolls	Mistereck *et al.*, 1992
Soy proteins in Spanish sausage products (chorizo, salchichón, mortadella and boiled ham), doughnut and soup stock cubes (skin test, RAST, bronchial and oral challenge)	Vidal *et al.*, 1997
Anaphylactic symptoms caused by pizza containing soy proteins	Vidal *et al.*, 1997

7. THRESHOLD DOSES

Published studies of threshold doses in soy allergic patients suffer from a number of methodological shortcomings such as selection bias, reported clinical reactions at the first challenge dose, severity of reactions, open administration of challenges, use of soy flour or soy milk and others (Sicherer *et al.*, 2000a). With these reservations in mind some general statements may be made.

The threshold dose for soy allergic patients with anaphylaxis is in the same order of magnitude (0.0013 mg of soy flour) as has been described in milk or egg allergic patients when the actual allergen contents of the allergic foods are compared (Bindslev-Jensen *et al.*, 2002).

Sicher *et al.* (2000a) report in atopic dermatitis patients that 28% reacted to the starting dose of 500 mg of dry hydrated foods or drink. There was not correlation between skin prick test wheal size, specific IgE and severity of clinical reaction.

The determination of threshold doses in patients with non-IgE-mediated late, mostly gastrointestinal, reactions is difficult to assess and has not been done in a prospective fashion. In these patients an elimination diet, followed by a reintroduction of the food in those individuals, which have responded favourably, is required.

It is unclear whether these patients may react to small allergen amounts similar to coeliac patients over a prolonged period of exposure.

8. CONCLUSION

Severe and/or fatal anaphylaxis reactions to soy and soy-containing foods seem to be rare. There are some reports, which indicate a possibly higher soy protein anaphylaxis rate in peanut allergic patients. Although possible, these reports have some methodological problems and minute peanut contaminations as triggers in these patients cannot be entirely ruled out.

Serologically and immunologically a number of cross-reacting peanut and soybean proteins have been described.

Threshold levels of triggering adverse reaction in soy allergic individuals are similar to milk and egg and reported to be in the low milligram range (0.0013 mg-500 mg) although studies that address these questions have not been performed in a satisfactory way. Studies which investigating the threshold levels of delayed reactions to soy proteins are not available and it is unknown whether those patients may react to smaller amounts during a prolonged period of exposure, similar to coeliac patients. Soy protein-based formulae are not indicated for cows' milk allergy prophylaxis.

XV. ALLERGY TO MILK AND DAIRY PRODUCTS INCLUDING LACTOSE

SUMMARY

Milk is a major allergenic food especially in infancy. Data on prevalence of milk allergy vary according to the different countries and studies but about 1% of the general population of adults and 2-3% of children can be considered approximate figures. Cows' milk allergy must not be confused with intolerance to lactose, which is a milk sugar and does not contain protein allergens. Cows' milk allergy is an adverse reaction which is mediated by immunological mechanisms. Lactose intolerance is a consequence of lactose maldigestion which is due to genetic intestinal lactase deficiency occurring in large sections of the general population. Components responsible for cows' milk allergy are proteins and most of milk proteins are potential food allergens, even those present at very low concentrations. Numerous allergenic structures (epitopes) have been identified that are widely spread along the protein molecules. Short peptide fragments may conserve significant part of the allergenicity of the whole protein. Some are released or become bioavailable after denaturation of the proteins or after degradation by proteolytic enzymes e.g. during digestion. Any milk product, i.e. containing native or denatured cows' milk protein or fragments derived thereof, may trigger an allergic reaction at amounts in the range of micrograms. Data available from double-blind, placebo-controlled food challenges do not permit to establish safe threshold levels which would protect allergic consumers. Technological treatments may alter the structure of cows' milk proteins but there are no indications that this will reduce the allergenicity or the allergen concentration to an extent to guarantee the safety for highly sensitive milk allergic individuals.

Due to high amino acid sequence homology and to the high frequency of immunological cross-reactions between proteins of milk from different ruminant species, these considerations may be applied to buffalos', goats' and ewes' milk.

Lactose intolerance is neither an allergic nor an immune-mediated disease. It results of a reduced capacity to digest lactose in lactase deficient adults. Doses less than 10 g per day are often tolerated by most lactase deficient adults. However residual amounts of cows' milk proteins that can still be present in lactose as contaminants from the production process of lactose might be harmful for allergic patients to milk.

1. BACKGROUND

Milk allergy is an adverse reaction to milk proteins of different mammalian species including cow, goat and ewe particularly, which is mediated by immunological mechanisms. It can be divided in IgE-mediated and non-IgE-mediated reactions which may involve other immunoglobulins, immune complexes and/or cell-mediated mechanisms. It should be clearly distinguished from non-immunological adverse reactions such as lactose intolerance, which is due to lactase deficiency occurring in large sections of the general population. Patients with milk allergy which is non-IgE-mediated may present clinically defined, chronic digestive symptoms. Most IgE-mediated milk allergy appears in young children in the first half year of life and in over 70% of cases spontaneously disappears within the first 6 years of life. The clinical picture can vary from mild to severe reactions involving the skin, respiratory tract, gastrointestinal tract or systemic reaction (anaphylactic shock).

2. FREQUENCY

Milk (cows' milk) is one of the very common and widespread allergens that first affect atopic children in their early life. It is an allergenic food for most of the populations regardless their geographic origin.

Data on prevalence are numerous and they greatly vary depending on the characteristics of the population studied. Major variables include, for example, geographic origin, genetic predisposition, age, and the diagnostic criteria used, e.g. self-reported allergy or diagnosis of allergy confirmed by *in vitro* and *in vivo* testing.

Table 17. Frequency of allergy and sensitivity to cows' milk in unselected children and adults

Population	Country	Prevalence of allergy/ sensitivity to cows' milk	Reference
403 children 299 children 480 children (0-3 years)	USA	1% (history, skin prick test) 2.2% (challenge test)	Bachman and Dees, 1957 Mueller, 1963 Bock, 1987
787 children <3 years	Canada	7.5% (open challenges)	Gerrard *et al.*, 1973
609 children	Isle of Wight (UK)	2.5% (case history)	Hide and Guyer, 1983
1158 children <1 year	The Netherlands	2.8% (challenge, elimination)	Schrander *et al.*, 1993
1348 children - 15 weeks	Turkey	1.6% (challenge, elimination)	Altintas *et al.*, 1995
1456 children	Isle of Wight	4.1% (skin prick test)	Dean, 1997
251 children	Estonia	1.2% at 6 months; 0.8% at 12 months (skin prick test)	Julge *et al.*, 1997
237 children	Estonia	12, 21, 26 and 23% at 0.5, 1, 2 and 5 years of age (RAST BLG >1) 1.7, 0.9 and 0% at 0.5, 1 and 2 years of age (skin prick test)	Julge *et al.*, 2001
620 children <2 years	Australia	2% (challenge tests)	Hill *et al.*, 1997 and 1999
1749 newborns	Denmark	2.2% (challenge/elimination) (decreasing to 1% in breast-fed infants)	Host *et al.*, 1988 Host and Halken, 1990
2721 children	Norway	3.2% prevalence at age of 2 years (skin prick test, open food challenge, double blind food challenge)	Eggesbo *et al.*, 2001b
502 adults	Iceland	1.2% (RAST)	Gislason *et al.*, 1999
1397 and 414 adults	Sweden	1% and 0.7% (RAST and case history)	Bjornsson *et al.*, 1996 Gislason *et al.*, 1999
33 110 adults	France	0.26% (2-step questionnaire)	Kanny *et al.*, 2001

In children the prevalence ranges from *ca.* 1-2% to 5% or even 7.5% as illustrated in Table 17. It is noteworthy that figures obtained through challenge tests/elimination and thus confirming a clinical allergy are generally 2-3 times lower than those recorded from questionnaire or *in vitro* serological tests (e.g. RAST tests).

Two to three percent thus appear to be a general percentage figure for the prevalence of milk allergy in the general population of children.

In unselected adults the prevalence among the general population has been reported to be around 1% as illustrated in Table 17.

The prevalence is increased in at risk groups of the population, i.e. in atopic and food allergic subjects (Table 18).

Table 18. Frequency of allergy and sensitivity to cows' milk in increased risk group of children from atopic background

Population	Country	Prevalence of allergy/sensitivity to cows' milk	References
142 and 378 food allergic children	France	9.2% (labial food challenge) 12% (food challenge)	Rancé *et al.*, 1997 and 1999b
74 age <3 years 111 age 3-19 years	USA	57% (double blind food challenge) 14% (double blind food challenge)	Bock and Atkins, 1990
165 patients with atopic dermatitis	USA	19% (skin tests); 9% (double blind food challenge)	Burks *et al.*, 1998a
107 children with atopic dermatitis	Germany	51% (double blind food challenge)	Niggeman *et al.*, 1999
44 food allergic children	Italy	22% (anaphylaxis)	Novembre *et al.*, 1998b

3. CLINICAL FEATURES

Symptoms of cows' milk allergy are classical symptoms of food allergy, including systemic reactions, and cutaneous, respiratory and gastrointestinal symptoms.

The onset of diseases is in most cases closely related to the time of introduction of milk. The majority of children have two or more symptoms from two or more organ systems, 50 to 70% have cutaneous symptoms, 50 to 60% gastrointestinal symptoms and approximately 20 to 30% respiratory symptoms. The gastrointestinal manifestations of cows' milk allergy reflect a continuum from clearly IgE-mediated to mixed reactions involving eosinophils as effector cells to clearly non-IgE-mediated reactions (Host *et al.*, 2002). Indeed, it should be noticed that clinical manifestations after consumption of cows' milk may involve other mechanisms than IgE-mediated cows' milk allergy. Cell-mediated or other immunopathological diseases may also be observed. Delayed reactions affecting mainly gastrointestinal tract occurs after 4 hours to several days after milk exposure and usually requires a volume higher than the volume required in IgE reactions. According to Niggeman (1999), immediate reactions occur in 64% of cases of cows' milk allergy in children while delayed reactions occur in 28% of cases and both reactions in 8%.

A variable but large proportion of sensitive children spontaneously recover of their allergy between 2 to 5 years of age. According to Schrander *et al.* (1992) from The Netherlands, oral tolerance to milk is acquired in 15%, 22%, 51% and 67% of the children at 1, 2, 3 and 4 years of age, respectively. In most countries the proportion of recovery ranges from *ca.* 33% to 67% of sensitive children at the age of 2-3 years. Figures are similar even if slightly higher in Denmark, i.e. 56%, 77%, 87%, 92% and 97% at 1, 2, 3, 5 and 10 and 15 years of age (Host *et al.*, 2002).

4. IDENTIFIED ALLERGENS

4.1 Structure, function and allergenic properties of main milk proteins

Milk composition changes during lactation. It is noteworthy that milk of ruminant species other than cow, e.g., buffalo, sheep, goat, but also many other species, including man, is constituted by the same or at least very homologous proteins, sharing the same structural, functional and biological properties, and associated in more or less similar proportions. However, human milk does not contain β-lactoglobulin.

Cows' milk contains about 30-35 g of proteins per litre. The action of chymosin (rennin), or the acidification of the milk to pH 4.6 enables two fractions to be obtained: lactoserum (whey), about 20% of the cows' milk proteins, and coagulum (curd), about 80% of the cows' milk proteins. Whey contains essentially globular proteins. The major ones, β-lactoglobulin (BLG) and α-lactalbumin (ALA), are synthesised in the mammary gland, while others, such as bovine serum albumin (BSA), lactoferrin (LF), immunoglobulins (Igs) come from the blood. In the coagulum, the casein fraction (CAS) comprises four proteins coded by different genes carried on the same chromosome: α_{S1}-, α_{S2}-, β-, and κ-caseins.

Table 19. Main characteristics of the major milk proteins

Proteins (Concentration % milk proteins)		**Concentration (g/L)**	**Molecular weight**	**No. amino acid residues per molecule**
Whey (20%) (~5 g/L)	BLG (10%) = Bos d 5	3-4	18.3 kDa	162
	ALA (5%) = Bos d 4	1-1.5	14.2 kDa	123
	Igs (3%) = Bos d 7	0.6-1.0	150 kDa	-
	BSA (1%) = Bos d 6	0.1-0.4	66.3 kDa	582
	LF (traces)	0.09	80 kDa	703
Whole Casein = Bos d 8 (80%) (~30 g/L)	α_{s1}-CAS (32%)	12-15	23.6 kDa	199
	α_{s2}-CAS (10%)	3-4	25.2 kDa	207
	β-CAS (28%)	9-11	24.0 kDa	209
	κ-CAS (10%)	3-4	19.0 kDa	169

Source: Wal, 1998 and 2002

The main characteristic that should be underlined is the multiplicity and diversity of proteins that are involved in cows' milk allergy. Sensitivities to the various cows' milk proteins have been demonstrated by Goldman *et al.* (1963 a and b), Gjesing *et al.* (1986) and Docena *et al.* (1996). Polysensitisation to several proteins occurs often and all milk proteins appear to be potential allergens.

Studies on large populations of allergic patients showed that most of the patients are sensitised to BLG (Bos d 5), CAS (Bos d 8), ALA (Bos d 4), BSA (Bos d 6), LF, and Igs (Bos d 7) (Kaiser *et al.*, 1990; Host *et al.*, 1992; Stoger and Wüthrich, 1993; Wal *et al.*, 1995 a and b; Bernard *et al.*, 1998; Restani *et al.*, 1999). A great variability is observed in the IgE response. CAS and BLG, as well as ALA, are major allergens. However, proteins present in

very low quantities, such as BSA, Igs, and especially LF, also appear to be important, since 35-50% of patients are sensitised to those proteins (Wal *et al.*, 1995a). In the last years, sensitivity to casein seems to have increased in terms of both frequency and intensity of the IgE response (Stoger and Wüthrich, 1993; Wal, 2002). Sensitisations to CAS, BLG and ALA are closely linked. In contrast, sensitivity to BSA appears to be independent, 50% of the patients being allergic to BSA regardless of their sensitivity to other milk allergens (Wal *et al.*, 1995b).

The major milk allergens present in lactoserum are BLG and ALA. BLG occurs naturally in the form of a 36 kDa dimer. It has no homologous counterpart in human milk. Each subunit corresponds to a 162 residues polypeptide. The molecule possesses two disulfide bridges and one free cysteine. This structure is responsible for the main physicochemical properties and also for interaction with casein during heat treatments. The relative resistance of BLG to acid and enzymatic hydrolysis allows the protein to be absorbed intact through the intestinal mucosa. The tertiary structure of BLG is known. It belongs to the lipocalin family and is considered a retinol-binding protein. Lipocalins have a high allergenic potential and several allergens of animal origin belong to this family. They share well-conserved sequence homologies in their N-terminus moiety where tryptophan at position 19 is always present, in spite of the mutations that have occurred during evolution. Crystallography studies revealed very similar folding, called β barrel structure, with the same arrangements of 8 (or 10) antiparallel β sheets (McKenzie *et al.*, 1972; Reddy *et al.*, 1988; Jakobsson *et al.*, 1985; Godovac-Zimmermann and Braunitzer, 1987; Papiz *et al.*, 1986; Brownlow *et al.*, 1997; Flower, 1996).

ALA is a monomeric globular protein of 123 amino acid residues with 14.4 kDa molecular weight and has four disulfide bridges. It is a regulatory component of the enzymatic system of galactosyl transferase responsible for the synthesis of lactose. It possesses a high affinity binding site for calcium, and this bond stabilises its secondary structure. The complete amino acid sequence of bovine ALA shows extensive homology with hen's egg white lysozyme but also with human ALA (Brew *et al.*, 1970; Browne *et al.*, 1969; Nitta and Sugai, 1989; Findlay and Brew, 1972).

Whole casein fraction constitutes the coagulum, i.e. the solid fraction of proteins obtained after coagulation of milk. Each individual casein, α_{S1}-, β-, α_{S2}- and κ-casein, represents a well-defined chemical compound but they cross-link to form ordered aggregates: micelles, in suspension in lactoserum. Their proportion in the micelles is relatively constant ca. 37, 37, 13 and 13%, respectively. Their distribution is not uniform within these micelles which comprise a central hydrophobic part and a peripheral hydrophilic layer where major sites of phosphorylation containing phosphoserine residues are presented, in relation with the calcium binding and transfer properties of caseins. α_{S1}-, α_{S2}-, β-, and κ-casein have little primary structure homology. Their functional properties also differ since three of them, α_{S1}-, α_{S2}-, and β-casein, appear to be calcium-sensitive, while κ-casein is not. However, the four caseins display common features that are quite unusual, differing greatly from other milk proteins. They are phosphorylated proteins, with a loose tertiary, highly hydrated structure (Schmidt, 1982). CAS is often considered poorly immunogenic because of this flexible, non-compact structure and because it is rapidly and extensively degraded by proteolytic enzyme during digestion. Caseins are not significantly affected by severe heat treatments but are indeed very susceptible to all proteinases and exopeptidases. Multi-sensitisations to the different caseins most often occur in patients sensitised to the whole casein fraction (Bernard *et al.*, 1998).

Several forms of caseins have been described which derived from naturally-occurring partial hydrolysis (e.g. by plasmin) and several of such peptide fragments derived from caseins conserve part of the allergenicity of the native protein (see section on epitopes below).

Due to the great variability of human IgE response, no single allergen or particular structure can account for a major part of milk allergenicity. Polysensitisation to several proteins occurs often; it is observed in ca. 75% of patients with cows' milk allergy, with a great variability of the IgE response both in specificity and intensity. Even if the proteins most frequently and most intensively recognised by IgE seem also to be the most abundant in milk, casein and β-lactoglobulin, all milk proteins appear to be potential allergens even those that are present in milk in trace amounts (e.g. lactoferrin). These are sometimes the only ones to be recognised and responsible for the clinical symptoms observed. In such cases confirmation of cows' milk allergy and identification of the responsible allergen requires very specific and sensitive immunochemical tests.

