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

(Request N° EFSA-Q-2005-084)

(adopted on 15 February 2006)

SUMMARY

Seafood is an important food category in Europe, comprising fish, crustaceans, and molluscs. Allergies to fish and crustaceans were dealt with in the NDA opinion 2004. Molluscs are a large and diverse group, which includes some 100,000 species living in salt water, in freshwater and on land. Some molluscs are an important food source. Molluscs are used as an added ingredient in some processed foods like soups and sauces and in products like surimi. Case reports and patient series indicate that several molluscs, e.g. snail, oyster, clams, mussels, squid, abalone and octopus, can cause food allergic reactions, some of them life-threatening. Prevalence of self-reported mollusc allergy ranges from about 0.15% (4/2716) in school children in France to about 0.4% (or 20% of all seafood allergic cases) in a household survey of 14,948 individuals in the US.

A major allergen of most molluscs is the abundant muscle protein tropomyosin, which has been well characterised in several mollusc species. Tropomyosin is an allergen also found in crustaceans, dust mites, and cockroach and other insects. Characterised tropomyosins in molluscs and different crustaceans show similarities but also significant differences in allergenic structures. In addition to tropomyosin, there is evidence that molluscs contain a number of other allergens, but these are poorly defined. Serological and clinical cross-reactivity between mollusc species, as well as between molluscs and crustaceans and house dust mite has been reported. Mollusc allergens do not cross-react with fish allergens. However, there is a possibility that patients reacting to fish infested with the parasite Anisakis might also react to molluscs due to cross-reactivity.

Mollusc allergenicity is not reliably reduced by food processing. Tropomyosin allergenicity is heat-resistant. Although the allergenicity of some other mollusc allergens appears to be destroyed by heat treatment, there are also reports of increased allergenicity after heating. There is little information on the lowest dose of mollusc allergen that can cause a clinical reaction. In one double blind placebo controlled food challenge study with dried snail reactions were observed in the low hundred-mg range.

KEY WORDS

Allergy, food, mollusc, tropomyosin, hemocyanin, clam, abalone, limpet, scallop, oyster, mussel, squid, cuttlefish, octopus.

BACKGROUND

Annex IIIa of Directive 2000/13/EC, as amended by Directive 2003/89/EC, establishes a list of ingredients that are known to trigger allergies or intolerances. The aforementioned Directive states that whenever the listed ingredients are used in the production of foodstuffs they must be labelled.

Article 6, paragraph 11 of the same Directive requests that the list in Annex IIIa shall be systematically re-examined and, where necessary, updated on the basis of the most recent scientific knowledge.

Furthermore, paragraph 11 states that Annex IIIa may be amended, in compliance with the procedure referred to in Article 20 (2), on the basis of a scientific opinion of the European Food Safety Authority.

TERMS OF REFERENCE

In accordance with Article 29 (1) (a) of Regulation (EC) N° 178/2002, the European Commission requests the European Food Safety Authority to provide a scientific opinion on the appropriateness for inclusion of molluscs and their eventual derived products in the list of food allergens set up in Annex IIIa of Directive 2000/13/EC, in the light of the most recent scientific evidence.

ASSESSMENT

The Panel decided to focus on the evidence basis upon which a decision on the appropriateness of inclusion under the Terms of Reference could be based. The Panel considers the decision whether or not to include molluscs a risk management task which is outside the remit of the Panel.

1. INTRODUCTION

There are more than 100,000 species of molluscs, which are second only to arthropods in the number and diversity of species. They live in salt water, fresh water and on land, and range in size from less than one millimetre to nearly 20 meters (giant octopus), and may weigh up to 900 kg (giant squid, *Architeuthis*). Molluscs are commonly classified into eight classes, of which three are of particular importance as food (Table 1). The largest class, *Gastropoda*, can be divided into three subclasses and counts more than 70,000 species (Hickman *et al.*, 2004). A mollusc species may have many subspecies.

Table 1. Taxonomic classification of molluscs encountered in the food allergy literature*

Phylum Mollusca	
Class *Gastropoda* Subclasses *Prosobranchia* (predominantly marine), *Opisthobranchia* (marine), *Pulmonata* (fresh-water and terrestrial)	Small abalone (*Haliotis diversicolor*), red abalone (*H. rufescens; H. rubra*), abalone (*H. midae*), northern disk abalone (*H. discus hannai*), whelk (*Hemifusus ternatana; Buccinum undatum; Neptunea arthritica*), limpet (*Patella piperata; P. vulgata*), grand keyhole limpet (*Fisurella maxima* Sowerby); land snail (*Euparipha pisana; Limax agrestis; Eobania vermiculata; Cernualla virgata; Helix aperta; H. terrestre; H. pomatia; H. aspersa aspersa; H. aspersa maxima; H. lucorum*), turban shell (*Turbo cornutus*)
Class *Bivalvia*	Green mussel (*Perna viridis*), blue mussel (*Mytilus edulis*), mussel (*M. galloprovincialis*), scallop (*Chlamys opercularis; C. nipponensis; Patinopecten yessoensis*), Pacific oyster (*Crassostrea gigas*), Eastern oyster (*C. virginica*), clam (*Lutraria philipinarum, Tapes decussates; T. japonica*), razor clam *(Tagelus plebius)*, cockle (*Cardium edule*), fan shell (*Pinna atropurpurea*)
Class *Cephalopoda*	Cuttlefish (*Sepia madokai, S. latimanus, S. officinalis*), squid (*Toradodes pacificus, Loligo edulis, L. vulgaris, L. japonica, L. forbesi, L. opalescens, L. pealei*), octopus (*Octopus luteus, O. vulgaris*)

*After Hickman *et al.*, 2004.