Cows' milk proteins are very heterogeneous with very few structural or functional common features. This heterogeneity is complicated by their genetic polymorphism resulting in several variants for each protein. These variants are characterised by point substitutions of amino acids or by deletions of peptide fragments of varying size or by post-translational modifications such as phosphorylation, glycosylation. All of these modifications and similar modifications generated through processing may affect the IgE-binding capacity and allergenicity (Malik *et al.*, 1988; Bernard *et al.*, 2000a).

4.2 Characterisation of epitopes on cows' milk proteins

No definite relationship can be established between structure and allergenicity. It appears that the three-dimensional structure is an important feature in cows' milk protein allergenicity. IgE-binding studies also show the presence of sequential epitopes and these linear epitopes are peptides of various size depending on the method used to isolate and purify them, but peptides as short as ca. 12-14 amino acid residues (i.e. about 1500 Da molecular weight) have been demonstrated to account for a significant part of the allergenicity of the whole molecule in some patients.

Using tryptic and synthetic peptides (Ball *et al.*, 1994; Selo *et al.*, 1999) or overlapping peptides (Heinzmann, 1999) sensitisation has been shown to involve many continuous epitopes that are widely spread all along the BLG molecule. The best recognised peptides, by more than 90% of the patients, are fragments (41-60), (102-124) and (149-162), each of them accounting for 10 to 15% of whole BLG immunoreactivity.

IgE-binding to native ALA and to large peptides confirmed the importance of conformational epitope(s). However, in several patients, reduced peptides exhibited a similar or a higher IgE-binding capacity than the native corresponding fragment, suggesting the existence of sequential epitope(s) exposed through protein denaturation. Moreover, IgE-binding sequences are also located in strongly hydrophobic regions of the ALA molecule where antigenicity is very unlikely to be predicted, and/or within parts of the molecule having a very high sequence homology with human ALA (Maynard *et al.*, 1997 and 1999).

Jarvinen *et al.* (2001) using overlapping decapeptides have identified numerous IgE- and IgG-binding epitopes of BLG and ALA. They confirmed the variability of the antibody responses to various regions of the molecules. Interestingly, the authors correlated the presence of IgE to

multiple linear epitopes with persistent (*vs* transient) milk allergy and suggested it could be a marker to identify the patients that might have a lifelong milk allergy.

Most allergic patients to CAS are sensitised to each of the 4 caseins. This likely results from a co-sensitisation to the different casein components after disruption of the casein micelles during the digestive process. However polysensitisation also appeared to be due to cross-sensitisation mechanisms and involves the only conserved regions which contain the major sites of phosphorylation. Interestingly, bovine β-casein induces a high IgE response despite it is also abundant in human milk and despite human and bovine β-caseins share a high sequence homology. It has been shown that conserved regions shared by both bovine and human β-caseins and particularly those comprising clusters of phosphorylated seryl residues are responsible for IgE cross-reactivity (Bernard *et al.*, 1998 and 2000b). Furthermore it is noteworthy that some of the major epitopes already characterised on α_S caseins are continuous epitopes that have also been located in hydrophobic regions of the molecule, where they are not accessible to antibodies unless the casein is denatured or degraded, as for instance during digestion (Spuergin *et al.*, 1996). Chatchatee *et al.* (2001) have observed differences between 2 groups of patients with persistent or transient cows' milk allergy. Sequences (69-78) and (173-194) were specifically recognised by IgE of 67% and 100% of the patients, respectively, over 9 years of age with persistent allergy but by none of the children less than 3 years old, who are likely to outgrow cows' milk allergy. In addition, sequences (69-78) were not found to be an IgG-binding epitope in any group. Several T cell epitopes have also been characterised and sequence (101-112) appears to be the core sequence comprised in the peptides which most frequently interact with HLA molecules in the immunoregulation of T cell responses to BLG (Sakaguchi *et al.*, 2002).

5. CROSS-REACTIVITIES

The same or closely homologous proteins and their variants are present in milk of other ruminant species. Of particular importance is the fact that the same caseins are present with high sequence homologies varying between 80% to more than 90%. As a consequence, a high IgE cross-reactivity between ewes', goats' and cows' milk casein occurs in most patients with cows' milk allergy (Dean *et al.*, 1993; Spuergin *et al.*, 1997; Bernard *et al.*, 1999; Restani *et al.*, 1999). However, the IgE response may also be species-specific with manifestations occurring after ingestion of sheeps' and goats' cheese but not cows' milk or other dairy products (Wüthrich and Johansson, 1995).

Adverse reactions have been reported in milk allergic patients-fed soy-based formulae as cows' milk substitutes. A 30 kDa glycinin-like protein from soybean that cross-react with cows' milk casein has been isolated and partially sequenced (Rozenfeld *et al.*, 2002).

6. POSSIBLE EFFECTS OF PROCESSING ON ALLERGENICITY AND DERIVED PRODUCTS

These considerations on the structure and properties of cows' milk proteins and on the structure and location of their IgE-binding epitopes, particularly the evidence of existence and importance of linear epitopes have implications on the incidence of technological /physiological processing on milk allergenicity.

Heat processing has no clear impact on cows' milk protein allergenicity. CAS is thermostable while BLG is thermolabile but it may be protected through interaction with casein. Therefore the results depend on temperature and time of heating but also on possible interactions within the food matrix. It must be emphasised that heat denaturation which leads to the loss of organised protein structures does not always result in a decreased allergenic potential. During heating, formation of aggregates may increase the allergenicity of the product. Heat treatment has only moderate effects on the reduction of allergenicity. Boiling of milk for a few minutes (2, 5 or 10 minutes) results either in no difference or in a reduction of about 50-66% of the positive reactions as compared to raw milk. Similar observations have been reported with raw *vs* pasteurised or homogenised and pasteurised milk (Gjesing *et al.*, 1986; Host and Samuelsson, 1988; Norgaard *et al.*, 1996; Werfel *et al.*, 1997).

It is generally accepted that the hydrolysis of milk proteins considerably reduces their allergenicity. However, several studies have shown that specific IgE from patients with milk allergy recognised enzymatic digestion products of whey proteins (i.e. BLG and ALA) or CAS and that the recognition of peptides may even be better than that of the intact molecule (Haddad *et al.*, 1979; Spuergin *et al.*, 1996; Maynard *et al.*, 1997; Selo *et al.*, 1998 and 1999).

Clinical studies have reported controversial results with hydrolyzed formulae, depending on the enzymes used and on the degree of hydrolysis. The incidences of reported adverse effects of allergic babies-fed partially or extensively hydrolyzed milk (either casein or whey) formulae range around 45-65% and 15% respectively (Oldaeus *et al.*, 1991; Ragno *et al.*, 1993; de Boissieu *et al.*, 1997). When partially hydrolysed formulae are concerned allergic reactions may be due to the presence of some residual native protein or of large fragments derived there from. When extensively hydrolyzed formulae are concerned, the allergic reaction may be triggered by short peptide fragments comprising IgE-binding epitopes that are released during the proteolysis.

7. THRESHOLD DOSES

There are few data on threshold levels available based on clinical studies using DBPCFC. Indirect indications on possible threshold doses have indirectly been obtained from reports of severe adverse reactions, including anaphylactic shocks that occurred after ingestion of minute amounts of dairy products. In addition, adverse reactions have also been reported after ingestion of foods containing unintentionally cows' milk protein and in which the amounts of contaminating cows' milk proteins have been afterwards quantified. These may be the so-called non-dairy products where residual amounts of cows' milk proteins used as processing aids may be present. Food allergens including BLG and other milk proteins are absorbed and excreted through breast milk and it may be responsible for adverse reactions in breast-fed infants (Gerrard and Shenassa, 1983 a and b; Jakobsson *et al.*, 1985). In reports where breast-fed babies experienced severe reactions, the concentrations of cows' milk proteins were as low as a few ng/mL of milk. They ranged from 0.5 to 50 ng/mL and reactions often reportedly occurred at about 5 ng/mL (Jakobsson *et al.*, 1985; Axelsson *et al.*, 1986; Machtinger and Moss, 1986; Host *et al.*, 1988; Sorva *et al.*, 1994).

Severe reactions have also been reported after ingestion of meat products containing CAS used for example as texturing agent at concentrations ranging from 1.1% to 0.04% (Malmheden Yman *et al.*, 1994). Frozen desserts containing unexpected trace amounts of whey proteins (9 μg/mL) have provoked anaphylaxis in a 3 year old boy (Laoprasert *et al.*,

1998). Cereal flour in baby food caused failure to thrive due to the "food quality" lactose which contained trace amounts of ALA (5 µg/g) (Fremont *et al.*, 1996).

8. LACTOSE INTOLERANCE

Lactose is a disaccharide which is exclusively present in milk and milk products where it is the main sugar; its concentration is about 5% and 7% in cows' and human's milk respectively. It is a major source of energy in the young infant. Lactose cannot be absorbed as such. It is normally hydrolysed in the small intestine by a lactase (β-galactosidase) to give glucose and galactose. Galactose is then rapidly converted into glucose in the liver. In lactose malabsorbers, the non-digested lactose reaches the large intestine where it is fermented by the colon microflora. The endogenous lactase in the enterocytes is regulated primarily by genetics in healthy individuals and it is not induced by chronic lactose consumption (Lisker *et al.*, 1975).

Lactose intolerance is neither an allergic nor an immune-mediated disease. It does not provoke anaphylactic reaction excepted in milk allergic patients who would then react to residual milk proteins but not to lactose. It results from a reduced capacity to digest lactose which may affect the dietary quality, e.g., low calcium intake. The maldigestion (incomplete digestion) of lactose is due to a reduced lactase activity in the small intestine. It can be due to an inherited deficiency in lactase which affects infants (MIM*223000). This form is rare and this document does not deal with prevention or management of this pathology.

The most common lactase deficiency affects adults (MIM#223100). Lactase activity naturally falls from infantile level to adult levels (a 10- to 20-fold reduction) between the age of 3 and 5 years in 75% of the world's population, while 25% of the population appears to maintain infantile levels of lactase in adulthood (Scrimshaw and Murray, 1988). Both male and female are equally affected. These figures are not representative of the EU population where "lactase persisters" are in majority.

In those individuals who are not able to fully digest significant amounts of lactose the non-absorbed lactose exerts an osmotic effect which induces secretion of water and sodium into the intestinal lumen. This increases the volume of the intestinal content and its transit. The lactose that reaches the large intestine is then fermented by the colon microflora. It results in the production of short chain fatty acids, primarily acetate and gases including hydrogen and methane in some individuals (Pouteau *et al.*, 1998). Those fermentation products are responsible for the clinical symptoms. Symptoms occur within 1 to 3 hours following consumption of a dose of lactose. They begin with stomach/intestinal distension sometimes accompanied by pain, then flatulence and borborygmi. In severe cases acute diarrhoea may follow when the concentration of osmotically active substrates and of fermentation end products increase in the bowel.

Lactose maldigestion itself is a normal physiological response to lactose consumption by most adult humans (as well as by any mammalians) and this does not mean that all suffer of lactose intolerance. Indeed most of the lactose maldigesters can support quantities of 7 to 10 g lactose without any objective symptoms (for review see Marteau *et al.*, 2002). Intolerance is the occurrence of gastrointestinal symptoms following the consumption of a single dose of lactose. The development of symptoms will occur when the quantity of lactose instantaneously present in the gut will much exceed the capacity of the lactase activity. It depends on the dose of lactose consumed, the transit of that lactose, the residual lactase activity and the capacity of the colon microflora to ferment the undigested lactose. There appears to be a capacity of the colon microflora to adapt and regulate fermentations and symptoms of intolerance are minimised through an acidified colon. Lactose concentration in the colon depends on the amount of ingested lactose itself but also on the composition of the whole diet. Exogenous lactase added to or produced in foods (for instance in fermented foods, yogurts) may enhance lactose digestion (Marteau *et al.*, 1990). It also depends on the dietary habits, e.g. how and how fast the lactose is consumed, whether it is consumed alone or with milk or diluted in a meal (Martini and Savaiano, 1988).

However it must be emphasised that the dose of lactose is critical in the elicitation of intolerance. The exact relationship between the dose, the maldigestion and the concentration of lactose that reaches the large intestine is certainly multifactorial but experiments where the production of hydrogen is measured have shown that a linear relationship exists in the range of typical serving sizes (6 to 20 g) (Hertzler *et al.*, 1996; Savaiano, 2002).

Clinical manifestations of lactose intolerance usually occur in the majority of maldigesters when large doses of lactose are consumed, for instance 50 g, and the proportion of intolerance decreases when the dose decreases (Vesa *et al.*, 2000). Doses less than 10 g (corresponding to 200 mL of milk) per day are often tolerated by most of the population of lactase deficient adults. As a consequence of regulation and Good Manufacturing Practice, the level of lactose that can be present in lactose-free foods should not be considered a danger for lactase deficient consumers. However residual amounts of cows' milk proteins that can still be present in lactose as contaminants from the production process of lactose might be harmful for allergic patients to milk.

9. CONCLUSION

Milk is a frequent and a major allergenic food. Most of cows' milk proteins are potential food allergens, even those present at very low concentrations. Numerous sequential epitopes have been identified that are widely spread all along the protein molecules. Short peptide fragments may conserve significant part of the allergenicity of the whole protein. Some are located in hydrophobic parts of the molecule and are released or become bioavailable after denaturation of the proteins or after their degradation by proteolytic enzymes, for example during digestion. Any milk product containing native or denatured cows' milk protein or fragments derived thereof may trigger an allergic reaction even those present in non-dairy foods, e.g. processing aids. Data available from double-blind placebo-controlled food challenges or from case reports do not permit to establish safe threshold doses nor to derive a level of exposure which could protect allergic consumers against a reaction to milk products present in their food in trace amount. There is no definite indication that technological treatments may alter the structure of cows' milk proteins or decrease their concentration to a sufficient extent to guarantee the loss of their allergenic potential, at least for a highly sensitive fraction of the

population of milk sensitive patients. Moreover due to the high frequency of immunological cross-reactions, these considerations may be applied to milk of species other than cows, such as buffalos, goats and ewes.

XVI. ALLERGY TO NUTS

SUMMARY

For the purpose of this Opinion, nuts are defined by the Labelling Directive 2003/89/EC (Annex IIIa). Nuts belong to several different botanical families. From an allergological point of view, however, they can be grouped together because of their similarity in allergenic properties, way of consumption, clinical picture provoked, and protein pattern. Together with peanut, nuts (particularly Brazil nut) are the most powerful food allergens, and they are able to elicit severe or even fatal anaphylactic reactions, even when ingested in small amounts. A few milligrams of nut proteins can provoke allergic reactions in sensitised subjects; which has been shown by double-blind, placebo-controlled oral provocation studies for hazelnut. Such allergenicity seems to be maintained also after heat treatment or industrial processing, as demonstrated for almond and cashew nut. Studies on hazelnut underlined that heat resistance can be restricted to some allergens: the 18 kDa hazelnut allergen, which is homologous to the birch pollen allergen Bet v 1, loses its allergenicity after roasting, while lipid transfer protein does not, and a particular subset of patients may react only to roasted hazelnuts. Except for hazelnut, sensitisation which is usually found in birch allergic patients, other nuts are not related to any pollen allergy. The main allergens in nuts are not cross-reacting with pollen allergens. They are represented by seed storage proteins like 2S albumins, legumins and vicillins.

1. BACKGROUND

Nuts include a wide variety of fruits; the most common are hazelnut, Brazil nut, walnut, pecan, almond, cashew nut, Macadamia nut and peanut. Nuts are increasingly consumed in many forms, varying from raw seeds to roasted snacks. In the USA, almonds rank first in per capita consumption of nuts, followed by pecans and walnuts. Nuts are one of the most important food allergens; their main characteristics will be approached for every single nut. A special chapter (Chapter XIII) is being dedicated to peanut because of its clinical relevance.

Hazelnut (*Corylus avellana*) belongs to the family of *Betulaceae*, a group of plants whose pollen is often responsible for respiratory symptoms; hazelnut differs from other nuts because patients who become sensitised to it are usually allergic to the pollen of birch-related trees, and develop hazelnut allergy secondarily to an immunological cross-reactivity between birch and hazelnut allergens.

Brazil nut (*Bertholletia excelsa*) does not belong to the subclass of *Rosidae*, like hazelnut, walnut, almond and cashew nut, but to the subclass of *Asteridae*, like sesame seeds and *Apiaceae* (carrot and celery).

English walnut (*Juglans regia*), like other nuts, belongs to the subclass of *Rosidae*, and, together with hazelnut, is a part of the order of Fagales.

Pecan nut (*Carya illinoinensis*) is closely related to walnut, as it belongs to the same family of *Juglandaceae*.

Almonds (*Prunus dulcis*), belongs to the *Rosaceae* family, which includes apple, pear and *Prunoideae* fruits (peach, apricot, plum and cherry).

Cashew nut (*Anacardium occidentale*) belongs to the *Anacardiaceae* family, together with pistachio and mango. It is globally popular and is appreciated for its flavour and texture. The world annual production is about 1,500,000 tons, one third of which coming from India.

Macadamia nut (*Macadamia integrifolia*) and Queensland nut (*Macadamia temifolia*) belong to the subclass of *Rosidae*, which include the majority of nuts; they are a member of the *Proteaceae* family.

Table 20. Taxonomy of nuts and other allergenic foods

Subclass	Order	Family	Subfamily	Tribe	Genus and species	Common name
Rosidae	Fabales	*Fabaceae*	*Papilionoideae*	*Phaseoleae*	*Glycine max*	Soybean
				Aeschynomeneae	*Arachis hypogaea*	Peanut
	Fagales	*Juglandaceae*			*Juglans regia*	English walnut
		Betulaceae			*Corylus avellana*	Hazelnut
	Sapindales	*Anacardiaceae*			*Anacardium occidentale*	Cashew nut
					Pistacia vera	Pistachio
					Mangifera indica	Mango
	Rosales	*Rosaceae*	*Amygdaloideae*		*Prunus dulcis*	Almond
			Maloideae		*Malus domestica*	Apple
			Prunoideae		*Prunus persica*	Peach
					Prunus armeniaca	Apricot
					Prunus domestica	Plum
					Prunus avium	Cherry
Asteridae	Ericales	*Lecythidaceae*			*Bertholletia excelsa*	Brazil nut
	Lamiales	*Pedaliaceae*			*Sesamum indicum*	Sesame seeds
	Apiales	*Apiaceae*			*Daucus carota*	Carrot
					Apium graveolens	Celery

2. FREQUENCY

2.1 Prevalence

Allergy to nuts is an emerging problem, whose importance has been highlighted by the institution of registers of anaphylaxis deaths kept since 1992 both in the UK and in the USA following the publication in the early 1990s of several cases of fatal food allergic reactions, nearly all caused by peanut (Sampson *et al.*, 1992). In the UK, out of the 37 food-induced fatalities reported from 1992 to 2000, 10 were attributable to peanut, 5 to walnut and 10 to other non-specified nuts (Pumphrey, 2000); in the USA, 32 fatal cases were reported, 20 caused by peanut, 3 by walnut, 2 by Brazil nut, 2 by pecan nut, 1 by pistachio and 2 by non-specified nuts (Bock *et al.*, 2001). An epidemiological study on the cases of anaphylaxis requiring emergency treatment in the Northwest of England reported 23 cases of reactions to nuts (Pumphrey and Stanworth, 1996). Also in a one-year observation study performed in a single emergency department of Brisbane, Australia, four cases of fatal food reactions were caused by nuts (Brown and McKinnon, 2001).

More recently, a study by Macdougall *et al.* (2002) reported 14 paediatric cases of severe food allergic reactions due to nuts, especially cashew nut (7 cases). The prevalence of clinical allergy to nuts (including hazelnut, walnut, Brazil nut, cashew and pistachio) and peanut is estimated at about 0.4% of the adult population of Great Britain (Emmett *et al.*, 1999; Tariq *et al.*, 1996) and at about 1.1% of the US adult population (Sicherer *et al.*, 1999). Nut sensitisation in children seems to be increasing (Sicherer *et al.*, 2003).

3. CLINICAL FEATURES

Data from a voluntary register of nut allergic patients (Sicherer *et al.*, 2001) show that the most frequent nut causing reactions is walnut (34%) followed by cashew (20%), almond (15%), pecan (9%), pistachio (7%), hazel, Brazil, Macadamia, pine and hickory (less than 5% each). Most patients (54%) are allergic to a single nut. Ingestion is the most common way of exposure, and the median time from exposure to reaction is 3 minutes. Interestingly, 23% of this population reported a coexisting allergy to peanut. The great majority of subjects react to their first known exposure to cashew nut, and half of them experience symptoms after minimal contact like smelling, touching or tasting the nut; most of the reported reactions were severe (Hourihane *et al.*, 2001).

Basically, different clinical reactions can be elicited by all nuts: they extend from lethal anaphylaxis to gastrointestinal and dermal symptoms. Severe, sometimes lethal anaphylaxis, has been described for hazelnut (Pastorello *et al.,* 2002a), Brazil nut (Pastorello *et al.*, 1998), walnut (Teuber *et al.*, 1998; Pumphrey, 2000; Bock *et al.*, 2001), pecan nut (Yunginger *et al.*, 1988; Bock *et al.*, 2001) and Macadamia nut (Sutherland *et al.*, 1999).

The most important gastrointestinal manifestation of nut allergy is the oral allergy syndrome, which has been confirmed by DBPCFC (Wensing *et al.*, 2002b; Ortolani *et al.*, 2000). Dermatitis can be elicited by cashew nut (Diogenes *et al.*, 1996; Hamilton and Zug, 1998; Rosen and Fordice, 1994; Marks *et al.*, 1984). This was also confirmed by DBPCFC (Bock and Atkins, 1989; Burks *et al.*, 1998a).

4. IDENTIFIED ALLERGENS

Most nut major allergens belong to the family of seed storage proteins, particularly to 2S albumins, which include the walnut allergen Jug r 1, the Brazil nut allergen Ber e 1, the cashew nut allergen Ana o 3, and two recently identified allergens of almond and hazelnut (Table 21).

The 2S albumins are widespread among different plant species and are contained in high quantities in seeds, as their main function is to provide a store of amino acids for use during germination and seedling growth. They also display antifungal activity, probably connected with the protection of seeds before and during germination. The 2S proteins belong to the prolamin superfamily, also including the trypsin/alpha-amylase inhibitors, which are important allergens in wheat and other cereals.

4.1 Hazelnut

Hazelnut is an interesting model of various patterns of IgE-mediated reactivity depending on the route of sensitisation and varying in different countries. Both pollen- and non-pollen-related allergens have been identified in hazelnuts. The first identified allergen was a protein of 18 kDa which is present in both hazelnut kernel and hazel pollen tissue, as a homologue of the major birch pollen allergen Bet v 1 (Hirschwehr *et al.*, 1992). This allergen, named Cor a 1, includes four isoforms: Cor a 1.01, 1.02 and 1.03 in hazel pollen, and 1.04 in hazelnut (Lüttkopf *et al.*, 2002). Cor a 1.04 is expressed in at least four sub-isoforms, which show different allergenic properties: the isoform Cor a 1.0404 is less allergenic, maybe because of the lack of a conformational epitope. By studying the IgE reactivity of the sera of patients with oral allergy syndrome, as diagnosed by DBPCFC in the study by Ortolani *et al.* (2000), Pastorello *et al.* (2002a) provided evidence that the 18 kDa protein was the major hazelnut allergen, as it was bound by IgE from 63 out of 65 DBPCFC positive patients. This protein was destroyed by heating and lost its allergenicity in roasted hazelnut. In the same study other hazelnut major allergens were identified: a 32 kDa 2S albumin, a 35 kDa legumin and a 47 kDa sucrose-binding protein belonging to the vicillin superfamily. A 9 kDa lipid transfer protein, which was a minor allergen in the DBPCFC positive patients, was found to be a significant allergen in a selected population of 7 patients with severe anaphylactic reactions to hazelnut without birch pollinosis. This heat-stable protein was not cross-reactive with birch pollen allergens, but with the peach major allergen.

Another hazelnut allergen unrelated to tree pollen was isolated and cloned by Beyer *et al.* (2002). It is an 11S globulin which was recognised by 12 out of 14 patients with a history of systemic allergic reactions to hazelnut such as urticaria, respiratory and gastrointestinal symptoms, 8 of which had no pollen-related symptoms nor specific IgE to hazel pollen (Beyer *et al.*, 2002). This protein may correspond to the 35 kDa legumin identified by the study by Pastorello *et al.* (2002a) already cited, and showed 49% amino acid sequence homology with peanut Ara h 3, although the clinical relevance is unclear.

4.2 Brazil nut

The first 2S albumin shown to be allergenic in nuts was the major allergen of Brazil nut Ber e 1. This methionine-rich protein was used in genetic engineering experiments to enhance the soy content of sulphur amino acids; in order to assess its safety, it was tested for allergenicity using sera from nine patients suffering from allergic reactions (oral allergy syndrome, angio-

edema, laryngeal oedema, bronchospasm) after ingestion of Brazil nut. Eight out of nine showed IgE-binding to the 2S albumin, which was thus to be considered a major allergen (Nordlee *et al.*, 1996). A subsequent study by Pastorello *et al.* (1998) confirmed that the 2S albumin was the major allergen of Brazil nut, as it was recognised by 11 out of 11 patients with documented history of anaphylactic shock or laryngeal oedema after ingestion of the nut. This study also demonstrated that the major allergen was actually responsible for these clinical pictures, as it was recognised by all the eleven patients with severe clinical symptoms to Brazil nut, and by none of the ten control patients, who were sensitised to the nut without symptoms (Pastorello *et al.*, 1998).

4.3 Walnut

The first allergen identified in walnut is another protein belonging to the 2S albumin family, synthesised as a precursor and then cleaved into a large subunit and a small subunit, joined by disulphide bridges (Teuber *et al.*, 1998). This allergen was cloned from a cDNA library from walnut somatic embryo, screened using serum from a walnut allergic patient, chosen from a selected population of 16 subjects who experienced life-threatening systemic allergic reactions to walnut. The recombinant walnut 2S albumin was found to be a major allergen in these patients, as it was recognised by 12 of them (75%). Moreover, the recombinant protein was able to inhibit IgE-binding to 14 kDa, 10-12 kDa and 5 kDa natural proteins of mature walnut kernel, probably corresponding to the intact 14 kDa heterodimeric protein and to the large and small subunits. This allergen, named Jug r 1, shows 46% amino acid sequence identity with the major allergen from Brazil nut Ber e 1. Its linear epitopes have recently been studied, with evidence of one linear immuno-dominant peptide (recognised by 15 out of 20 walnut allergic patients) and data suggesting for one or more conformational epitopes (Robotham *et al.*, 2002).

Using the same population of patients and the same recombinant techniques as for Jug r 1, Teuber *et al.* (1999) also identified a second major allergen in walnut, the vicillin-like protein Jug r 2, recognised by 9 of 15 walnut allergic subjects. The vicillin is synthesised as a pro-protein of 66 kDa molecular weight, and then cleaved at a highly hydrophilic sequence containing repeated motifs, to remove a short hydrophobic leader peptide and give a 48 kDa mature protein. Despite its high amino acid sequence identity (70%) with peanut vicillin Ara h 1, this allergen does not cross-react with homologous peanut proteins.

4.4 Almond

Allergy to almond has been poorly studied until recently. Pasini *et al.* (2000) found a low correlation between symptoms to ingested almond and positive CAP-FEIA for detection of specific IgE antibodies. By analysing patients with positive CAP test but negative history, and patients with life-threatening anaphylactic reactions with false-negative CAP test, they found that the CAP-FEIA positivity to almond was due to the presence of two 50-62 kDa glycoproteins with little immunological significance. Meanwhile, the 37 kDa allergen responsible for severe reactions was not represented in the *in vitro* test; this protein, however, was not characterised at molecular level.

Another study by Poltronieri *et al.* (2002) identified two new IgE-binding almond proteins in serum from patients responsive to almond in oral food challenge tests: a 45 kDa gamma conglutinin (a dimer compound formed by a 30 kDa heavy chain bearing the IgE-binding epitopes plus a 15 kDa light chain) and a 12 kDa 2S albumin, showing at the C terminal 80%

amino acid sequence similarity to homologous proteins from English walnut and Brazil nut. These two proteins, however, seem to be almond-specific, as their ability to bind IgE from almond allergic patients' sera was not inhibited by walnut and hazelnut extracts.

Table 21. Nut allergens associated with clinical reactions

Allergen	Allergenic source	Family	Molecular weight*	Population studied	Authors
Ber e 1	Brazil nut	2S albumin	9 kDa	9 patients with history of severe systemic allergic reactions after ingestion of Brazil nut, positive SPT and RAST	Nordlee *et al.*, 1996
				11 patients with either anaphylactic shock or laryngeal oedema	Pastorello *et al.*, 1998
Not assigned	Almond	-	37 kDa	2 patients with laryngeal oedema	Pasini *et al.*, 2000
Not assigned	Almond	Conglutinin γ (storage globulin)	45 kDa	5 patients with positive history, SPT and RAST, DBPCFC or open food challenge with raw almond	Poltronieri *et al.*, 2002
		2S albumin	12 kDa		
Jug r 1	Walnut	2S albumin	15-16 kDa	16 patients with a well-documented history of life-threatening systemic allergic reactions to walnut	Teuber *et al.*, 1998
Jug r 2	Walnut	Vicillin	48 kDa (pro-protein 66 kDa)		Teuber *et al.*, 1999
Cor a 1.04	Hazelnut	Rnase (Bet v 1 homologue)	18 kDa	25 patients with allergic reactions to tree pollens and intolerance to hazelnut	Hirschwehr *et al.*, 1992
				43 patients with positive DBPCFC to hazelnut	Lüttkopf *et al.*, 2002
				65 patients with positive DBPCFC and 7 patients with a history of severe anaphylaxis to hazelnut	Pastorello *et al.*, 2002a
Cor a 2	Hazelnut	Profilin	14 kDa	25 patients with allergic reactions to tree pollens and intolerance to hazelnut	Hirschwehr *et al.*, 1992
Not assigned	Hazelnut	2S albumin	32 kDa	65 patients with positive DBPCFC and 7 patients with a history of severe anaphylaxis to hazelnut	Pastorello *et al.*, 2002a
Not assigned	Hazelnut	Legumin	35 kDa		
Not assigned	Hazelnut	Sucrose-binding protein (vicillin super-family)	47 kDa		
Cor a 8	Hazelnut	Lipid transfer protein	9 kDa		
Cor a 9	Hazelnut	11S globulin	40 kDa	14 patients with hazelnut-induced systemic reactions and positive RAST	Beyer *et al.*, 2002
Ana o 1	Cashew nut	Vicillin	53 kDa	20 patients with life-threatening reactions to cashew nut and positive RAST	Wang *et al.*, 2002
Ana o 2	Cashew nut	Legumin	31-35 kDa (large subunit)	15 patients with life-threatening reactions to cashew nut and positive RAST	Teuber *et al.*, 2002
Ana o 3	Cashew nut	2S albumin	6-12 kDa		

* Reported molecular weight

4.5 Cashew nut

García *et al.* (2000) described three cashew allergic patients who showed IgE reactivity to several proteins, with the strongest IgE-binding at 15, 30 and 60 kDa. These allergens however were not purified and sequenced.

The first allergens identified in cashew nut are seed storage proteins: in fact, in a population of 15 patients with life-threatening allergic reactions to cashew, the most relevant allergens were a legumin and a 2S albumin, recognised each by 11 patients (73%); a minor allergen, recognised by 5 patients (33%) was identified as a sucrose-binding protein homologue (7S globulin), which belongs to the vicillin group (Teuber *et al.*, 2002). Two of these allergens, the legumin-like protein and the vicillin-like protein, are characterised at molecular level. The legumin-like major allergen has already been described by Sathe *et al.* (1997) and corresponds to anacardein, also called CMP (cashew major protein), a 13S multimeric globulin which is the predominant soluble globulin in cashew and constitutes about 50% of the total seed proteins. It is made up of several polypeptides of 46-53 kDa, which upon reduction separate into two subunits of 30-37 and 18-24 kDa, respectively (Sathe, 1994; Sathe *et al.*, 1997); the larger one is a cashew major allergen (Teuber *et al.*, 2002).

The vicillin-like allergen is a 7S globulin which represents about 5% of the extractable proteins. It was cloned by screening a cDNA library with sera from patients with severe anaphylactic reactions to cashew: the recombinant protein was recognised by 50% out of 20 cashew allergic patients and by 25% out of 8 cashew tolerant patients. Epitope mapping revealed 11 linear IgE-binding epitopes, 3 of which were immunodominant; none of the epitopes showed significant sequence homology with those of the peanut vicillin allergen Ara h 1 (Wang *et al.*, 2002).

5. CROSS-REACTIVITIES

5.1 Clinical cross-reactivity

While allergy to vegetable foods is often due to a primary sensitisation to pollens by inhalation, with subsequent reactivity to related foods containing cross-reacting allergens, allergy to nuts is almost exclusively due to a true food sensitisation, not pollen-mediated. Only allergy to hazelnut can be due to sensitisation to birch pollen or, less frequently, to mugwort pollen (Hirschwehr *et al.*, 1992; Caballero *et al.*, 1997).

An additional problem is the possibility of cross-reactions between peanut and nuts or among nuts themselves. As already mentioned, allergy to nuts is characterised by a high frequency of life-threatening anaphylactic reactions, so when allergy to a single nut is demonstrated, the patient is often advised to avoid the entire nut group. It is still unknown whether this avoidance is justified or if it is an overstatement, as only few studies have dealt with cross-reactivity among nuts. Some studies highlighted the strong association between peanut and nut allergy, with a prevalence of multiple nut sensitivity of about 40% in nut allergic patients (Ewan, 1996); in a randomly sampled population, on the contrary, only 2.4% of peanut and nut allergic subjects reported symptoms with more than one variety (Sicherer *et al.*, 1999).

5.2 Cross-reacting allergens

5.2.1 Hazelnut

The major allergen Cor a 1 was demonstrated to be cross-reactive with the birch pollen major allergen Bet v 1 (Hirschwehr *et al.*, 1992). In a recent study, the 18 kDa allergens from hazelnut kernel and hazel pollen tissue were cloned using sera from 43 patients with positive DBPCFC to hazelnut (Lüttkopf *et al.*, 2002). Four recombinant variants of the major hazelnut allergen Cor a 1.04 were produced, which surprisingly showed only 63% identity and partial IgE cross-reactivity with the major hazel pollen allergen Cor a 1.01, but 85% identity with the major birch pollen allergen Bet v 1, thus demonstrating that the epitopes of hazelnut Cor a 1.04 are less related to hazel pollen than to birch pollen.

Another allergen displaying cross-reactivity with a birch pollen homologous allergen is hazelnut profilin; the clinical relevance of this immunological cross-reactivity seems however low (Hirschwehr *et al.*, 1992; Wensing *et al.*, 2002c).

Other minor allergens were described in hazelnut, which do not cross-react with Bet v 1 or other pollen-derived allergens: the heat-stable hazelnut lipid transfer protein was shown to be reactive with the peach lipid transfer protein (Pastorello *et al.*, 2002a), which is a major allergen in this fruit as in other *Prunoideae* fruits like apricot, cherry and plum. The main characteristic of this allergen is the lack of clinically significant cross-reactivity with homologous allergens in pollens; this is also confirmed for hazelnut lipid transfer protein.

5.2.2 Walnut

Walnut is one of the few nuts for which cross-reactivity with peanut has been investigated. Teuber *et al.* (1999), using sera from two walnut allergic patients, demonstrated that cross-reactive proteins occur within these two foods. However, the major allergen Jug r 2, despite its high amino acid sequence identity (70%) with the peanut vicillin Ara h 1, does not cross-react with homologous peanut proteins (Teuber *et al.*, 1999).

5.2.3 Almond

Few studies are available on the cross-reactivity of almond with other nuts. Using sera from 2 subjects with systemic allergic reactions to coconut, Teuber and Peterson (1999) found that almond extract was able to inhibit IgE-binding to the 37 kDa walnut and coconut allergens. Thus, the 37 kDa almond allergen described by Pasini *et al.* (2000), which causes severe anaphylactic reactions to almond, could be responsible for molecular cross-reactivity between almond and other nuts; it has not been demonstrated whether this reactivity is clinically significant.