In the literature dealing with food allergy, as well as in the literature giving food consumption data, molluscs are often grouped together with crustaceans under the term *shellfish*, and the implicated species are not listed at all or are sometimes given but without quantitative data. However, *Mollusca* constitute a separate *Phylum*, whereas *Crustacea* (shrimp, lobster, crab, and crayfish) constitute a class under another phylum, *Arthropoda* (Levinton, 2001; Hickman *et al.*, 2004). Data on allergy to "shellfish" only are not informative when assessing the allergenicity of molluscs. Therefore, only literature giving information specifically on molluscs as a group or on specific species of molluscs has been considered.

2. MOLLUSC CONSUMPTION AND ALLERGY IN EUROPE

2.1 Mollusc consumption

Molluscs are common food in most European countries. Consumption data specifically for molluscs are sparse. According to FAO food balance sheet data for "Molluscs and other products" amounts available for consumption during 2000-2002 in European countries range from zero to 22 g/d (FAO, 2006; http://www.fao.org/faostat/foodsecurity/index_en.htm). The most popular mollusc food item seems to be oyster, with an annual consumption of more than 3 million tonnes annually worldwide (Leung and Chu, 2001). In some countries, squid is an important seafood in terms of volume consumed. In Europe, squid is consumed in particular in the Mediterranean region, Portugal, and the Atlantic island communities of Spain like the Canary Islands. Great regional differences in terms of mollusc species and quantities consumed are likely. Molluscs are widely used in gourmet food. Their use as an added ingredient appears to be limited, but they can be found in some processed foods like soups and sauces and in products like surimi.

2.2. Routes of exposure

Food allergic reactions after ingestion of molluscs are the major concern. However, it should be noted that for molluscs, like for crustaceans and finfish, skin allergy (contact urticaria) and respiratory allergy may also develop (NDA, 2004). In some mollusc-processing workplaces, respiratory as well as cutaneous allergy to various molluscs has been a considerable problem (Garcia-Abujeta *et al.* 1997; Tomaszunas *et al.*, 1988; Goday Buján *et al.*, 1991; Desjardins *et al*. 1995; Glass *et al.*, 1998; Goetz and Whisman, 2000; Tabka *et al.*, 1998). Some cases of mollusc-elicited occupational asthma have been verified by specific inhalation challenges (Desjardins *et al.*, 1995; Goetz and Whisman, 2000). Also in the non-workplace setting, inhalation of vapour from cooking squid (Carillo *et al.*, 1992), mussels (Nava *et al.*, 1983) or snail (Guilloux *et al.*, 1998) has been reported to trigger immediate respiratory symptoms.

3. FREQUENCY

3.1.1 Prevalence of mollusc allergy

3.1.1.1 Data on mollusc allergy - considerations on reliability

For the interpretation of data published, it must be kept in mind that test positivity (e.g. skin prick test (SPT) or serum specific IgE) does not mean that clinical food allergy is also present (NDA, 2004). Further, it must be pointed out that adverse reactions to molluscs, in particular gastroenteritis-like symptoms may be caused by infectious agents or toxins e.g. algal toxins in blue mussels (Aasen *et al.*, 2005; Hungerford, 2005; Myrmel *et al.*, 2004; Prato *et al.*, 2004; Lopata and Potter, 2000), which is a source of error particularly in studies of self-reported reactions against molluscs.

For assessment of cross-reactivity, it should be noted that the *in vitro* techniques often do not allow a clear distinction whether IgE to two different foods is present as a result of independent sensitisation to the two foods (i.e. dual sensitisation), or because IgE resulting from sensitisation to one food also reacts with another food (i.e. cross-reactivity). Furthermore, when several foods cross-react, there may be different allergens on the molecular level causing cross-reactivity between the different pairs of food, as has been demonstrated with snail, mite and shrimp (van Ree *et al.*, 1996a).

In studies on sensitization and clinical reactivity, because molluscs often do not cross-react, the absence of reaction to one or a few molluscs used for testing does not exclude the presence of sensitization and clinical allergy to other molluscs.

The Panel could only identify two published reports of double blind placebo controlled food challenge (DBPCFC) with molluscs, one positive report (2/4 patients positive) with snails (Pajno *et al.*, 2002) and one with only negative results (0/9 patients positive) with squid (Castillo *et al*., 1997).

3.1.1.2 Allergy to molluscs in the general population

In a cross-sectional, questionnaire-based survey of food allergy in 3500 non-challenged children in 150 classes from eight schools in a French city (Rancé *et al.*, 2005), information was obtained for 2716 children. Two cases (0.8% of allergic cases) reported allergy to mussels, one case (0.4% of cases) to snails and one case to oysters, giving a population prevalence of self-reported mollusc allergy of about 0.15% (4/2716).