Two other almond allergens, a 45 kDa gamma conglutinin and a 12 kDa 2S albumin, show no cross-reactivity with the homologous proteins from walnut and Brazil nut, despite 80% amino acid sequence similarity (Poltronieri *et al.*, 2002).

5.2.4 Cashew nut

In a study by Fernández *et al.* (1995), an IgE cross-reactivity between cashew and pistachio was demonstrated by RAST inhibition, but the involved allergens were not identified.

5.2.5 Macadamia nut

Sutherland *et al.* (1999) demonstrated that some allergens of Macadamia nut, particularly that of about 17 kDa molecular weight, are cross-reactive with homologous allergens of hazelnut; no cross-reactivity with peanut was found.

6. POSSIBLE EFFECTS OF FOOD PROCESSING ON ALLERGENICITY AND DERIVED PRODUCTS

Hazelnut, which is often used in pastry goods, partially loses its allergenicity after roasting. In a study by Pastorello *et al.* (2002a), the IgE-binding pattern of raw and roasted hazelnut was considered: it was found that the major allergen Cor a 1 was destroyed by heating and lost its allergenicity in roasted hazelnut, while the minor allergen lipid transfer protein, recognised by a distinct subset of patients without birch pollinosis, was heat-resistant. Very recently some patients of this study were re-evaluated by DBPCFC with roasted hazelnut in order to confirm that, as demonstrated *in vitro*, also *in vivo*, roasting destroys the major hazelnut allergen Cor a 1.04 to which all the patients were sensitised. Nevertheless, 5 out of 17 patients studied reacted with mild oral allergy syndrome to the challenge (Skamstrup Hansen *et al.*, 2003). In birch pollen allergic patients also allergic to Cor a 1.04, roasting can decrease but not abolish clinical sensitivity in highly allergic patients.

Wei *et al.* (2003) studied the resistance of some nut allergens, particularly the cashew major protein, to some of the most commonly employed industrial processing methods, such as roasting, blanching, autoclaving and microwave heating. By ELISA technique they demonstrated that cashew major protein lost its IgE-binding capacity upon heating (Wei *et al.*, 2003). This decrease appears to be due to a heat-induced reduction in protein solubility rather than to destruction of certain epitopes.

In some cases thermal treatment does not reduce the allergenicity of a nut, but on the contrary enhances it. This was seen in pecan nut, which upon heating develops new allergens as a result of a Maillard reaction (Berrens, 1996); this effect of roasting was also described in peanut. These modifications have a clinical significance: an anaphylactic reaction to cooked pecan nuts was reported by a patient who showed specific IgE antibodies exclusively against allergenic determinants present in aged or heated pecan, but not in fresh pecans (Malanin *et al.*, 1995).

7. THRESHOLD DOSES

Nut-induced fatalities have recently been described after the ingestion of foods apparently free from nuts, so it is likely that a few micrograms may produce very severe reactions (Ortolani *et al.*, 2000; Wensing *et al.*, 2001).

For hazelnut, doses of about 7 and 10 mg of hazelnut protein have been reported to induce respiratory or gastrointestinal symptoms in allergic patients (Malmheden Yman *et al.*, 1994; Wensing *et al.*, 2001). A European multicentre study demonstrated that very low doses of hazelnut can elicit symptoms, as the mean provocative dose during DBPCFC ranged from 1.4 g to 15.3 g (Ortolani *et al.*, 2000). In a DBPCFC study on 29 hazelnut allergic patients, threshold doses for eliciting subjective reactions varied from 1 mg up to 100 mg of hazelnut

protein (equivalent to 6.4-640 mg of hazelnut meal), while objective symptoms were observed in two patients after 1 and 1000 mg, respectively. On the basis of a dose-response curve it could be extrapolated that 50% of this population reacted to doses of 6 mg or less of hazelnut protein, corresponding to about 40 mg of hazelnut (Wensing, *et al.*, 2002b).

8. CONCLUSION

Nuts are a common cause of systemic allergic reactions which can be life-threatening. Multiple nut sensitivity is frequent, but cross-reacting allergens have not yet been identified. Roasting can reduce hazelnut allergenicity but not abolish it even in patients sensitised to Bet v 1; and roasting does not affect hazelnut allergenicity in patients sensitised to lipid transfer protein. Threshold doses derived from clinical studies of patients with oral allergy syndrome are very variable and do not allow the determination of a threshold dose for allergic consumers.

XVII. ALLERGY TO CELERY

SUMMARY

Celery is a frequent cause of pollen-related food allergy, particularly in European countries. In Switzerland and France about 30-40% of patients with food allergy are sensitised to celery root and about 30% of severe anaphylactic reactions to food were thought to be due to celery according to the patient's history. Symptoms of celery allergy comprise local oropharyngeal reactions, and a number of more severe manifestations including life-threatening systemic anaphylaxis. Allergy to celery root is highly associated with birch and mugwort pollen sensitisation. The serological IgE cross-reactivity with celery is due to at least three classes of allergenic proteins: Api g 1, a 17 kDa protein homologous to Bet v 1, the major birch pollen allergen; Api g 4, the celery 15 kDa profilin; the cross-reactive carbohydrate determinants (CCD), 32-70 kDa allergens. Recently, a 58 kDa allergen, has been included in the IUIS nomenclature as Api g 5.

Celery root is often consumed in a processed form as a cooked vegetable or as a spice. Thermal processing does not completely deplete allergenicity of celery. Celery allergic patients should be instructed to avoid spice mixtures or prepacked food possibly containing celery spice, since allergenicity of celery powder is comparable to that of raw celery. The heat resistance of celery allergens decreases in the following order: cross-reactive carbohydrate determinants > Api g 4 > Api g 1. Five of six patients with positive DBPCFC to cooked celery were sensitised to profilin and/or cross-reactive carbohydrate determinants.

Few studies were performed to determine the minimum tolerable dose in celery sensitised patients, which demonstrated that local oropharyngeal symptoms are elicited by low doses of celery (0.7-2.7 g), while systemic symptoms arose only after ingestion of doses ranging from 7.5 to 31 g. Threshold doses were similar also for cooked celery, while celery spice was able to induce systemic symptoms at very low doses of 0.16-5.85 g.

1. BACKGROUND

Celery (*Apium graveolens*) belongs to the *Apiaceae* family (or *Umbelliferae*) (see Table 20). The celery plant is composed of a root or tuber, also called celeriac, and sticks, that are used in nutrition and diagnostics.

Celery was cultivated by the Greeks and Romans and has been widely used both as a food and in medication since the Middle Ages.

Celery is not only consumed raw in salads, but also as cooked vegetable, as a constituent of sauces and soups, because of its aromatic flavour, and as a spice which is a common ingredient in various processed foods.

Celery can induce allergic reactions of immediate type, from oral contact urticaria to anaphylactic shock. About 30% of patients with oral allergy syndrome are allergic to celery. The first evidence for IgE celery specific antibodies in sensitised subjects was reported by Kaupinen (1980) using skin prick tests, then by Dechamp *et al.* (1984) and Pauli and Bessot (1985). Diagnostic tests like skin tests with raw celery and active allergen extracts have high

positive predictive values, while the negative predictive values are low. Usually the frequency of sensitisation to celery tuber is higher than to celery stick in celery allergic subjects.

2. FREQUENCY

2.1. Prevalence

Allergy to celery is one of the most frequent pollen-related food allergies in certain European countries such as Switzerland, France and Germany. In Switzerland approximately 40% of patients with food allergy are sensitised to celery root, some with severe anaphylactic reactions (Wüthrich *et al.*, 1990). In a French study of patients attending a specialist allergy clinic reported that about 30% of 580 food allergic patients showed specific IgE to celery, and a considerable number of severe food reactions appear to be due to celery (André *et al.*, 1994). In Germany, 70% of patients with a pollen-related food allergy have a positive skin prick test or RAST to celery (Jankiewicz *et al.*, 1996). In Italy about 10% of 262 patients with oral allergy syndrome to fresh fruit and vegetables had a clinical history of allergic reactions to celery. About 3% of the 262 patients with oral allergy syndrome experienced severe symptoms to celery such as laryngeal oedema (Ortolani *et al.*, 1988).

3. CLINICAL FEATURES

Ingestion of celery may lead to intolerance reactions in humans, a fact that has been known for almost 70 years. The first case of an allergic reaction to celery root was observed by Jadassohn and Zaruski in 1926 in Zurich. Forsbeck and Ros in 1979 described the first anaphylactic shock occurring after celery ingestion. Kaupinen in 1980 reported 14 severe reactions after ingestion of this food. The first who described the coexistence of celery allergy with sensitisation to certain pollens was Wüthrich and Dietschi (1984).

Whereas other pollen-related allergies to fruits often induce mild symptoms (oral allergy syndrome), allergic reactions to celery are frequently more severe (Ortolani *et al.*, 1988; Wüthrich *et al*., 1990; André *et al.*, 1994).

Pauli and Bessot (1985) and Pauli *et al.* (1988) reported the clinical and biological characteristics of 20 patients, observed between 1974 and 1984, who presented allergic clinical symptoms after ingestion of raw celery in salad. Urticaria and angio-edema were observed in 18/20 cases, respiratory disorders in 7/20 and systemic anaphylaxis in 4/20 cases (vascular collapse in three cases and loss of consciousness in one case). Most of the patients who were intolerant to raw celery stated that they could tolerate the cooked vegetable. Symptoms occurred after ingestion of cooked celery in three patients only.

In 2000, Ballmer-Weber *et al.* recruited 32 patients with a history of allergic reactions to celery (oral allergy syndrome, urticaria/angio-edema, gastrointestinal and respiratory symptoms) to perform a DBPCFC with raw celery root. Out of the 32 patients, 22 were responders, 11 of them showing only symptoms localised to the oral cavity, and 10 showing systemic reactions. The two-step procedure of DBPCFC allowed the identification of over 50% of allergic patients when they produced only local oral symptoms. Four patients with a negative DBPCFC result complained of oral allergy syndrome in the open provocation. This finding raises the issue of the accuracy of DBPCFC as the standard for the diagnosis of food

allergy because a subset of patients may only produce oral allergy syndrome if there is direct contact between the incriminated food and the oral mucosa.

In the same study, the authors evaluated the current diagnostic procedures in patients with celery allergy and found great variations in the suitability of commercially available products for diagnostic purposes which were changed to be due to protein and phenol content of the extracts.

4. IDENTIFIED ALLERGENS

Celery allergens identified to date are the birch-related Api g 1 (Breiteneder *et al.*, 1995), a 16 kDa protein homologous to Bet v 1 (Ebner *et al.*, 1995; Schoning *et al.*, 1995) and Api g 4 (Vallier *et al.*, 1992), the celery profilin; the cross-reactive carbohydrate determinants also seem to be allergenic (Fotisch *et al.*, 1999) (Table 23). A 60 kDa allergen was also described but its function was not determined (Heiss *et al.*, 1996). Recently, Ganglberger *et al.* (2000) described two new high molecular weight allergens of celery, recognised by the sera of five patients with positive case histories, skin prick tests and RASTs to celery and birch. These 55 and 58 kDa proteins represent members of a protein family not described so far, as no homologous sequences were found in the databases. The 58 kDa allergen, included in the IUIS nomenclature as Api g 5, may correspond to the 60 kDa allergen found by Heiss *et al.* (1996). Bublin *et al.* (2003) found the complete abolition of binding of serum IgE from all 14 patients tested by chemical deglycosylation of the Api g 5 glycoprotein allergen and observed that native Api g 5 other than the deglycosylated protein completely inhibited the IgE-binding to high molecular weight allergens in protein extracts from birch pollen, mugwort pollen and celery. These results confer convincing evidence that IgE directed to cross-reactive carbohydrates are capable of eliciting allergic reactions *in vivo*.

In patients with allergy to celery demonstrated by DBPCFC (Lüttkopf *et al.*, 2000) Api g 1 was the major allergen, recognised by IgE from 13 of 22 patients (59%). Another major allergen was cross-reactive carbohydrate determinants, determined by IgE reactivity in 12 of 22 patients (55%); celery profilin was recognised by IgE from 5 of 22 patients (23%).

Vieths *et al.* (1995a) demonstrated that in patients with a birch pollen/celery sensitisation the major allergen was Api g 1, while in patients with mugwort pollen/celery sensitisation, the major allergen was profilin. Api g 1 has been characterised, cloned and produced as a recombinant protein; it shows a 40% identity and 60% similarity to Bet v 1, and shares several B-cell epitopes with this allergen (Breiteneder *et al.,* 1995). Vallier *et al.* in 1992 showed that the panallergen profilin, a cross-reacting protein in many kinds of plant foods, is involved in celery allergy. Recently, a new isoform of the celery major allergen, Api g 1.0101 has been described. This isoform displayed cross-reactivity with another isoform, Api g 1.0101 and to Bet v1 from birch pollen, but a weaker IgE-binding capacity than Api g 1.0101 (Hoffmann-Sommergruber *et al.*, 2000). Identification and characterisation of such hypoallergenic isoforms may represent safer tools for immunotherapy, since they bear a lower risk of IgE-mediated anaphylactic side effects. Another promising approach has been suggested by Neudecker *et al.* (2003). These authors found that a site-directed mutagenesis (for example Glu 45 to Trp 45 or Lys 44 to Glu 44) in the structure of the P-loop region, known IgE epitope of Bet v1, produced a modulation of IgE-binding to the celery major allergen Api g 1.0101 for a subgroup of celery allergic patients with birch pollinosis.

Table 23. Celery allergens

Allergens	Allergenic source	Family	Molecular weight	Population studied	Authors
Api g 1	Celery	hom: Bet v1	17 kDa	1) Api g 1 from celery tuber extract and recombinant Api g 1 bind IgE of all 10 sera of celery allergic patient selected on the base of case history and positive SPT 2) Cross-reactivity with Bet v 1 was proven by cross-inhibition experiments	Breiteneder *et al.,* 1995
Api g 4	Celery	Profilin	15 kDa	1) Recognition by rabbit antiserum raised against the 15 kDa celery of birch recombinant profilin 2) IgE by sera of 2 birch profilin and mugwort allergic patients bind the purified 15 kDa celery allergen	Vallier *et al.*, 1992
Cross-reactive carbo-hydrate determi-nants	Celery	N-glycan containing α1,3-fucose and β1,2-xylose	32-70 kDa	7 celery allergic patients selected on the basis of a positive case history presented IgE against cross-reactive carbohydrate determinants	Fotisch *et al.*, 1999
Api g 5	Celery	no homologous sequences were found in the databases	55-58 kDa	Sera of 5 patients with positive case history, SPT and RAST to birch pollen and celery show IgE-binding to blotted celery extract and isolated celery antigens (58-63 kDa)	Gangleberger *et al.*, 2000

Vallier *et al.* (1992) showed that the allergen profilin is involved in celery allergy. Profilin belongs to a ubiquitous family of actin phosphatidylinositol 4,5-bisphosphate-binding proteins, involved in signal transduction from the outer cell membrane to the inner cell and regulating the actin polymerisation in non-muscle cells. The celery profilin, Api g 4, seems to be particularly important in patients allergic to celery with a birch-mugwort-celery sensitisation (Wüthrich *et al.*, 1990; Bauer *et al.*, 1996; Scheurer *et al.*, 2000).

The cross-reactive carbohydrate determinants, structures containing α1,3-fucose and β1,2-xylose attached to proteins via N-glycoside linkages, are highly immunogenic in mammals; some celery allergic patients exclusively display IgE-binding to these determinants of molecular weight >45 kDa. However, any clinical significance of cross-reactive carbohydrate determinants-specific IgE is still a matter of debate (Fötisch *et al.*, 1999; Aalberse, 1998).

5. CROSS-REACTIVITIES

Three different structures have been found to be responsible for the cross-reactions between pollen and plant foods, namely Bet v 1 and related plant proteins, profilin, and carbohydrate determinants.

The open reading frame of the cDNA of Api g 1 codes for a protein of 153 amino acids with a molecular mass of 16.2 kDa and 40% identity (60% similarity) to the major allergen of birch

pollen, Bet v 1 (Breiteneder *et al.,* 1995). Both Api g 1 and Bet v 1 belong to a class of intracellular PR proteins, as revealed by sequences in the protein databases. These proteins are known to be strongly up-regulated in plants by pathogens or by treatment of cell cultures with microbial elicitors and are present in monocots and dicots from various taxonomically unrelated plant families (Warner *et al.*, 1992). In particular, the deduced amino acid sequence of Api g 1 showed a striking similarity of 79.1% (61.4% identity) to the two parsley PR proteins, PcPR1-1 and PcPR1-3 (Somssich *et al.*, 1988). Celery and parsley belong to the same plant family, the *Apiaceae*.

Celery profilin, Api g 4, has high sequence identity (71-82%) to known allergenic plant profilins. Its IgE cross-reactivity with the minor birch pollen allergen Bet v 2, identified as a profilin by Valenta *et al.* (1992), explains the birch-mugwort-celery syndrome. Other allergenic profilins were also identified and sequenced in pollen from olive tree, Ole e 2; timothy grass, Phl p 11; bermuda grass, Cyn d 12; sunflower, Hel a 2 (Asturias *et al.,* 1997 and 1998a) and various foods such as soybean, Gly m 3; peanut, Ara h 5; pear, Pyr c 4 and cherry, Pru av 4 (Scheurer *et al.,* 2000 and 2001). All these profilins may be responsible for allergenic cross-reactivity between celery and other foods or pollens.

In a publication (Lüttkopf *et al.,* 2000) the allergens recognised by IgE from 22 patients with a positive DBPCFCs to celery were identified and compared with those recognised by patients with a negative challenge test, and the cross-reactivities with pollen allergens were determined. The major allergen Api g 1 (16 kDa) was recognised by 59% of patients; other major allergens were cross-reactive carbohydrate determinants, recognised by IgE of 55% of patients. Celery profilin, Api g 4, was recognised by IgE from 23% of patients. IgE-binding to all 3 structures in celeriac extract was inhibited by birch pollen extract, whereas mugwort pollen extract could only inhibit IgE reactivity to Api g 4 and cross-reactive carbohydrate determinants. In the same study, cross-inhibitions with extracts of birch pollen, mugwort pollen, timothy grass pollen, and lychee, demonstrated the ubiquitous presence of cross-reactive carbohydrate determinants and profilin, while Api g 1 was only cross-reactive with birch pollen. No Api g 1 homologue was found in mugwort pollen, grass pollen, lychee fruit and cooked celeriac, as shown by the lack of inhibition with these extracts. Homologues of Api g 4 and cross-reactive carbohydrate determinants were also present in tree pollen and pollens from weeds, *Graminaceae* and other plant families.