Sicherer *et al.* (2004) performed a nation-wide random cross-sectional telephone survey of prevalence of seafood allergy in the United States using a standardised questionnaire. Fish or shellfish allergy was determined according to report of convincing symptoms and physical evaluations, using a classification system with 9 categories for type of evidence and level of likeliness. A total of 5529 households completed the survey (67.3% participation rate), representing a census of 14,948 individuals. Although “shellfish” was used as a major food group, some data are given specifically on molluscs. Sixty-seven persons reported reactions to scallops, clams, oysters, or mussels, representing about 0.4% of the study population (67/14,948) and 20% of all doctor-diagnosed or “convincing” seafood allergy.

Self-reporting usually gives a too high estimate of food allergy prevalence (Altman and Chiaramonte, 1997).

3.1.1.3 Prevalence of mollusc allergy - patient series of adults and children

In studying adult food allergic patients in Gran Canaria, Spain, Castillo *et al.* (1996) found that of 142 food sensitised individuals, 120 reported clinical symptoms after ingestion of one or more foods. Of these, 33 (27.5%) reported allergy to squid (second most common food allergen after shrimp), 12 (10%) to oyster, 10 (8%) to clam, and 10 (8%) to mussel.

Among 163 severe food allergic reactions mainly from France reported by the Allergy Vigilance Network (about 300 participating allergologists) in 2001, six (3.8%) were caused by molluscs (Moneret-Vautrin *et al.*, 2002). In a similar report of 107 lethal or near-lethal reactions for 2002, the term “shellfish” is used without further specifications, except that five cases of reactions to snails are specified (4.7% of all reactions) (Moneret-Vautrin *et al.*, 2004).

Crespo *et al.* (1995a) studied 355 children with food allergy in Spain. Based on clinical history, skin prick tests and specific IgE, molluscs caused 1.6% of 608 allergic reactions. Of the 355 presumed food allergic patients, 24 reacted to shellfish; 23 of these were allergic to crustaceans and 10 to molluscs, and 9 of 10 children allergic to molluscs were also allergic to crustaceans.

In a survey of food hypersensitivity among allergy clinic patients in Baltic countries (n=1139; 17 clinics from 15 cities), participants filled in a questionnaire in which 86 foodstuffs were listed. Hypersensitivity to clam was indicated by 6.2%, to oyster by 3.2%, and to snail by

1.4% of the participants. It should be noted that fewer than 50% of the patients had ever eaten clam, oyster or snail (Eriksson *et al.*, 2004).

In a report on 67 consecutive anaphylaxis cases from a immunology/allergy centre in Singapore (Thong *et al*., 2005), 30 cases were caused by food, and 11 of these (36.7%) were caused by molluscs (limpet and abalone).

4. CLINICAL FEATURES

4.1 General clinical manifestations of mollusc food allergy

Zinn *et al.* (1997) studied in detail symptoms reported in a cohort of 105 individuals who had experienced adverse reactions to various seafoods. Symptoms were found almost identical for crustaceans, molluscs and fish, consistent with findings by O'Neil *et al.* (1993) and Daul *et al.* (1993), and largely similar to allergic reactions reported to other foods (NDA, 2004). However, there are a number of reports on house dust mite-allergic patients who appear to present with severe asthma as a dominant symptom after ingestion of molluscs (Carrillo *et al.*, 1991 and 1994; Castillo *et al.*, 1994; Azofra and Lombardero, 2003). Case reports suggest that asthmatic symptoms are particularly common in reactions against snails in mite-sensitised patients (de Maat-Bleeker *et al.*, 1995; Amoroso *et al.*, 1988; Azofra and Lombardero, 2003; Pajno *et al.*, 2002; Meglio *et al.*, 2002; De la Cuesta *et al.*, 1989; van Ree *et al.*, 1996a; Peroni *et al.*, 2000; Tomás *et al.*, 1997).

Anaphylactic reactions and death have been reported in mollusc-allergic patients (Table 2).

Molluscs, specifically cuttlefish (Caffarelli *et al.*, 1996), squid (Dohi *et al.*, 1991), abalone (Dohi *et al.*, 1991), *Sulculus Supetexta* and *Turbo Cornutus* (Juji *et al.*, 1990), oyster (Maulitz *et al.*, 1979) and snail (Longo *et al.*, 2000) have been implicated in food-dependent exercise-induced anaphylaxis.

Table 2. Case reports and patient series of allergic reactions upon ingestion of molluscs

Mollusc species	Patients	Symptoms	Reference
Limpet	Five patients (two females, three males) age 23-46 yrs	Anaphylactic reaction, dominated by bronchospasm	Azofra and Lombardero, 2003, Spain
Limpet	Three males, one female, age 8 – 24 yrs	Anaphylaxis, dominated by bronchospasm	Carillo *et al.*, 1994, Spain
Limpet	Woman 22 yrs, man 29 yrs	Urticaria, angioedema, severe asthma, anaphylaxis	Carrillo *et al.*, 1991, Spain
Limpet	Male age 20, female age 26	Anaphylaxis	Joral *et al.*, 1997, Spain
Limpet	Five patients	Severe asthma, anaphylaxis	Castillo *et al.*, 1994, Spain
Limpet	Two patients	Asthma	De la Cuesta *et al.*, 1989, Spain
Limpet, abalone	Eleven patients	Anaphylaxis	Thong *et al.*, 2005, Singapore

Grand keyhole limpet	Boy 3 years, boy 9 years, boy 11 years, girl 11 years, man 27 years	Angioedema; urticaria; anaphylaxis, respiratory failure	Morikawa *et al.*, 1990, Japan
Grand keyhole limpet and abalone	Three females, 8 males, 7-49 years	Shock, respiratory failure, dyspnoea, urticaria, angioedema, vomiting	Maeda *et al.*, 1991, Japan
Abalone	Girl 17 years	Exercise-induced generalized symptoms	Dohi *et al.*, 1991, Japan
Clam	One patient	Anaphylaxis	Moneret-Vautrin *et al.*, 2002, France/Belgium
Clam	10 cases	Clinical hypersensitivity	Castillo *et al.*, 1996, Spain
Cockles	One patient	Anaphylaxis	Moneret-Vautrin *et al.*, 2002, France/Belgium
Snail	Two males age 12 and 13	Severe asthma	Tomás *et al.*, 1997, Portugal
Snail	Two children	Asthma, urticaria, vomiting	Meglio *et al.*, 2002, Italy
Snail	Girl 16 years	Asthma, generalized urticaria	De Maat-Bleeker *et al.*, 1995, The Netherlands
Snail	One case	Death, anaphylaxis	Pumphrey, 2004, Great Britain
Snail	Twenty-eight patients	Anaphylaxis, asthma, urticaria, rhinitis	van Ree *et al.*, 1996a, The Netherlands
Snail	One student	Death, anaphylaxis	Wu and Williams, 2004, China (Hong Kong)
Snail	Five females, five males, 11-70 years	Severe bronchospasm, angioedema, urticaria, diarrhoea	De la Cuesta *et al.*, 1989, Spain
Snail	Four patients 9-50 years	Asthma	Grembiale *et al.*, 1996, Italy
Snail	Seven patients	Asthma, face oedema, anaphylactic shock	Guilloux *et al.*, 1998 France
Snail	Boy 14 years	Exercise-induced anaphylaxis, seizures	Longo *et al.*, 2000, Italy
Snail	Girl 12 years	Urticaria, swelling of the lips, anaphylaxis	Peroni *et al.*, 2000, Italy
Snail	Three girls 9, 12, 13 years, one boy 12 years	Severe asthma, anaphylaxis	Pajno *et al.*, 2002, Italy
Snail	Boys 8 and 10 years	Facial oedema, asthma	Petrus *et al.*, 1997, France
Snail	Seven patients	Anaphylaxis, asthma, urticaria, facial oedema, swelling/ induration at former Der p hyposensitisation site	Vuitton *et al.*, 1998, France
Snail	Four patients	Anaphylaxis	Didier *et al.*, 1995, France
Snail	Twelve patients	Severe anaphylaxis, asthma, dyspnoea	Banzet *et al.*, 1992, France
Snail	36 patients, boys and girls, 10 to 18 years	Asthma	Palma Carlos *et al.*, 1985, Portugal
Snail	One patient	Anaphylaxis	Moneret-Vautrin and

			Kanny, 1995, Belgium/France
Snail	One patient	Anaphylaxis	Moneret-Vautrin *et al.*, 2002, Belgium/France
Snail	Five patients	Anaphylaxis or laryngeal oedema	Moneret-Vautrin *et al.*, 2004, Belgium/France
Snail	Fourteen patients	Severe asthmatic crisis , urticaria, angioedema	Ardito *et al.*, 1990, Italy
Oyster	Three patients	Anaphylaxis	Moneret-Vautrin *et al.*, 2002, Belgium/France
Oyster	Male 31 years	Exercise-induced food anaphylaxis	Maulitz *et al.*, 1979, USA
Oyster	Female 54 years	Anaphylaxis shock	González Galán *et al.*, 2002, Spain
Oyster	12 cases	Clinical hypersensitivity	Castillo *et al.*, 1996, Spain
Mussel	Eleven adults age 18-50 years	Angioedema, urticaria, nausea/vomiting/diarrhoea, rhinitis, asthma	Nettis *et al.*, 2001, Italy
Mussel	Two children	Anaphylaxis	Novembre *et al.*, 1998, Italy
Mussel	Ten cases	Clinical hypersensitivity	Castillo *et al.*, 1996, Spain
Mussel	One case	Anaphylaxis	Pastorello *et al.*, 2001, Italy
Mussel	One case	Anaphylaxis	Cianferoni *et al.*, 2001, Italy
Scallops	Grouped with patients reacting to shrimp and crab, no specific data	Anaphylaxis	Kemp *et al.*, 1995, USA
Turban shell	Girl 17 years	Exercise-induced anaphylaxis	Juji *et al.*, 1990, Japan
Squid	Five males, two females, 14-42 years	Asthma, urticaria/angioedema, nausea/vomiting/ diarrhoea, rhino-conjunctivitis	Carillo *et al.*, 1992, Spain
Squid	33 cases	Clinical hypersensitivity	Castillo *et al.*, 1996, Spain
Squid	One child	Anaphylaxis	Miyake *et al.*, 1987, Japan
Squid	Boy 6 years	Angioedema, asthma	Petrus *et al.*, 1999, France
Squid	Girl 18 years	Exercise-induced generalized symptoms	Dohi *et al.*, 1991, Japan
Cuttlefish	Girl 14 years	Dyspnoea, hoarseness, facial and neck swelling, diffuse urticaria	Caffarelli *et al.*, 1996, Italy
Cuttlefish	Girl 10 years, two occasions	Urticaria, anaphylaxis	Shibasaki *et al.*, 1989, Japan