5.1 Clinical cross-reactivity

Sensitisation to celery is frequently associated with birch and/or mugwort pollinosis, hence the term “birch-mugwort-celery-syndrome”. There is evidence that birch pollen and celery allergy are highly related in Central Europe, while celery allergy is most frequently related to mugwort pollen allergy in Southern Europe. Moreover, allergies to carrot and spices, predominantly of the umbelliferous family, are strongly associated to celery allergy, known as “celery-carrot-mugwort-spice syndrome”. This syndrome is frequently described in the German literature (Wüthrich and Dietschi, 1985).

Ballmer-Weber *et al.* (2000) have found that all patients with positive DBPCFC for celery were sensitised to either birch (91%) or mugwort (64%) pollen. The authors found that 2 out of 22 patients did not shown any sensitisation to birch pollen; also in a previous study performed in Swiss patients, 8% of celery allergic subjects were not sensitised to rBet v 1 or rBet v 2 (Wüthrich and Straumann, 1997). These observations are in contrast with those by

Hoffmann-Sommergruber *et al.* (1999) who reported that patients with a history of celery allergy in Central Europe in general are allergic to birch pollen.

Wüthrich *et al.* (1990) hypothesised that the association between celery and birch is due to a common thermolabile allergen while the common allergen between celery and mugwort is thermostable. A confirmation of this assumption is the fact that RAST with cooked celery extract results negative in patients with birch allergic rhinitis, while it remains positive in those with mugwort allergic rhinitis.

The only studies carried out in patients with celery allergy confirmed by positive DBPCFC pointed out a sensitisation to carrot in 77% of patients with CAP >0.7 kU/L (Ballmer-Weber *et al.*, 2000), and the coexistence of symptoms to other birch-related foods, like apple, hazelnut and potatoes (Lüttkopf *et al.*, 2000).

A study by Ortolani *et al.* (1988) reported a statistically significant association between celery and fennel allergy in patients with oral allergy syndrome to vegetable foods.

6. POSSIBLE EFFECTS OF FOOD PROCESSING ON ALLERGENICITY AND DERIVED PRODUCTS

Jankiewicz *et al.* (1997) investigated the immunochemical stability of celery allergens after various treatments such as microwaving, drying, gamma-irradiation, ultra high pressure and high voltage impulse, and demonstrated that the cross-reactive carbohydrate determinants are the most heat-stable allergens, followed by profilin and Api g 1.

An interesting finding is that the risk of clinical reactivity to cooked celery cannot be deduced from the sensitisation pattern to individual celery allergens. Even though the majority of patients reacting to cooked celery recognise the heat-stable allergens Api g 4 and cross-reactive carbohydrate determinants, a minority of them are exclusively sensitised to Api g 1, which is more labile. The Api g 1 sensitised patients react to higher doses of cooked celery compared to the other patients.

EAST inhibition data from Ballmer-Weber *et al.* (2002) are in accordance with the study of Jankiewicz *et al.* (1997), showing that Api g1 is the most labile allergen in celery, that celery profilin Api g 4 is more stable under thermal processing, and that the IgE reactivity of cross-reactive carbohydrate determinants is not affected by heating. In this study 12 patients were selected with history of allergic reactions to raw or raw and cooked celery. Eleven patients underwent DBPCFC with cooked celery, of which 8 had a positive history of allergic reaction after consumption of cooked celery, and 3 had a negative history to the cooked vegetable but reported adverse reactions to raw celery. In 6 out of 8 patients with a positive case history the cooked celery allergy was confirmed by DBPCFC procedure: 5 of these complained of oral allergy symptoms; one had flush, cough and dyspnea. Five patients of 11 were non-responders to the drink containing cooked celery. Heat treatment of celery did not lead to unequivocal reduction of allergenicity.

There were no patients complaining of allergy to cooked celery without symptoms to the raw vegetable. This may indicate that no neoallergens are created by the heating process, and that residual activity of the native celery allergens is responsible for the allergenic activity of the cooked vegetable (Ballmer-Weber *et al.* 2002).

In 5 of the recruited 12 patients DBPCFC was performed with celery spice, dried and pulverised celery: all the 5 patients reacted to the active drink and were regarded to be responders and presented reactions comparable to symptoms observed during DBPCFC with raw extract (2 oral allergy syndrome, 3 more severe symptoms). It is interesting to note that these symptoms occurred at a lower provocation dose after ingestion of celery spice compared to the raw celery, probably because of a high protein content of celery spice (about 4.5 times higher than raw celery) (Ballmer-Weber *et al.* 2002).

The authors concluded that: 1) for some subjects celery allergic reactions to cooked celery will take place even when high temperatures are used; and 2) celery spice is allergenic for those with an allergy to raw celery (Ballmer-Weber *et al.* 2002).

7. THRESHOLD DOSES

Ballmer-Weber *et al.* (2000) described DBPCFCs for 32 patients with the history of an allergic reaction to celery. Twenty-two patients produced symptoms to the active preparation: 11/22 complained about symptoms strictly localised to the oral cavity (oral allergy syndrome). The mean provocation dose to elicit oral allergy syndrome was 1.3±1.4 g of celery (minimum 0.7 g). Eleven patients showed systemic reactions. The mean provocation dose to elicit a systemic reaction was 19.3±11.8 g. The 8 non-responders underwent an open challenge with celery, and 4 of them had oral allergy syndrome with 5 g of celery.

The same authors confirmed allergy to cooked celery in six out of 8 patients with a positive case history (Ballmer-Weber *et al.*, 2002). Five of these complained about oral allergy syndrome during the local mucosal challenge ("spit" phase) at the mean provocation dose of 1.26±0.44 g of cooked celery. In one patient symptoms were systemic and occurred at the provocation dose of 34.5 g of cooked celery. DBPCFC with celery spice has been performed in 5 of the recruited patients: all of them were responders. Two had oral allergy syndrome at the provocation dose of 1.6 g of celery spice, three had systemic symptoms during the local mucosal challenge at 0.16 g, 0.32 g and 5.85 g of celery spice

8. CONCLUSION

Celery tuber is an important source of food allergens in Central Europe. The majority of the celery allergic patients have reactions to raw celery and less frequently to cooked celery. Celery root could be found as food ingredient in several prepacked food as it is widely used in the food industry because of its aromatic flavour.

There are no sufficient data to determine a safe threshold dose. Only two studies reported the threshold doses eliciting local symptoms (0.7-2.7 g) or systemic symptoms (7.5-31 g), which were similar for crude and cooked celery. Celery spice is bioactive and weight/weight basis can trigger systemic symptoms also at low doses (0.16-5.85 g).

XVIII. ALLERGY TO MUSTARD

SUMMARY

Mustard is made from the seeds of one or more species of mustard plants. It is in common use all over Europe, but the specific preparation of mustards as well as the amount consumed appears to vary from region to region. Mustard is used with a variety of foods, and often is present in various foods and sauces as food ingredient.

Allergic reactions to mustard, including severe anaphylactic reactions, are well documented in clinical and laboratory studies. Mustard allergy is also encountered in small children.

Information on the prevalence of mustard allergy and incidence of severe reactions caused by mustard comes from patient series. The larger patient studies suggest that mustard allergy may account for 1-7% of food allergies in the regions they were done. In two studies of patients with anaphylactic reactions, mustard was the fifth and sixth most common food causing anaphylaxis. While some studies claim that mustard is among the most common food allergens in certain European regions, reliable data on mustard allergy are missing for most European countries.

The major allergens of mustard have been isolated and characterised. The two major allergens described are very similar and belong to the 2S albumin storage proteins, like other seed and nut allergens. Mustard allergens are heat-resistant and resistant to enzymatic degradation, and therefore are not markedly affected by food processing.

Clinical cross-reactivity with other foods appears not to be a problem.

Provoking doses of allergen in mustard allergic patients are in the high microgram range as determined in DBPCFC studies. Allergic reactions in several case reports appeared to be triggered by small amounts of mustard, like contaminated cooking utensils.

1. BACKGROUND

The mustard plant belongs to the *Cruciferae* (*Brassicaceae)* family. Mustard used in food is often a mixture of seeds from two or more species of *Brassicaceae*, like *Sinapis alba* L. (yellow mustard), *Brassica nigra* (black mustard), and *Brassica juncea* L. (oriental mustard). Flour from the yellow species (*Sinapis alba*) is used most commonly in Europe, while oriental mustard (*Brassica juncea*) is used most commonly in the United States and Japan.

Mustard consumption in different countries varies according to local food habits. According to Rancé (2000), France has the largest consumption of mustard in Europe, followed by Germany and Great Britain, but the basis for this ranking is not given.

Mustard is used on some meat dishes, like hot-dogs and hamburgers, but is very often an added ingredient in sauces, salads and other foods. For example, mayonnaise as well as ketchup may contain mustard. Mustard also is used in various traditional remedies, among other things to stimulate appetite, and as a laxative, expectorant and antiseptic agent for the treatment of various gastrointestinal, respiratory and skin diseases (Rancé, 2000).

A first case of anaphylaxis supposedly caused by mustard was described in 1980. Later, there have been a number of reports on cases and patient series from France and Spain, and also some from Sweden, Finland and Italy. However, a large proportion of the documentation on mustard allergy still comes from one European country. Two DBPCFC studies documenting mustard allergy and anaphylactic reactions to mustard have been published (Rancé and Dutau 2002; Morisset *et al.,* 2003b). Mustard allergy is a problem not only in adults, but is also found in small children, and several of the patient series are paediatric studies. One group of authors (Rancé *et al.*, 2000) link the early occurrence of mustard allergy to early consumption in baby foods. Several authors claim that mustard allergy is becoming more frequent in France, but the documentation for this is limited (Rancé *et al.*, 2000; André *et al.* 1994).

2. FREQUENCY

2.1 Frequency data

In an often cited study from Finland (Niinimäki and Hannuksela, 1981), a series of 1120 atopic and 380 non-atopic patients were skin tested (scratch method) for allergy to spices. Of the 1038 atopics tested with curry (mean age 22.4 years, range 1-80), 181 (17.4%) were positive, whereas of 335 non-atopics only 1 (0.3%) was positive. Mustard is a component of curry, and when 71 of the curry-positive persons were skin tested (scratch method) with curry components, 23 (32.4%) (2.2% of all tested atopics) had positive reactions to mustard, number four in frequency after other curry ingredients coriander (59.2%), caraway (54.9%), and cayenne (45.1%). However, of all the patients, only five reported clinical symptoms when eating seasoned food. Among these were two children reporting erythema and pruritus in the perioral region and the sensation of a lump in the larynx when eating mustard. Similar symptoms were reported by some of 35 non-selected patients in the same study after oral mucosal challenge with mustard (throat symptoms 3 cases, lip pruritus and/or oedema 2 cases, face erythema/itching 1 case, oral cavity pruritus 1 case).

In France, André *et al.* (1994) studied 580 patients (480 adults, 100 children) with a "pathological reaction" to food, 60 of which presented severe, near-fatal reactions. The authors found, based on specific IgE, mustard to be responsible for 11% of food allergy reactions and 3% of the anaphylactic reactions.

André *et al.* (1994) found mustard allergy to be increasing in France in comparison to previous studies. Moneret-Vautrin and André (1983) had found sensitisation to mustard in 1% of patients, while the numbers (André *et al.*, 1994) for 1984-1986 (n=149) were 5%, for 1987-1989 (n=192) 14%, and for 1990-1992 (n= 239) 11%.

Moneret-Vautrin (2001) reports on 707 children under 15 years with food allergies. Allergy to mustard was registered in 1.1% of the allergic children. In the same study, the frequency of allergy to mustard in 196 adults was 0.84%.

Moneret-Vautrin and Kanny (1995b) did a multicentre survey of food-induced anaphylactic shocks in France. Of 81 reported cases, 2 were identified as being caused by mustard.

Rancé *et al.* (1999b) studied 378 children with challenge-proven (labial or single blind placebo controlled) food hypersensitivity, and found that five allergens accounted for 82% of

confirmed food hypersensitivity in this patient study: egg (51.8% of confirmed hypersensitivity), peanut (34.3%), milk (11.6%), mustard (8.9%), and codfish (7.1%).

In another report, of 544 children with positive skin prick test and/or specific IgE and positive food challenge (labial or single blind), 49 were positive to mustard which accounted for 6% of the reactions. One child had experienced an anaphylactic reaction to mustard, with generalised symptoms. Among the five predominant allergens, mustard in this paediatric study came in fifth as a cause of anaphylaxis, after peanut (9 cases), egg (8 cases), milk (5 cases), cod (2 cases), and mustard (1 case) (Rancé *et al.*, 1999a).

In another study from the same group, mustard skin prick tests were systematically performed on atopic children attending a hospital as outpatients with a clinical history suggesting food allergy (Rancé *et al.*, 2000). A total of 3600 mustard skin prick tests were performed, 15 out of 36 skin prick test positive patients reacted to mustard in single-blind placebo-controlled food challenge.

Leanizbarrutia *et al.* (1988b) in Spain found 18.2% to be skin test positive to mustard in a patient population (n= 269) who visited their allergy clinic for other reasons. Among a subpopulation of pollen skin test positive patients from the same study, 54.2% were skin test positive to mustard, while a mite sensitive subpopulation did not differ from the patient population as a whole (19.4% *vs* 18.2%). Of the 49 mustard skin test sensitive patients, only 4 (8%) complained of symptoms associated with mustard ingestion.

Castillo *et al.* (1996) found that mustard allergy constituted 7% of food allergy consultations (8 of 120) in the Canary Islands (Spain). Diagnosis was based on an unequivocal history, together with positive skin prick test or serum specific IgE (CAP-System), and absence of symptoms after mustard elimination from the diet.

Rancé and Dutau (2002) performed a DBPCFC study of 163 children with asthma and symptoms of food allergy (age 2-9 years, mean 5.5 years). Of 385 tests performed, 250 (65%) were positive. Mustard accounted for 6.9% of the allergic reactions.

Morisset *et al.* (France) (2003b) performed DBPCFC with mustard on 24 skin test positive patients and single-blind placebo-controlled food challenge on additional 6 skin test positive patients (age 3-20 years). Seven of 30 food challenges were considered positive, indicating that 23.3% of the skin test positive patients had clinical allergy to mustard. Skin tests were performed with four different allergen preparations, and specific serum IgE to mustard was determined. Skin test reactivity or serum specific IgE levels were not predictive for reaction to challenge in this study. Patients were selected only for skin test positivity and not for symptoms, but were recruited from a hospital clinic. Patients studied may thereby have been selected for relatively severe, symptomatic cases and it seems inappropriate to combine patients into one study with different diagnostic criteria.

The presence of mustard allergy in small children may be taken as an indication of primary sensitisation to mustard in at least some food allergies. Also, the relative absence of cross-allergies with other foods (see below) supports the notion that most clinical mustard allergies are due to primary mustard sensitisation. However, reported cross-reactivities with pollens and with other members of the *Cruciferae* (*Brassicaceae*) family may influence the prevalence of specific IgE and skin test positivity and lead to an overestimation of the

prevalence of sensitisation to mustard, and may possibly also influence the occurrence of oral allergy syndrome-like symptoms elicited by mustard.

Another factor contributing to a possible overestimation of mustard sensitisation and allergy, as determined by skin prick testing or labial provocation challenge, is the presence of irritating substances in mustard that may cause false positive allergy-like reactions.

Data on the prevalence of mustard sensitisation, clinical mustard allergy and the incidence of severe allergic reactions to mustard in the general population are missing, so that we have to rely on fairly rough estimates and extrapolations from patient populations.

3. CLINICAL FEATURES

3.1 General clinical manifestations

Generally, the pattern of allergic and general symptoms after ingestion of mustard appears to be similar to the symptoms due to other foods. Clinical symptoms reported from single-blind placebo-controlled food challenge studies, DBPCFC studies and case histories include the full spectrum from anaphylactic shock to oral allergy syndrome to subjective symptoms: burning sensation, swelling of lips and tongue, facial oedema, laryngeal oedema, dysphonia, difficulty in breathing and swallowing, asthma, nausea, generalised urticaria, rhino-conjunctivitis, and atopic dermatitis (Monreal *et al.,* 1992; Rancé *et al.*, 1999a and 2000; Rancé and Dutau, 2002; Caballero *et al.*, 2002 and 1997).

3.2 Natural history of mustard allergy - early sensitisation

In the study of Rancé *et al.* (2000 and 2001) 8 of the 15 mustard food allergic children were under 3 years of age. Mustard is, according to the authors, "a probable hidden allergen in the usual infant food diet". Sensitisation *in utero* and through mother's milk may be a possibility (Niinimäki *et al.*, 1989). The latter authors reported positive skin prick test in 3 infants aged 12-18 months, breastfed until the age of 11 months, who had never consumed mustard. This may represent a possible parallel to the early sensitisation to peanut and sesame seed. Niinimäki and Hannuksela (1981) tested for allergy to spices in a series of 1120 atopic and 380 non-atopic patients. Mustard was tested separately in patients with a positive skin test reaction to curry. The mustard prick test was positive in 23 of 71 subjects tested (32.4% of cases), and was accompanied by clinical signs in two cases. These were two paediatric observations of erythema and pruritus in the perioral region and the sensation of a lump in the larynx after eating mustard. Guillet and Guillet (2000) report that the prevalence of mustard allergy increases in children with age.

Little is known about the natural history of mustard allergy.

3.3 Case reports

A number of case reports contain informative data with regard to symptoms, offending foods, etc. These are listed in Table 24.