4.2 Natural history of mollusc sensitisation

There are a few data on the age of onset and the lifetime course of mollusc allergy, but a number of case reports and patient series (Table 2) suggest that many of the reactions occur in school age children and young adults. The youngest case reported was a three year-old boy reacting to grand keyhole limpet. Thus, the age distribution of mollusc allergy appears to be different from that seen with eggs and milk and some other foods associated with onset of allergy in early infancy, probably due to the later introduction of molluscs into the diet.

5. IDENTIFIED MOLLUSC ALLERGENS

The protein tropomyosin has been documented as a major allergen in many molluscs (Leung *et al.*, 1996; Chu *et al.*, 2000) as well as crustaceans (Shanti *et al.*, 1993; Daul *et al.*, 1994; Leung *et al.*, 1994), and a minor allergen in one mollusc, i.e. snail (Asturias *et al.*, 2002). Tropomyosin is a water soluble and heat stable 34-36 kDa protein (Subba Rao *et al*., 1998) which has numerous isoforms (Rethinasamy *et al.*, 1997; Ishimoda-Takagi *et al.*, 1986) but is highly conserved and present in muscle and non-muscle cells.

Tropomyosin has been identified as an allergen and has to varying degrees been characterised at the protein and DNA levels in a number of mollusc species (formal allergen name in bold when given): garden snail (*Helix aspersa*) **Hel as 1** (Asturias *et al.*, 2002); clam (*Chlamus nobilis*) **Chl n 1** (Chu *et al.*, 2000); oyster *(Crassostrea gigas)* **Cra g 1** (Ishikawa *et al.*, 1997; 1998a; 1998c; Leung and Chu, 2001); abalone (*Haliotis discus, H. midae, H. rufescens, H. asinia*) **Hal d 1**, **Hal m 1**, **Hal r 1** (Lu *et al.*, 2004; Chu *et al.*, 2000; Lopata *et al.*, 1997); mussel (*Perna viridis)* **Per v 1** (Chu *et al.*, 2000); fan shell (*Pinna atropurpurea*) (Leung and Chu, 1998); scallop (*Mimachiamys nobilis*) **Mim n 1** (Lu *et al.*, 2004; Leung and Chu, 1998; Goetz and Whisman, 2000); squid (*Todarodes pacificus*) **Tod p 1** (Miyazawa *et al.*, 1996; Leung and Chu, 1998); octopus (*Octopus luteus*) (Leung and Chu, 1998); common whelk (*Buccinum undatum*) (Lee and Park, 2004; Leung and Chu, 1998); turban shell (*Turbo cornutus*) **Tur c 1** (Ishikawa *et al.*, 1998b).

Variation in tropomyosin epitopes, suggesting the presence of both common and more or less species-specific epitopes (Ayuso *et al.*, 2002a; Leung and Chu, 2001; Chu *et al.*, 2000; Ishikawa *et al.* 1998a, 1998b), is discussed below. Tropomyosin allergen sequences can be retrieved from the GenBank database (Chu *et al.*, 2000).

Importantly, tropomyosin is not the only mollusc allergen. Evidence for non-tropomyosin allergens has been reported in oyster (*Crassostrea gigas*) (Leung *et al.*, 1996; Leung and Chu, 1998), abalone (*Haliotis discus hannai*) (Choi *et al.*, 2003; Morikawa *et al.*, 1990; Maeda *et al.*, 1991), limpet (Azofra and Lombardero, 2003; Morikawa *et al.*, 1990; Maeda *et al.*, 1991), fan shell (Leung and Chu, 1998); scallop (Leung *et al.*, 1996; Leung and Chu, 1998; Goetz and Whisman, 2000; Goetz and Whisman, 1990), squid (*Todarodes pacificus*) (Leung *et al.*, 1996; Leung and Chu, 1998), cuttlefish *(Sepia esculenta)* (Lin *et al.*, 1993), octopus (Leung *et al.*, 1996; Leung and Chu, 1998), common whelk (*Buccinum undatum*) (Lee and Park, 2004; Leung *et al.*, 1996; Leung and Chu, 1998), pen shell (*Pinna atropurpurea*) (Leung *et al.*, 1996) and snail (Amoroso *et al.*, 1988; Guilloux *et al.*, 1998; Asturias *et al.*, 2002; Martins *et al.*, 2005). In snail, tropomyosin is a minor allergen (Asturias *et al.*, 2002). Some of the putative non-tropomyosin allergens (e.g. hemocyanin (Mistrello *et al.*, 1992; Morikawa *et al.*, 1990; Maeda *et al.*, 1991; Juji *et al.*, 1990; Koshte *et al.* 1989), myosin heavy chain (Martins *et al*., 2005), arginine kinase (Yu *et al.*, 2003; Binder *et al.*, 2001) and amylase (Azofra and Lombardero, 2003)), may constitute more or less "private" allergens (Carrillo *et al.* 1991, 1994; Azofra and Lombardero, 2003), whereas other non-tropomyosin candidate allergens may be more broadly cross-reactive allergens.

6. CROSS-REACTIVITIES

6.1 Cross-reactivity between molluscs

Epitopes of high similarity have been demonstrated in tropomyosins from different mollusc species (Ivanciuc *et al.*, 2002; Ayuso *et al.*, 2002a; Leung and Chu, 2001; Chu *et al.*, 2000; Ishikawa *et al.* 1998a, b), and many examples of cross-reactivity between molluscs *in vitro*, in skin prick testing and clinically have been published (Asturias *et al.*, 2002; Choi *et al.*, 2003; Chu *et al.*, 2000; Tanaka *et al.*, 2000; Martinez *et al.*, 1997; Maeda *et al.*, 1991; Miyake *et al.*, 1987; van Ree *et al.*, 1996a, b; Wu and Williams, 2004; Lopata and Potter, 2000; Lopata *et al.*, 1997; de Maat-Bleeker *et al.*, 1995). In the presence of reactivity to one mollusc species, reactivity to other species can therefore be expected. However, cross-reactivity between molluscs has often been found to be limited. Clinical cross-reactivity between only a limited number of mollusc species has often been observed, and molluscs in terms of cross-reactivity tend to fall into a number of loosely defined clusters that to some extent but not always reflect the taxonomic classification (Carrillo *et al.*, 1991, 1992, 1994; Shibasaki *et al.*, 1989; Castillo *et al.*, 1994; Azofra and Lombardero, 2003; de la Cuesta *et al.*, 1989; van Ree *et al.*, 1996a; Lee and Park, 2004; Choi *et al.*, 2003).

Among individuals reacting to scallops, clams, oysters, and mussels (n=67) in the study of Sicherer *et al.* (2004), 34 (51%) reacted to 1, 13 (19%) to 2, 5 (8%) to 3, and 15 (22%) to all four species. In the study of Wu and Williams (2004) from Hong Kong, the sensitisation rate to different molluscs in 70 patients sensitised to shellfish was 13% for scallop, 16% for clam, 21% for oyster, 32% for abalone, and 45% for limpet. Further, whereas sensitisation to scallop gave a probability of sensitisation to limpet of 0.88, sensitisation to oyster gave a probability of sensitisation to clam of only 0.33. These numbers, as well as numbers from other studies (Zinn *et al.*, 1997; Lopata *et al.*, 1997) and the clinical observations referred to above, indicate substantial differences in clinical allergy as well as sensitisation to different molluscs in the same individual, consistent with limited cross-reactivity (Wu and Williams, 2004).

Studies at the molecular level, demonstrating that mollusc tropomyosins do not always have IgE-binding epitopes in the same regions, support the notion that cross-reactivity between molluscs is limited compared to cross-reactivity between crustaceans (Chu *et al.*, 2000; Ishikawa *et al.*, 1998a, b, c, and 1999).

In relation to land and sea snails, van Ree *et al.* (1996a) found no significant difference in allergens between land and sea snails, whereas Vuitton *et al.* (1998) found that only 4 out of 7 patients with clinically manifest allergy to terrestrial snails also reported reactions upon eating sea snails.

6.2 Cross-reactivity between molluscs and crustaceans

Cross-reactivity between molluscs and crustaceans clinically (Castillo *et al.*, 1997; Carrillo *et al.*, 1992; Vuitton *et al.*, 1998; Ishikawa *et al.*, 1998 a and b; Ishikawa *et al.*, 1999; Lehrer and

McCants, 1987) or serologically (Asturias *et al*., 2002; Carillo *et al.*, 1992; Shibasaki *et al.*, 1989; McCants and Lehrer, 1986; Goetz and Whisman, 1990, 2000; Leung and Chu, 1998, 2001; Leung *et al.*, 1996; Miyazawa *et al.*,1996; Koshte *et al*., 1989; Santos *et al*., 1999; Petrus *et al.* 1999; Lee and Park, 2004; Lin *et al.*, 1993; Choi *et al.*, 2003; DeWitt *et al.*, 2004; van Ree *et al.*, 1996b; Wu and Williams, 2004) is reported in many publications and must be expected in medical practice.