Table 24. Case reports of mustard allergy

Patient	Symptom	Food	Reference
Young male	Anaphylaxis, shock during intradermal test	Pizza margherita	Panconesi *et al.*, 1980
Female age 40	Atopic eczema, acutely vesicular	Mustard	Meding, 1985
Female age 25	Urticaria, angioneurotic oedema	Mustard, mayonnaise	Widström and Johansson, 1986
Female age 24	Anaphylaxis, acute pancreatitis	Hamburger with mustard	Carrillo *et al.*, 1987
Female age 32	Allergic contact dermatitis (Type IV)	Mustard in salad	Dannaker and White, 1987
Three fish-stick workers	Contact urticaria	Mustard in flour-flavour mixture	Kavli and Moseng, 1987
Female age 59	Anaphylactic shock	Fork stained with mustard	Leanizbarrutia *et al.*, 1988b
Male age 50	Anaphylactic shock	Sandwich with mustard sauce	Leanizbarrutia *et al.*, 1988a
Females age 47 and 15	Anaphylaxis	Meal with mustard	Vidal *et al.*, 1991
Boy age 11 Girl age 14	Anaphylaxis	Mustard sauce	Monreal *et al.*, 1992
Male age 31 Male age 32	Angio-edema, urticaria, dyspnoea, sneezing etc	Mustard sauce or seeds	Malet *et al.*, 1993
Female age 38	Anaphylaxis	Chicken dips with mustard	Kanny *et al.*, 1995
Male age 43 Male age 19 Female age 17	Severe systemic reactions	Mustard/mustard sauce	Jorro *et al.*, 1995
Three children	Allergic urticaria/angio-edema	Relishes with mustard, paprika, cumin, and anise.	Eseverri *et al.*, 1999
Female age 52	Urticaria, lip and tongue oedema, dyspnoea	Fillet of anchovy in sauce containing mustard	Valsecchi *et al.*, 2000

4. IDENTIFIED ALLERGENS

Table mustard usually is a blend of flour obtained from the two different species yellow mustard (*Sinapis alba* L.) and oriental mustard (*Brassica juncea* L.). The relative amounts of the two types vary. Other mustard species may sometimes be added, and table mustard contains also other components.

Mustard contains a number of irritating substances such as isothyocyanates in *B. nigra*, sinalbin in *S. alba*, and capsaicin. Capsaicin releases substance P, which may cause non-IgE-mediated mast cell degranulation (Rancé *et al.*, 2000). Thus, mustard contains substances that may trigger non-immune reactions mimicking allergic reactions, causing false positive skin test reactions and making the interpretation of labial provocation tests with mustard uncertain (Rancé, 2000).

The major allergens of yellow mustard, Sin a 1, and of oriental mustard, Bra j 1, have been characterised and were found to be closely related in structure (see below). Sin a 1 was the first food allergen to be cloned and expressed by molecular biology techniques (González de la Peña *et al.*, 1993 and 1996). Other mustard allergens remain to be characterised. There are no data indicating a difference in allergenicity of the different mustard species.

Mustard allergens described until now are seed storage proteins (Table 25). Seed storage proteins generally constitute more than 10% of the total protein isolated from seed. Mustard allergens Sin a 1 and Bra j 1 belong to the 2S albumin storage proteins. Several important allergens from various seeds and nuts are 2S albumins.

Sin a 1 from *Sinapis alba* is a 14 kDa-protein, whereas the Bra j 1 major allergen is a 16-16.4 kDa protein (Menéndez-Arias *et al.*, 1987 and 1988; González de la Peña *et al.*, 1991). Sin a 1 has been reported to have close similarity to other 2S albumins, such as those isolated from rapeseed, castor bean and Brazil nut. Sin a 1 is composed of two disulphide-bonded polypeptide chains of 39 and 88 amino acids (Menéndez-Arias *et al.*, 1988), derived by proteolytic cleavage from a single precursor. Sin a 1 has been cloned and expressed by molecular biology techniques (González de la Peña *et al.*, 1993 and 1996). Recombinant Sin a 1 was recognised by rabbit polyclonal sera and mouse monoclonal antibodies, as well as by IgE in sera from mustard sensitive individuals.

González de la Peña *et al.* (1991) isolated and characterised the mustard allergen Bra j 1. Sera from mustard sensitive individuals (10 sera) were found to contain specific IgE for both Sin a *1* and the 2S fraction of *Brassica juncea*. Further, six monoclonal antibodies and a rabbit polyclonal serum specific for Sin a 1 recognised the 2S fraction of *Brassica juncea*. The 2S fraction of *Brassica juncea* exhibited an inhibiting capacity similar to Sin a 1 itself in competition assays. The amino acid composition of Sin a 1 and Bra j 1 showed great similarity but with differences in some amino acids. The authors conclude that Bra j 1and Sin a 1 are very closely related allergens. Bra j 1 was been found to be closely related to Sin a 1 in structure also by Monsalve *et al.* (1993).

Table 25. Identified mustard allergens

Allergen	Source of allergen	Family	Molecular weight	Population/source of antibodies	Authors
Sin a 1	*Sinapis alba* (yellow mustard)	2S albumin (seed storage protein)	14 kDa	Sera from 8 mustard-allergic individuals	Menéndez-Arias *et al.*, 1987 and 1988; González de la Peña *et al.*, 1993 and 1996
Bra j 1	*Brassica juncea* (oriental mustard)	2S albumin (seed storage protein)	16 kDa	Sera from 10 individuals, high levels of histamine release and binding to crude mustard extract and purified allergen Sin a 1	González de la Peña *et al.*, 1991; Monsalve *et al.*, 1993

5. CROSS-REACTIVITIES

The *Cruciferae (Brassicaceae)* family includes a number of common vegetables, like cabbage, cauliflower, Chinese cabbage, Brussels sprouts, broccoli, turnip, rutabaga and radishes, and fodder crops like rape (Monreal *et al.*, 1992). Palomares *et al.* (2002) made

recombinant rapeseed 2S pronapin precursor protein, and found that it bound IgE in sera from mustard (Sin a 1) allergic patients, as well as a Sin a 1-specific polyclonal rabbit antiserum and IgE in serum from a rapeseed allergic patient. This indicates a potential cross-reactivity between mustard and rapeseed, which has been reported by Meding (1985), Widström and Johansson (1986), Caballero *et al.* (1994), and Ortolani *et al.* (1993). The serological cross-reactivity needs to be borne in mind in case of future introduction of rapeseed protein into foods.

However, cross-sensitivity between *Brassicaceae* species (except among mustards) seems to be rare (Ortolani *et al.*, 1998; Rancé *et al.*, 2000). Blaiss *et al.* (1987) described anaphylactic shock after ingestion of cabbage in a multi-sensitive patient. Skin tests were positive for mustard, cabbage, broccoli and cauliflower, but the paper gives no information about clinical history with regard to mustard intolerance, and no challenge with mustard was performed. Caballero *et al.* (2002) studied 29 patients with symptoms suggestive of mustard allergy. Only one patient had mild symptoms after ingestion of cabbage and had a positive reaction to skin prick testing with cauliflower and cabbage. Some patients had positive reactions to cauliflower and cabbage but tolerated them in their diet. Rancé *et al.* (2001) in a study of 36 mustard sensitive children noted a single case of sensitisation to radishes, without clinical expression. Leanizbarrutia *et al.* (1988a) reported negative skin test results to cauliflower, Brussel sprouts, collard and turnip in two patients with probable anaphylactic reactions and skin test positivity to mustard.

No clear-cut report of clinical cross-reactivity between mustard and foods other than *Brassicaceae* species has been found. Asero *et al.* (2002) report on a male patient that had experienced an anaphylactic reaction presumably due to sunflower seeds in bread. Pre-incubation of the patient's serum with mustard extract completely removed specific IgE against the lower range of sunflower allergens (including 2S albumins). The authors recommend that sunflower seed allergic patients should always be tested also with mustard. A Brazil nut allergy case reported by Bartolomé *et al.* (1997) showed serological cross-reactivity to mustard. Some allergen similarity of mustard, rapeseed, sunflower and Brazil nut has been described (major allergens are 2S storage proteins). In the patients studied by Caballero *et al.* (2002) (29 patients with symptoms suggestive of mustard allergy), more than 50% of the patients had symptoms suggestive of allergy to other vegetable foods, and only two patients had sensitivity exclusively to mustard. Castillo *et al.* (1996) found a significant association between allergies to nuts and spices.

A relationship between mustard allergy and pollen allergy has been found by some investigators. In the patients studied by Caballero *et al.* (2002), mugwort sensitisation was significantly more frequent in the mustard allergic patients than in a population of patients with pollinosis ($p<0.0001$). Hypersensitivity to nuts has been shown to be associated with mugwort pollen sensitisation (Caballero *et al.*, 1994) and cross-reactivity between hazelnut and mugwort pollen has been described with immunochemical techniques (Caballero *et al.*, 1997). Previously the same authors had reported a significant association between mustard sensitisation and *Compositae* pollen sensitivity in children (Caballero *et al.*, 1994). Niinimäki and Hannuksela (1981) noted a statistically significant correlation between positive skin test results to mustard and to flower pollens (correlation value 0.38, $p<0.01$). The same authors in a later paper (Niinimäki *et al.*, 1989) reported evidence for cross-allergy between birch pollen and spices. Leanizbarrutia *et al.* (1988b) in a subpopulation of grass pollen skin test positive patients from a patient population of 269 allergics, found that 54.2% were skin test positive to mustard, while a mite sensitive subpopulation did not differ from the patient population as a

whole (19.4% *vs* 18.2%). This may suggest cross-reactivity between grass pollen and mustard seeds. However, convincing immunological and clinical cross-reactivities between seed proteins and pollen protein, and structure homology have not been reported.

Laboratory tests (REIA) were not able to show cross-reactivity between mustard and caraway, coriander, pepper and paprika, while a high concentration of celery inhibited some patients' sera (Domínguez *et al.*, 1990).

6. POSSIBLE EFFECTS OF FOOD PROCESSING ON ALLERGENICITY AND DERIVED PRODUCTS

Recombinant Sin a 1 was found to be highly resistant to trypsin digestion (like the native mustard allergen) (González de la Peña *et al.*, 1996). Native mustard allergen has been found to show high heat, acid and alkali treatment stability, together with high resistance to proteolytic degradation by trypsin, chymotrypsin, and pepsin (Domínguez *et al.*, 1990). Pronounced resistance of mustard allergens to high temperature has also been reported by others (Leanizbarrutia *et al.*, 1988 a and b). Thus, the major mustard allergens do not appear to loose significant activity during food processing.

The lipid matrix of oil may possibly increase the allergenicity of mustard allergens (Onaderra *et al.*, 1994).

7. THRESHOLD DOSES

Leanizbarrutia *et al.* (1988a) describe anaphylactic shock in a patient who had sucked on a fork stained with a small amount of mustard. Panconesi *et al.* (1980) and Widström and Johansson (1986) reported that the amounts of mustard ingested were very small in the cases with anaphylactic reactions that they observed, and two of the five cases probably arose from accidental contamination of food by cooking utensils.

In single-blind placebo-controlled food challenge with mustard powder, Rancé *et al.* (2000) tested 36 skin prick test positive patients. Fifteen patients were positive. The authors gave progressive doses of mustard: 1, 5, 10, 20, 50, 100, 250, and 500 mg. They found that the cumulative reactive dose varied from 1 mg (lowest dose tested) to 936 mg (highest dose given). The mean cumulative reactive dose was 153 mg of mustard.

Taylor *et al.* (2002) report from a round-table conference, where Rancé reported a lowest provoking dose determined by DBPCFC of 1 mg ground mustard seed, corresponding to 0.3 mg of protein.

Morisset *et al.* (2003b) performed DBPCFC with mustard seasoning on 24 skin test positive patients. Six additional patients were given single-blind placebo-controlled food challenge. Increasing doses of 10, 30, 100, 300 and 900 mg were given every 20 minutes, to a total cumulative dose of 1340 mg. Seven patients tested positive. The lowest dose that triggered a reaction in these patients was 40 mg (one patient), according to the authors corresponding to 13.5 mg of mustard seeds, roughly equivalent to 0.8 mg of proteins.

8. CONCLUSION

Mustard is commonly used all over Europe. Mustard allergy and anaphylactic reactions to mustard have been documented by DBPCFC studies. The major allergens have been well characterised. Frequency estimates for mustard allergy are mainly based on patient series. It appears that in France, food allergy to mustard is among the more common food allergies, accounting for about 1-7% of food allergy. Apart from Spain, the documentation on mustard allergy from other countries is scarce. The major allergens of mustard are heat-resistant and are not much affected by food processing. Protein doses provoking reactions in mustard allergic patients can be in the high microgram range, although threshold doses have not been established. No specific detection method for mustard allergens has been described.

XIX. ALLERGY TO SESAME

SUMMARY

Sesame seeds represent a potent food allergen. Over the past few years, the number and severity of reactions to dietary sesame has increased, probably because of a growing use of sesame seeds and sesame oil in foods. Sesame allergy is common in Eastern countries like Israel, where it is the third most common cause of IgE-mediated food allergy, and is becoming frequent also in European countries. Its prevalence ranges from 0.7% to 1.2%. Allergy to sesame seeds starts early in childhood, due to the use of sesame-containing foods in the diet of infants as a source of protein and iron. Sesame allergens are often associated with particularly severe reactions with a high risk of anaphylaxis. Sesame has also been described as an occupational sensitiser for bakers and other exposed workers.

Sesame allergens were only recently identified. Ses i 1, a sulphur-poor 2S albumin with about 40% homology to allergens of sunflower seeds, Brazil nut and castor bean was the first identified allergen in sesame seeds. Recently, 4 other allergens have been identified: Ses i 2, a sulphur-rich 2S albumin; Ses i 3, a 7S vicillin-type globulin; a 34 kDa allergen, homologous to seed maturation protein and a 78 kDa allergen, homologous to embryonic abundant protein.

Oral challenge-based studies revealed that both sesame seeds and sesame oil can cause allergic reactions in sensitised patients, with threshold doses ranging from 30 mg to 10 g of sesame seed, and few millilitres (1-5 mL) of sesame oil. The interaction between sesame allergens and the lipid matrix in sesame oil may increase allergenicity and may cause reactions to few milligrams of sesame proteins.

1. BACKGROUND

Sesamum indicum is a plant originally from tropical Africa, which is now universally cultivated for its seeds. It is the most important species in the *Sesamum* genus of the *Pedialaceae* family; the annual worldwide production is around 2 million tons. The seeds are used in several food products, especially in bakery products, fast-foods, “health foods”, vegetarian and ethnic dishes; the oil obtained from the seeds is used for cooking and salad dressing in Oriental, Chinese and South American cuisines, and is also employed by the pharmaceutical industry as a vehicle of medications for intramuscular injection.

Allergy to sesame seeds has been increasingly reported in recent years, maybe because of their increasing consumption. In some countries sesame is one of the major causes of food allergy: in Israel, where sesame seed-based foods (halva and tehina) are included in the diet of infants and young children as a source of proteins and iron, sesame is the third common cause of IgE-mediated food allergy and the second most common cause of anaphylaxis (Dalal *et al.* 2002 and 2003).

2. FREQUENCY

2.1 Prevalence

Until recently, sesame allergy was considered rare in Western countries.

In recent years, reports of allergic reactions to sesame seeds or sesame oil have become more frequent, as noted by Kanny *et al.* (1996), who found 24 cases reported in literature from 1950 to 1996, and observed three cases from 1990 to 1994, as against six cases in 1995. Swiss authors reported five sesame allergic patients (1.2%) among 402 food allergic patients seen between 1978 and 1991 (Kägi and Wüthrich, 1991).

Data from Australia and Asian countries also show a significant increase in the frequency of sesame-induced allergic reactions (Sporik and Hill, 1996; Hill *et al.*, 1999).

3. CLINICAL FEATURES

An anaphylactic reaction to sesame seed was first reported in 1950 in a patient who had ingested a Turkish honey-based confectionery (halva), which contains sesame seeds and sesame oil (Rubinstein, 1950). Since then, there have been many reports of anaphylactic shock (Kägi, 1991; Asero *et al.*, 1999, James *et al.*, 1991) or generalised skin rash (Eberlein-Konig *et al.*, 1995; Blamoutier and Denimal, 1992). Kanny *et al.* (1996) described nine patients who experienced severe systemic reactions or anaphylactic reactions after ingestion of sesame-containing foods. They reacted to labial provocation tests and to double-blind or single-blind placebo-controlled food challenges with sesame flour or oil given in small quantities (7-10 g).

Many other cases of severe allergic reactions to sesame have been reported, especially in populations at particular risk. Pajno *et al.* (2000) described the case of a coeliac patient with systemic reactions after sesame ingestion, confirmed by DBPCFC; Levy and Danon (2001) reported 10 cases of sesame allergic children with atopic dermatitis, who experienced reactions after ingestion of sesame-containing foods (halva and tehina).

Occupational hypersensitivity can occur in bakers after prolonged exposure to sesame seeds, causing asthma, rhinitis and contact urticaria (Keskinen *et al.*, 1991). There have also been reports of reactions to sesame oil, which is used in cooking, and in cosmetics (Pecquet *et al.*, 1998) and pharmaceutical products such as injectable neuroleptics and vitamin D (Chiu and Haydik, 1991; Birnbaum *et al.*, 1997).

4. IDENTIFIED ALLERGENS

Sesame seeds contain about 50% oil and 20% proteins. The major protein of sesame, alpha-globulin, is the insoluble 11S globulin, which constitutes 60-70% of the seed protein, while the major soluble protein fraction is represented by the 2S albumins, which account for 25% of the total protein content. The 7S vicillin, a less abundant protein in sesame, constitutes, together with the 11S protein, the Osborne globulin fraction (Tai *et al.*, 1999).

Two studies compared the allergenicity of different varieties of sesame seeds (white, brown and black sesame). The first by Kolopp-Sarda *et al.* (1997) used sera from 10 sesame sensitised individuals and found that six of them recognised a 25 kDa allergen of brown sesame; other allergens of 30 and 14 kDa were recognised by a minority of patients. The second by Fremont *et al.* (2002) used sera from 6 sesame sensitised patients, with or without clinical reactivity, for an immunoblotting experiment with white sesame. The allergenic

pattern was similar for symptomatic and asymptomatic subjects; ten allergens were revealed, with more intense IgE-binding to those around 12-13 and 22-23.5 kDa. None of these allergens were characterised.

The first allergen identified and sequenced in sesame seeds by Pastorello *et al.* (2001) was a 2S albumin with about 40% homology to allergens of sunflower seeds, Brazil nut and castor bean (Table 26). This seed storage protein was recognised as a major allergen by ten patients with severe systemic reactions to sesame seeds (urticaria/angio-edema, laryngeal oedema, gastrointestinal symptoms, asthma, hypotension or anaphylactic shock). All ten patients showed high levels of sesame specific IgE and highly positive skin prick tests with both fresh seeds and commercial extracts. An Israelian study evaluating 24 subjects with symptoms and specific IgE to sesame, confirmed the previous findings: 22 out of 24 patients recognised the 14 kDa 2S albumin precursor, which was the only major allergen identified (Wolff *et al.*, 2003). The reacting epitope was found on the peptide corresponding to the residues 24-94. Some minor sesame allergens, of higher molecular weight, were also revealed.

Table 26. Sesame seed allergens

Allergen	Family	Molecular weight	Population studied	Authors
Ses i 1	2S albumin (sulphur-poor)	10 kDa	10 patients (7 boys, aged 4-11 years, and 3 women, aged 23-36 years) with severe anaphylactic reactions, positive SPT and specific IgE to sesame seeds	Pastorello *et al.*, 2001
Ses i 2	2S albumin (sulphur-rich)	7 kDa	20 patients (1 adult and 19 children aged 2-15) with systemic reactions and positive IgE to sesame seeds	Beyer *et al.*, 2002
Ses i 3	7S vicillin-type globulin	45 kDa		
Not assigned	Seed maturation protein	34 kDa		
Not assigned	Embryonic abundant protein	78 kDa		

A study by Beyer *et al.* (2002) confirmed the allergenicity of sesame 2S albumins. Using sera from 20 sesame allergic individuals, they identified another allergenic 2S albumin, which is sulphur-rich, and 47% homologous and 35% identical to that identified by Pastorello *et al.* (2001) which is sulphur-poor. In the Beyer's study the sulphur-rich protein is a major allergen while the sulphur-poor behaved like a minor one. In the same study, the authors demonstrated that a 7S vicillin-like globulin is a major allergen of sesame, recognised by 75% of patients; this protein showed 41% homology to the walnut allergen Jug r 2 and 36% homology to the peanut allergen Ara h 1. Moreover, it seemed to share an IgE-binding site with Ara h 1, as one of the 22 known IgE-binding epitopes of Ara h 1 showed 80% homology with the corresponding area of the sesame vicillin. Lastly, the authors identified two high-molecular weight allergens homologous to a seed maturation protein and to an embryonic abundant protein of soybean; neither protein was previously described as a food allergen in other plants.

5. CROSS-REACTIVITIES

Few data are available on the clinical and immunological cross-reactivity of sesame seeds. Sesame seems to be cross-reactive especially with tree nuts and members of the *Leguminosae* family (soybean and peanut). Some sesame allergic patients have positive prick test with soybean and peanut (Dreborg, 1991). Beyer *et al.* (2002) showed sequence homology between an IgE-binding site of the major peanut allergen Ara h 1 and the 7S globulin of sesame seed: that might explain cross-reactivity in certain patients. Voks *et al.* (1993) found the existence of common allergenic structures in sesame seed, hazelnut and rye grain.

6. POSSIBLE EFFECTS OF FOOD PROCESSING ON ALLERGENICITY AND DERIVED PRODUCTS

One of the most common sesame-derived products is sesame oil, which is extensively used in cooking and in pharmaceutical and cosmetic industries. Morisset *et al.* (2003a) found that anaphylactic shock can be induced in adults by sesame oil. The authors noted that subjects reacting to a few milligrams of proteins in sesame oil, reacted only to 100 mg up to 7 g of sesame seeds. This striking difference in reactive protein quantities may indicate an interaction between sesame allergens and the lipid matrix, which may considerably increase allergenicity. Such an interaction has been demonstrated with the mustard major allergen (Onaderra *et al.*, 1994).

7. THRESHOLD DOSES

In a study by Morisset *et al.* (2003a) data from 12 positive oral challenges to sesame seed were analysed: haemodynamic modifications and respiratory symptoms were observed in 8% and in 42% of the oral challenges to sesame, respectively. A cumulative reactive dose below or equal to 65 mg of solid food (equivalent to 12.4 mg of sesame proteins) was found in 8% of sesame allergic patients. The lowest reactive threshold was observed at less than 30 mg of sesame seed. Five out of six blinded oral challenges with sesame oil were positive: two patients had an anaphylactic shock with 1 and 5 mL, respectively.

8. CONCLUSION

Allergy to sesame seeds has been increasingly reported in recent years, especially in countries like Israel where exposure occurs early in life. Sesame seeds are potential allergens and can cause severe anaphylactic reactions. Oral challenge-based studies revealed that both sesame seeds and sesame oil elicit reactions in sensitised patients, with threshold doses ranging from 30 mg to 10 g of sesame seed, and few millilitres (1-5 mL) of sesame oil. In a significant percentage of sesame allergic patients, a few milligrams of sesame protein are enough to cause severe symptoms. A threshold dose for highly sensitive patients has not been established.

XX. ADVERSE REACTIONS TO SULPHITES

SUMMARY

Sulphites, or sulphiting agents, are defined as sulphur dioxide (SO_2) and several inorganic sulphite salts that may liberate SO_2 under appropriate conditions. They can occur naturally in foods as a consequence of fermentation, but they are also added to foods as preservatives. The prevalence of sulphite sensitivity in the general population is unknown, but it appears to be rare among non-asthmatics. Most reactions to sulphites are characterised by severe bronchospasm, which can occur within minutes after ingestion of sulphite-containing foods. The smallest concentration of sulphites able to provoke a reaction in sensitive individuals has not been determined. Use of sulphating agents in fruits and vegetables intended to be consumed raw appears to be responsible for several cases of sulphite-induced bronchospasm. This led to the prohibition of sulphite use in raw fruits and vegetables by the United States Food and Drug Administration, which also requires labelling of foods containing sulphiting agents in concentrations of 10 ppm (10 mg/kg) or more. Though the threshold for sensitivity reactions may be even lower, the regulation is based on the fact that the assay used to detect the level of sulphites in foods is not sensitive enough to detect amounts less than 10 mg/kg.

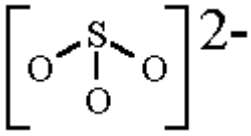
1. BACKGROUND

Although adverse reactions to ingested sulphites were first reported in 1976 (Prenner and Stevens, 1976), it was not until after 1980 that reports on sulphite sensitivity became sufficiently common to attract the attention of the scientific community, consumer groups and regulatory agencies (Gunnison and Jacobsen, 1987). Despite the increased amount of data that has accumulated on sulphites as the interest of the medical community on the issue has grown in recent years, several questions regarding prevalence, pathogenesis and best protection of sulphite-sensitive patients remain to be settled.

1.1 Chemistry and possible sources of exposure to sulphites

Sulphites, or sulphiting agents, are defined as sulphur dioxide and several inorganic sulphite salts that may liberate SO_2 under appropriate conditions. These include sodium and potassium metabisulphites ($Na_2S_2O_5$, $K_2S_2O_5$), sodium and potassium bisulphites ($NaHSO_3$, $KHSO_3$) and sodium and potassium sulphites (Na_2SO_3, Na_2SO_3) (Simon, 1998). The structural formula of the sulphite ion is given in Figure 1.

Figure 1. Structural formula of the sulphite ion



Sulphites are a well-recognised part of the human natural and artificial environment. Considerable quantities of them are generated in the body by normal catabolic processing of sulphur-containing compounds, notably the amino acids cysteine and methionine (Cooper, 1983; Gunnison and Jacobsen, 1987). In foods, sulphites can occur naturally as a consequence of fermentation (e.g. during the fermentation of wine) (Taylor *et al.*, 1986), but they are also

added to foods and used as preservatives, a practice that has been implemented for centuries (Bush *et al.*, 1986a). Sulphites can also be found in medications, including those used for the treatment of allergic reactions (Nicklas, 1989). Finally, individuals are daily exposed to SO_2 as part of the air pollution mix.

1.2 Sulphites in foods

As previously indicated, sulphites can occur naturally in foods as a consequence of fermentation, but they are also added to foods as preservatives. Though sulphites were already used in antiquity by Egyptians to cleanse their wine vessels, their first recorded use as food preservatives occurred in 1664, when cider was stored in flasks of SO_2 to retard spoilage. In the United States, SO_2 has been widely used since the late 1800 and the sulphite salts since 1920; they were first used in the manufacture of wine and beer and their use expanded to several other products (Bush *et al.*, 1986a).

Sulphites provide a number of useful attributes when applied to foods: they inhibit enzymatic browning (especially in fresh fruits and vegetables, shrimps and raw potatoes), as well as non-enzymatic browning (especially in dried foods and dehydrated vegetables) (Taylor *et al.*, 1997; Simon, 1998). They also have antimicrobial activity (as in wine and beer), dough-conditioning properties (as in frozen pies and pizza crusts) and bleaching effects (as in maraschino cherries) and they are used as processing aids in beet sugar (Bush *et al.*, 1986a; Simon, 1998). The E numbers that correspond to various sulphating agents used as food additives are given in Table 27.

Table 27. E numbers corresponding to sulphiting agents

E number	Sulphiting agent
E 220	sulphur dioxide
E 221	sodium sulphite
E 222	sodium hydrogen sulphite
E 223	sodium metabisulphite
E 224	potassium metabisulphite
E 226	calcium sulphite
E 227	calcium hydrogen sulphite
E 228	potassium hydrogen sulphite

The levels of sulphites contained in foods range from under 10 mg/kg (e.g. frozen doughs, corn syrup, jellies) to 60 mg/kg (e.g. fresh shrimp, pickles, fresh mushrooms) to 100 mg/kg (e.g. dried potatoes, wine vinegar). The highest levels of sulphites (up to 1000 mg/kg) can be found in dried fruit, wine, fruit juices (e.g. lemon, lime, grape) and certain freshly prepared sauces available from retailers (Simon, 1998). In Table 28, Lester (1995) presents a list of certain foods by level of their total content in sulphites (naturally-occurring plus added).

The SCF (1994) identified a no observed effect level (NOEL) of 70 mg/kg body weight/day of sulphur dioxide equivalents (SDE) for gastric irritation in animals. Based on this and on a safety factor of 100, the Committee estimated an Acceptable Daily Intake (ADI) of 0-0.7 mg SO_2/kg body weight that would ensure that gastric reactions will not occur in man. It explicitly stated, however, that "a numerical ADI would not prevent the occurrence of sulphite-induced asthma" and that it considered that "EC labelling regulations should ensure that the presence of added sulphites in foods and non-alcoholic beverages is always indicated

in the list of ingredients". It also expressed its concern that no such labelling was required for alcoholic beverages and recommended that "the presence of added sulphite should be declared on labels of alcoholic beverages."

Directive 95/2/EC[3] on food additives other than colours and sweeteners states maximum levels of sulphites expressed as SO_2 in mg/kg or mg/L and relates this to the total quantity available from all sources for several foods and beverages. It is also stated that an SO_2 content of not more than 10 mg/kg or 10 mg/L is not considered to be present.

Table 28. Estimated total SO_2 levels in foods which have been treated with sulphites

Sulphite level			
Undetectable (<10 mg/kg)	**Low (10-49.9 mg/kg)**	**Moderate (50-99.9 mg/kg)**	**High (>100 mg/kg)**
Malt vinegar Dried cod Canned potatoes Beer Dry soup mixes Soft drinks Instant tea Pizza dough (frozen) Pie dough Sugar (esp. beet sugar) Gelatine Coconut Fresh fruit salad Domestic jams and jellies Crackers Cookies Grapes High fructose corn syrup	Pectin Shrimp (fresh) Corn syrup Sauerkraut Pickled peppers Pickled cocktail onions Pickles/relishes Corn starch Hominy Frozen potatoes Maple syrup Imported jams and jellies Fresh mushrooms	Dried potatoes Grape juice (white, white sparkling, pink sparkling, red sparkling) Wine vinegar Gravies, sauces Fruit topping Maraschino cherries	Dried fruit (excluding dark raisins and prunes) Lemon juice (non-frozen) Lime juice (non-frozen) Wine Molasses Sauerkraut juice

Source: Lester, 1995

1.2.1 Methods of measurement of sulphites in foods

Several methods for measuring sulphite residue levels in foods are available (Fazio and Warner, 1990; Su and Taylor, 1995). Levels of sulphating agents in foods are usually expressed as SO_2 equivalents. Variations of the Ripper method (Ripper, 1892) are used to detect free SO_2. The Monier-Williams method (Monier-Williams, 1927) measures total SO_2, which includes the same substances detected by the Ripper method plus some combined forms of sulphites. Neither method is entirely satisfactory since non-sulphite substances may interfere in the analyses, some combined forms may not be measured under the assay conditions and others, which do not pose a risk, may be detected (Bush *et al.*, 1986a).

[3] European Parliament and Council Directive 95/2/EEC on food additives other than colours and sweeteners. Official Journal of the European Union L 061, 18.03.1995, p. 1-40.

The United States Food and Drug Administration (FDA) regulation, which requires food manufacturers and processors to disclose the presence of sulphiting agents in concentrations of 10 parts per million (i.e. 10 mg/kg) or more, is based on the fact that the assay used to detect the level of sulphites in food is not sensitive enough to detect amounts less than 10 mg/kg in all foods, but the threshold for eliciting reactions in sulphite sensitive individuals may be even lower.

1.2.2 Estimation of average daily exposure to sulphites from foods

Assessment of consumer exposure to sulphites in foods is inadequate. This is partially due to the shortcomings of the methods of measurement. Storage and preparation of food also affects the final amount of consumed sulphites.

Though the actual consumption may vary widely, based on individual dietary practices, average daily sulphite consumption in the United States has been estimated to be 19 mg of SDE (297 μmol of sulphite), with the 99th percentile of the population consuming daily 163 mg of SDE (~2.5mmol of sulphite) (FDA, 1985). In France, researchers have estimated an average daily intake of 20 mg SO_2, reaching 31.5 mg/day among consumers of cider, beer and wine (Mareschi *et al.*, 1992). As a working framework, the average daily consumption is less than 20 mg of SDE (Lester, 1995).

It should be noted that sulphites are frequently used in restaurant foods as preservatives. A report has estimated that an average restaurant meal may contain 25 to 100 mg of sulphites (Simon, 1989).

2. FREQUENCY

The prevalence of sensitivity to sulphating agents in the general population is unknown (Bush *et al.*, 1986b). Estimates of the percentage of asthmatics characterised as sensitive to oral sulphite challenge range from less than 4% up to 66% (Table 29).

The probable reasons for the wide disparity are the difference in the physical form of the orally administered sulphating agent (i.e. solution *vs* encapsulated, the former generating more positive reactions than the latter, due to inhalation of volatilised SO_2), the differences in the selection criteria of the sample populations submitted to oral challenges and the non-uniformity in the upper limit of challenge dose as well as in the minimum decrement in pulmonary function accepted as evidence of sulphite-induced bronchoconstriction. In addition, adequate placebo challenges have not been carried out in some studies (Gunnison and Jacobsen, 1987).

The SCF stated that the percentages of sulphite sensitivity reported in the literature (1-4% among all asthmatics and 5-10% among steroid-dependent asthmatics) are probably overestimates based on patients referred to allergy clinics (SCF, 1994). The FDA estimates that one out of a hundred people are sulphite sensitive, and that 5% of those who have asthma are also at risk of suffering an adverse reaction to the substance (FDA, 1996).

The average age of the individual who experiences asthma after exposure to sulphites is 40 years, and sensitivity is reportedly higher among women (Gunnison and Jacobsen, 1987,

Simon, 1989). It is uncommonly reported in preschool children, perhaps because their diets include fewer foods with high sulphite content and do not include wine (Lester, 1995).

Table 29. Prevalence of sulphite sensitivity among asthmatics

Study sample	% of sulphite sensitive	Oral challenge protocol	Reference
14 patients with history of "orange drink" asthma among 272 asthmatics	6%	Single-blind	Freedman, 1977
61 steroid-dependent asthmatics without history of "restaurant" asthma	8%	Single-blind	Simon *et al.*, 1982
15 asthmatics with history of food asthma	7%	Single-blind	Koepke and Selner, 1982
29 asthmatic children	66%	Single-blind	Towns and Mellis, 1984
134 among 1073 asthmatics (selection criteria unspecified)	37%	Not mentioned	Buckley *et al.*, 1985
120 non-steroid-dependent asthmatics	4% [0.8%]	Single-blind (and subsequent double-blind in positive respondent subgroup)	Bush *et al.*, 1986b
83 steroid-dependent asthmatics	19% [8.4]		
61 steroid-dependent asthmatics	4.5%	Double-blind	Prieto *et al.*, 1988
20 children with steroid-dependent asthma	30% [20%]	Single-blind (and subsequent double-blind in positive respondent subgroup)	Sanz *et al.*, 1992
37 asthmatic children	43%	Double-blind	Steinman *et al.*, 1993
20 asthmatics	60%	Not mentioned	Arai *et al.*, 1998
24 "wine asthmatics"	17%	Double-blind oral challenge	Vally and Thompson, 2001

3. CLINICAL FEATURES

3.1 Symptoms

Most reactions to sulphites are characterised by bronchospasm, occasionally severe, which can occur within minutes after ingestion of sulphite-containing foods. In restaurants, the sudden choking sensation may incorrectly be attributed to aspiration of food (Nicklas, 1989). Bradycardia, flushing and prominent gastrointestinal symptoms (Sheppard *et al.*, 1980; Schwartz, 1983), as well as urticaria, angio-edema, hypotension (Prenner and Stevens, 1976; Habenicht *et al.*, 1983; Schwartz, 1983) and shock (Lester, 1995) have also been observed.