However, in individual patients, cross-reactivity between molluscs and crustaceans is not always found. Of 61 clinically mollusc allergic individuals, only 8 (13%) showed IgE-binding against crustaceans or fish (Lopata and Jeebhay, 2001). Analogous to the separation of molluscs into clusters with limited cross-reactivity, cross-reactivity between molluscs and crustaceans is often restricted to a few species of crustaceans and molluscs. There are several reports of individuals reacting clinically to both to some mollusc and some crustacean, for example squid and shrimp (Carrillo *et al.*, 1992), while tolerating other crustaceans and molluscs, and a similar situation is reported with regard to serological cross-reactivity (Carrillo *et al.*, 1992, 1994; Castillo *et al*., 1994; Ishikawa *et al.*, 1998b). In the study of Sicherer *et al.* (2004), only forty-one persons (14%) with shellfish allergy reported allergy to both one or more crustaceans and one or more molluscs. Wu and Williams (2004) at a university clinic performed skin testing with different molluscs and crustaceans in 84 consecutive patients reporting shellfish allergy. Of 70 patients with positive shellfish tests, roughly one third (25) were sensitised to crustaceans only, roughly one third (27) were sensitised to both crustaceans and molluscs, and roughly one third (18) were sensitised to molluscs only. Ninety percent of patients were dust mite sensitised, which may have increased apparent cross-reactivity between crustaceans and molluscs. Notably, even in the presence of cross-sensitisation to other molecules (van Ree *et al.*, 1996b), sensitisation to tropomyosin in one allergenic food, e.g. snail, is not always accompanied by sensitisation to tropomyosin in another allergenic food, e.g. shrimp.

The extent to which mollusc allergy may be secondary to crustacean allergy (or mite or cockroach allergy, is somewhat uncertain, but the numbers cited above indicate that it is limited. However, Laffond Yges (1996) reports on 38 patients, of which 25 were found to be sensitized to both crustaceans and molluscs, 12 to crustaceans only, and only 1 to molluscs only. Further, Crespo *et al.* (1995b) found that of 24 shellfish allergic children, 23 were allergic to crustaceans, while 10 were allergic to molluscs. Nine of the ten children allergic to molluscs were, according to the authors, equally allergic to crustaceans. These findings suggest that crustacean allergy could sometimes be the primary allergy in patients reacting against molluscs. However, cross-inhibition studies sometimes suggest dual sensitisation (Goetz and Whisman, 1990, 2000; van Ree *et al.*, 1996a). Primary mollusc sensitisation has been described in relation to cross-reactivity with mites (Martins *et al*., 2005; van Ree *et al.*, 1996a).

The limited cross-reactivity between molluscs and crustaceans may reflect a substantial diversity of mollusc allergens, which complicates also cross-reactivity patterns.

6.3 Cross-reactivity of molluscs with house-dust mite, cockroach and helminths

A number of examples of concomitant clinical hypersensitivity or *in vitro* IgE-binding between crustaceans, molluscs, insects, arachnids and nematodes suggest that there is one or more cross-reactive allergens causing clinical cross-reactivity between invertebrates of the phyla *Mollusca* and *Arthropoda* and insects like cockroach (Azofra and Lombardero, 2003;

Ayuso *et al*., 2002b; Banzet *et al.*, 1992; Barberio *et al*., 1992; Carrillo *et al.*, 1991, 1992; Castillo *et al*., 1994; Crespo *et al*., 1995b; Martinez *et al*., 1997; Pascual *et al*., 1997; Petrus *et al.*, 1997, 1999; Reese *et al.*, 1999; van Ree *et al.*, 1996a and b; O'Neil *et al*., 1985; Witteman *et al*., 1994; Koshte *et al*., 1989). Clinically relevant cross-reactivity between molluscs and house-dust mite has been described in particular for snail and mite (see below), but also for e.g. limpet and mite (Azofra and Lombardero, 2003). Sometimes crustaceans are also involved, and the term "mite-crustacean-mollusc-syndrome" (Milben-Krustazeen-Mollusken-Syndrom in German) has been used (Kütting and Brehler, 2001).

The dust mite allergens Der p 10, Lep d 10 and Der f 10 (Aki *et al.*, 1995; Asturias *et al.*, 1998) and cockroach (*Periplaneta americana* and *Blatella germanica*) allergens Per a 7 and Bla g 1 (Asturias *et al*., 1999; Santos et *al*., 1999; Pomes *et al*., 1998) are tropomyosins. Tropomyosin is a major allergen in mites (Aki *et al*., 1995). Tropomyosins of several mollusc, mite, and insect species share similar epitopes, and show an overall sequence identity of about 50-60% (Ivanciuc *et al*., 2002; Leung and Chu, 2001; Santos *et al*., 1999; Aki *et al*., 1995; Daul *et al*., 1994; Leung *et al*., 1994; Shanti *et al*., 1993; Jeong *et al*., 2004; Ayuso *et al*., 2002a and b). However, as indicated above, there are a number of not yet well described non-tropomyosin allergens in molluscs, and there are indications that these non-tropomyosin allergens play sometimes a dominant role in cross-reactivity between molluscs and house-dust mite, cockroach and other insects (Guilloux *et al*., 1998; van Ree *et al*., 1996a).