3.2 Diagnosis

A careful history, though important in detecting sulphite sensitivity, is not sufficient to make the diagnosis and skin testing (prick puncture or intradermal technique) can identify only a small fraction of patients. Therefore, various challenge protocols have been developed. For sensitivity to ingested sulphites, the substances used for the challenges are usually contained in opaque capsules. False negative results may occur if the sensitivity has to do with inhaled sulphites. On the other hand, as a high proportion of asthmatics (unlike non-asthmatics) are sensitive to inhaled sulphur dioxide, challenges with acid solutions may produce false positive

results if the dosage is high (Simon, 1998). The standard practice has been to use challenges for asthmatics below 100 mg/mL of sulphites. As for subjects with a history suggesting sulphite-induced urticaria or anaphylaxis, capsule challenges up to a maximum dose of 200 mg are used (Simon, 1996).

4. PATHOGENESIS

The pathogenesis of adverse reactions to sulphites has not been clearly documented. Three possible mechanisms have been invoked: an IgE-mediated reaction, a sulphite-induced cholinergic response and low levels of the enzyme sulphite oxidase (Bush *et al.*, 1986a; Nicklas, 1989; Lester, 1995).

There have been reports of positive skin tests to sulphites, *in vitro* mediator release or passive sensitivity transfer (Prenner and Stevens, 1976; Twarog and Leung, 1982; Wolf and Nicklas, 1985; Yang *et al.*, 1986; Simon and Wassserman, 1986; Boxer *et al.*, 1988), all of them suggesting that some reactions to sulphites may in part be IgE-mediated. A specific antibody, however, has never been identified (Lester, 1995) and the majority of studies have not been able to demonstrate and IgE-mediated mechanism (Gunnison and Jacobsen, 1987; Nicklas, 1989). It appears that IgE-mediated mechanisms, if they do exist, are very rare in sulphite sensitive individuals or are limited to a subset of patients (Bush *et al.*, 1986a; Sainte-Laudy *et al.*, 1994).

The bronchoconstrictive effect of inhaled SO_2, mediated by parasympathetic nerve endings in the bronchi, has been studied with respect to environmental pollutants. Whether gastroesophageal reflux of SO_2 causes bronchospasm in sulphite sensitive patients is not clear. It has been hypothesised that it is also possible to mechanically distend the stomach, produce a cholinergic response and stimulate release of gastrin and other active mediators in sulphite sensitive patients (Nicklas, 1989).

Low levels of the mitochondrial enzyme sulphite oxidase have been demonstrated in some sulphite sensitive patients (Jacobsen *et al.*, 1984; Stevenson and Simon, 1984). Absorbed sulphites are added to those produced endogenously and increase the demand placed on the enzyme sulphite oxidase. It is possible that when this demand is not met, sulphite sensitive patients exhibit symptoms.

Finally**,** it has also been hypothesised that a number of food additives, including sulphites, induce intolerance because of their aspirin-like properties (Williams *et al.*, 1989) and an association between respiratory reactions to aspirin and those to sulphites has been reported (Sabbah *et al.*, 1987; Hassoun *et al.*, 1994).

5. POSSIBLE EFFECTS OF FOOD PROCESSING ON SENSITIVITY AND DERIVED PRODUCTS

The amounts of sulphites initially used to treat foods do not reflect residue levels after processing. Storage and preparation of food also affects the final amount of consumed sulphites. Mechanisms of loss include volatilisation to SO_2 in acidic conditions, leaching, autooxidation, as well as the irreversible reactions with food constituents (Gunnison and Jacobsen, 1987).

Sulphites can react with food constituents, including sugars, proteins and lipids, to form combined sulphites. Some of these reactions are reversible, while others are not. The former lead to compounds that may serve as reservoirs for free sulphite, while the latter remove sulphites permanently from the pool of available free SO_2. Since free SO_2 is the most likely cause of adverse reactions to sulphiting agents, these chemical reactions have significant implications regarding foods which may cause difficulty in sensitive patients (Bush *et al.*, 1986a; Simon, 1998). The likelihood of a particular food provoking a reaction depends upon the ratio of free to bound sulphite. For example, lettuce has few components to which sulphites can react, therefore most of the sulphite in lettuce remains in the free inorganic state and this explains why lettuce (salad bars) seems to provoke sulphite sensitive reactions frequently (Martin *et al.*, 1986; Simon, 1998). In contrast, sulphites added to shrimp and potatoes tend to be bound and are not as likely to produce reactions in sulphite sensitive subjects.

6. THRESHOLD DOSES

Toxicity studies in non-asthmatic individuals have been conducted primarily through oral challenges and inhalation studies (Bush *et al.*, 1986a). Small numbers of individuals have ingested doses of up to 400 mg of SO_2 equivalents per day, without adverse effect (Taylor *et al.*, 1986). However, doses of 4 to 6 g per day predictably caused nausea, vomiting, gastric irritation and occasional gastrointestinal bleeding (Schwartz, 1984; Bush *et al.*, 1986a). Prenner and Stevens (1976) confirmed sulphite sensitivity in a patient after the ingestion of a total dose of 10 mg of $NaHSO_3$ solution. Case reports of positive oral challenges with encapsulated sulphites have been made for doses of 10 mg (Schwartz, 1983) and 25 mg (Habenicht *et al.*, 1983). Challenge studies in larger number of non-asthmatics with a risk for adverse reactions to sulphites have failed to identify a significant number of reactors (Meggs *et al.*, 1985; Sonin and Patterson, 1985; Bush *et al.*, 1986a).

Among asthmatics, the amount of sulphite required to produce a response also varies and quantities as low as 1 to 5 mg of ingested potassium metabisulphite have been reported to provoke a reaction in sulphite sensitive asthmatics (Stevenson and Simon, 1981). Ingestion of sulphited solutions is more likely to precipitate asthma than ingestion of encapsulated sulphites, perhaps due to inhalation of volatilised SO_2 (Bush *et al.*, 1986b).

According to Simon (1989), most sulphite sensitive individuals will react to ingested metabisulphite in quantities ranging from 20 to 50 mg (Simon, 1989; Lester, 1995). However, threshold levels have not been systematically assessed and the smallest concentration of sulphites able to provoke a reaction in a sensitive person is unknown.

7. CONCLUSION

The prevalence of sulphite sensitivity in the general population is unknown, but it appears to be rare among non-asthmatics. Estimates of the percentage of asthmatics characterised as sensitive to oral sulphite challenge range from less than 4% up to 66%. The SCF stated that the reported prevalences of 1-4% among all asthmatics and 5-10% among steroid-dependent asthmatics are probably overestimates based on patients referred to allergy clinics (SCF, 1994). Most reactions to sulphites are characterised by severe bronchospasm, which can occur within minutes after ingestion of sulphite-containing foods. In restaurants, the sudden choking

sensation may incorrectly be attributed to aspiration of food. Assessment of consumer exposure to sulphites in foods is inadequate, as the currently available measurement methods have several shortcoming and the amounts of sulphites initially used to treat the foods do not reflect residue levels after processing. The average daily sulphite consumption has been estimated to be approximately 20 mg of sulphur dioxide equivalents. It should be noted, however, that sulphites are frequently used in restaurant foods as preservatives and an average restaurant meal may contain sulphites well in excess of 25 mg. Most sulphite sensitive individuals will react to ingested metabisulphite in quantities ranging from 20 to 50 mg. Use of sulphating agents in fruits and vegetables intended to be consumed raw appears to be responsible for several cases of sulphite-induced bronchospasm. This led to the prohibition of sulphite use in raw fruits and vegetables by the USA FDA, which also requires labelling of foods containing sulphiting agents in concentrations of 10 mg/kg or more. Though the threshold for sensitivity reactions may be even lower, the regulation is based on the fact that the assay used to detect the level of sulphites in foods is not sensitive enough to detect amounts less than 10 mg/kg. However, threshold levels have not been systematically assessed and the smallest concentration of sulphites able to provoke a reaction in a sensitive person is unknown.

GENERAL CONCLUSIONS

Based on the reviewed literature, it is concluded that there is ample evidence to support the inclusion of the following foods, food components, and food ingredients and the derived products into the list of food allergens (Annex IIIa of Directive 2003/89/EC): cereals containing gluten, fish, crustaceans, egg, peanut, soy, milk, lactose, nut, celery, mustard, sesame seed, and sulphites. These are the most common food allergens which are generally resistant to food processing and they have the capacity to trigger an allergic reaction in an allergic consumer if they are added to foods. This list should be kept under review in the light of changing food practices and emergence of new clinical observations and other kind of scientific information.

The doses of allergens capable of triggering food allergic reactions are variable and can be very small, i.e. in the milligram or microgram range. The information currently available is insufficient to draw firm conclusions regarding the lowest dose that could cause an adverse effect (threshold). Processing of foods, and the matrix of the foods, can influence allergenicity, but the data available do not indicate that food processing influences allergenicity in a predictable fashion. For these reasons, a system of risk evaluation based on the assessment of no observed adverse effect levels (NOAEL) does not apply currently.

The technical issues related to the detection of low levels of allergens in food remain unresolved. If a threshold level could be established, analysis of foods for traces of potential food allergens and declaration are crucial for consumer protection. Although many test systems are in use and commercially available for food allergen analysis, most of which rely on immunochemical methods, major technical problems remain. These are related for example to insufficient extraction, detection limits outside the range of clinical sensitivity, insufficient specificity due to cross-reaction and insufficient interlaboratory reproducibility

The possibility that specific derivatives of the food allergens listed in Annex IIIa of the Directive are unlikely to trigger an allergic reaction needs to be evaluated on a case by case basis.

GLOSSARY AND ABBREVIATIONS

ADHD	Attention deficit-hyperactivity disorder (*qv*)
ADI	Acceptable Daily Intake
Adrenaline	A catecholamine that occurs naturally in the body and is also used for the treatment of anaphylaxis. Also known as epinephrine
ALA	α-lactalbumin
Albumin	A large water-soluble protein
Allergen	Substance, usually a protein, capable of inducing an allergic response
Allergy	Immune response in sensitive individuals which results in an adverse reaction
Anaphylaxis	Acute form of allergy characterized by urticaria (*qv*), shortness of breath, rapid fall in blood pressure and swelling of the throat and lips. Without immediate treatment, which consists of intramuscular injection of adrenaline (*qv*), anaphylaxis can be fatal
Anaphylactic shock	See anaphylaxis
Angio-edema	Presence of fluid in subcutaneous tissues or submucosa, particularly of the face, eyes, lips and sometimes tongue and throat, occurring in an anaphylactic reaction
Antibody	Immunoglobulin which is specific for an antigen or allergen
Antigen	Substance recognized by the immune system
APC	Antigen-presenting cells
Asthma	Chronic inflammatory disease of the airways which renders them prone to narrow too much. The symptoms include paroxysmal coughing, wheezing, tightness and breathlessness. Asthma may be caused by an allergic response or may be induced by non-immunological mechanisms
Atopic dermatitis	Disease of the skin characterized by itching and dry and lined skin
Atopy	Predisposition to IgE production associated with allergy to several common allergens
Attention deficit-hyperactivity disorder	Condition characterized by inattentiveness, over activity and/or impulsiveness
BLG	β-lactoglobulin
Blind	In epidemiology, this term is used in relation to the knowledge the observer or the observed individual or patient has of any intervention. In a double-blind trial neither the observer nor the observed individual knows which treatment is being given. In a single-blind trial only the observed individual is unaware of which treatment is being given
B lymphocytes	Bursa-equivalent lymphocytes. After maturation into plasma cells they produce antibodies (immunoglobulins) during humoral responses in immunological reactions. They were first discovered in the Bursa of Fabricius in the chicken; hence the name
BSA	Bovine serum albumin

CAP (also ImmunoCAP)	A system designed for sandwich immunoassays and immunometric assays. Solid-phase bound allergens are allowed to react with antibodies in the serum sample and specific IgE antibodies are detected by labelled anti-IgE antibodies.
CAP-FEIA	CAP-system IgE fluoroenzyme immunosorbent assay for detection of specific IgE antibodies
CAS	Casein fraction
CCD	Cross-reactive carbohydrate determinants
CMP	Cows' milk protein
Coeliac disease	Disease characterized by damage to the small intestinal wall and intolerance of gluten, a protein present in wheat flour
Cytokine	Mediators that are produced by a variety of cell types which influence immune and inflammatory responses
DBPCFC	Double-blind placebo-controlled food challenge (*qv*)
Dermatitis	Inflammation of the skin
Dermatitis herpetiformis	Skin disease often associated with gluten-sensitive enteropathy
Disaccharidases	Enzymes catalyzing the hydrolysis of disaccharides (sugars) to their constituent monosaccharides (e.g. lactase)
Double-blind placebo-controlled food challenge	An *in vivo* test in which the patient and doctor do not know which food is being tested until after the tests and the recording of responses have been completed. Often regarded as the standard for testing for allergenicity
EAST	Enzymeallergosorbent test
ELISA	Enzyme-linked immunosorbent assay: a sensitive technique for the detection and measurement of compounds, especially proteins
Enzymes	Proteins which catalyse chemical reactions in the body
Epinephrine	See Adrenaline
Epitope	Peptide sequence within an antigenic molecule which is recognized by either lymphocytes or antibodies
FEIA	Fluorescence enzyme immunoassay, see ELISA
Food additive	Substance added to food to facilitate some part of the processing or manufacture of the foodstuff or to impart a particular characteristic; they can be classified according to the purpose for which they are used into, for example, acidity regulators, antioxidants, food colours
Food allergy	Adverse reaction to food, mediated by immunological mechanisms
Food intolerance	General term for adverse reaction to food and food ingredients. In this opinion, the term is restricted to presumed non-immunological reactions to food and food ingredients
Globulin	A large globular water-soluble protein
Glycoproteins	Proteins conjugated with a carbohydrate group
GMP	Good Manufacturing Practice
Histamine	Decarboxylation product of the amino acid histidine. It is an important inflammatory mediator in allergy and in other circumstances and it is

	also involved in “pseudoallergy”
HLA	Human Leukocyte Antigen. The major human histocompatibility complex. They are complex glycoproteins on the surface of cells which give us our individual immunological identity
HPLC	High Performance Liquid Chromatography
Hypersensitivity	Heightened responsiveness induced by allergic sensitization. There are several types of response including that associated with allergy (see immediate-type hypersensitivity)
ICD	International Classification of Diseases
IFN-γ	Interferon gamma, produced by Th1 and other cells, antagonizes IgE antibody production
IgA	One of the five main classes of human immunoglobulin. IgA is involved in mucosal protection
IgE	One of the five main classes of human immunoglobulin. IgE is involved in allergy and anaphylaxis as well as protecting against intestinal parasites. IgE-mediated hypersensitivity is characterized by the speedy release of mediators such as histamine
IgG	One of the main classes of human immunoglobulin
IL	See Interleukins
Immediate-type allergy/hypersensitivity	IgE-mediated hypersensitivity characterized by release of mediators such as histamine
Immunogen	Substance capable of eliciting an immune response
Immunological tolerance	Specific immunological unresponsiveness or altered responsiveness resulting from exposure to antigen
Incidence	The number of new cases of a disease that occur during a particular time in a defined population
Interleukins	Soluble polypeptide mediators, produced by activated lymphocytes and other cells during immune and inflammatory response
IUIS	International Union of Immunological Societies
LOAEL	Lowest Observed Adverse Effect Level
LF	Lactoferrin
LTP	Lipid Transfer Protein
Mast cells	Cells found predominantly in connective tissue, although a specialized population of mast cells is found in mucosal sites (e.g. the gut and lung). Following degranulation, mast cells release preformed and newly synthesized mediators of inflammation, including histamine
Migraine	Type of headache, characterized by usually being unilateral and/or being accompanied by visual disturbance
MIM	Mendelian Inheritance in Man
Neoallergen	Allergen (*qv*) formed during the processing of food
NOAEL	No Observed Adverse Effect Level
NOEL	No Observed Effect Level
Open challenge	In the context of adverse reactions to food, challenging the patient with

	the food suspected to cause the adverse reaction, without any attempt to hide the nature of the challenge from the observer or the patient
OAS	Oral Allergy Syndrome
Otitis media	Inflammation of the middle ear
Parvalbumin	Low molecular weight albumin
PCR	Polymerase Chain Reaction
Prevalence	Total number of cases of a disease in existence at a certain time in a designated population (including new and old cases)
RAST	Radioallergosorbent test; a test for the measurement of specific IgE antibodies in the blood
REIA	Reverse enzyme immunoassay
Rhinitis	Inflammation of the nasal passages, resulting in runny nose
Rhinoconjunctivitis	Rhinitis (*qv*) combined with inflammation of the conjunctiva
SDE	Sulphur dioxide equivalents
SDS-PAGE	Sodium dodecylsulphate polyacrylamide gel electrophoresis, a biochemical method for determination of molecular weights of polypeptides and proteins
Serotonin	Vasoactive decarboxylation product of the amino acid tryptophan, also known as 5-hydroxytryptamine
SPT	Skin Prick Test. A clinical test of allergenic reactivity and allergenicity commonly used in allergy clinics
Sensitisation	Immunological response to an allergen or food protein which does not necessarily lead to a clinical reaction
T helper cells	T cells which help B lymphocytes to produce antibodies. Two principle subtypes exist. Th1 cells (*qv*) produce IFN-γ amongst others cytokines and antagonize the IgE responses. Th2 cells (*qv*) produce interleukins that promote IgE production and allergic sensitization
T lymphocytes	Thymus-dependent lymphocytes which, amongst other functions, help B lymphocytes during immunological responses and provide protection from intracellular microbial infection. Distinct subpopulations have been characterized - see T helper cells
Th1 cells	T helper lymphocytes of the type 1 subgroup which produce cytokines such as IFN-γ. In general, their actions antagonize the IgE response
Th2 cells	T helper lymphocytes of the type 2 subgroup which produce cytokines that promote IgE hypersensitivity reactions
TNF-α	Tumour necrosis factor α
Tropomyosin	Muscle protein
Urticaria	An intensely itchy rash which results from inflammation and leakage of fluid from the blood into the superficial layers of the skin in response to various mediators. Synonyms are “hives” or “nettle rash”

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