6.3.1 Snail and mite allergy

A strong association between house dust mite allergy and snail allergy has been reported by several investigators (Palma Carlos *et al*., 1985; Ardito *et al*., 1990; Martins *et al*., 2005; Banzet *et al*., 1992; Barberio *et al*., 1992; De Maat-Bleeker *et al*., 1995; Azofra and Lombardero, 2003; Peroni *et al*., 2000; van Ree *et al*., 1996a and b; Didier *et al*., 1995; Pajno *et al*., 2002, 1994; Vuitton *et al*., 1998; Longo *et al*., 2000), and can be said to be part of the "mite-shellfish-mollusc-syndrome" (see above). Although there is evidence for primary sensitisation to snail in some patients (Martins *et al*., 2005; van Ree *et al*., 1996a), primary sensitisation appears most often to be against mite in the studies referred to. Asthma appears to be a prominent symptom after ingestion of snails, perhaps reflecting the airways as the primary reactive organ in mite allergy.

Tropomyosin (Der p 10) appears to play only a minor role in snail-mite cross-reactivity (Guilloux *et al*., 1998; van Ree *et al*., 1996a and b; Asturias *et al*., 2002). Multiple allergens seem to be involved (van Ree *et al*., 1996a; Martins *et al*., 2005), e.g. possibly Der p 4 (amylase), Der p 5, Der p 7, and hemocyanin (Mistrello *et al*., 1992). Hemocyanin occurs in two *Phyla* of invertebrates (*Mollusca* and *Arthropoda*) and represents 90-98% of hemolymph protein content (Ellerton *et al*., 1983).

6.3.2 The fish parasite Anisakis simplex and mollusc allergy

Since about 1990, fish parasites of the genus *Anisakis* have been recognised to contribute to fish-related allergic reactions including anaphylaxis (Kasuya *et al*., 1989, 1990; Audicana *et al*., 1995; Purello-D'Ambrosio *et al*., 2000). 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. Whereas in most cases live *Anisakis* larvae may be necessary to induce sensitisation (Baeza *et al*., 2004), reactions can be elicited even when the fish is cooked. An association between *Anisakis* sensitisation and *Ascaris*, *Daphnia*, chironomids, dust mites, Atlantic shrimp, and German cockroach has been described (Kennedy *et al.*, 1988; Pascual *et al.*, 1997; Johansson *et al.*, 2001). There are clinical observations suggesting cross-reactivity between crustaceans (shrimp), molluscs (oysters in the case cited) and *Anisakis* (González Galán *et al.*, 2002). There is, in theory, a possibility that individuals reacting clinically to fish because of *Anisakis* infestation might also react to mollusc (and possibly crustaceans), which would appear as fish-mollusc cross-reactivity.

The notion of clinical cross-reactivity between molluscs, crustaceans and *Anisakis*, possibly caused in part by tropomyosin, is supported also by *in vitro* immunological studies (Pascual *et al.*, 1997; Martinez *et al.*, 1997; Asturias *et al.*, 2000) and sequence homology data (Ayuso *et al.*, 2002b; Ivanciuc *et al.*, 2002). However, *Anisakis* has several other allergenic molecules than tropomyosin (Martinez *et al.*, 1997; Arlian *et al.*, 2003; Caballero and Moneo, 2004; Moneo *et al.*, 2005), and cross-reactivity between *Anisakis* and dust mites (Johansson *et al.*, 2001) apparently is not due to tropomyosin (Bernardini *et al.*, 2005).

6.4 Absence of cross-reactivity between mollusc and vertebrate tropomyosins and myosins

Vertebrate tropomyosins appear to be non-allergenic (NDA, 2004; Leung and Chu, 2001). Invertebrate myosins, found to be a major snail allergen, did not cross-react with chicken, pig, rabbit, cow and horse myosins (Martins *et al.*, 2005).

7. POSSIBLE EFFECTS OF FOOD PROCESSING ON ALLERGENICITY, AND DERIVED PRODUCTS

Tropomyosin allergenicity has been reported to be heat-resistant (Leung *et al.*, 1994, Tabka *et al.*, 1998, Castillo *et al.*, 1994, Guilloux *et al.*, 1998, Ishikawa *et al.*, 1997; Miyazawa *et al.*, 1996). Some putative mollusc allergens other than tropomyosin have been reported to be destroyed by heating (Vuitton *et al.*, 1998; Lee and Park, 2004). Reported clinical allergic reactions against molluscs often were elicited by food prepared by heating, and it appears that mollusc allergenicity is not reliably reduced by heat treatment and food processing.

A number of reports suggest that mollusc allergenicity may be increased by heating (Carrillo *et al.*, 1991, 1992, 1994; Goetz and Whisman, 2000; Ishikawa *et al.*, 1997; Lopata and Potter, 2000; Castillo *et al.*, 1994; Nettis *et al.*, 2001), and one study on scallop tropomyosin (Nakamura *et al.*, 2005) identifies the Maillard reaction as one mechanism for increased IgE-binding capacity after heating.

8. METHODS OF ANALYSIS

8.1 Identification

Lopata *et al.* (2002) developed a monoclonal antibody assay for species-specific identification of abalone for forensic purposes. A PCR technique was developed for similar purposes (Sweijd *et al.*, 1998).

8.2 Quantification

There appear to be no commercially available assays for the quantification of mollusc tropomyosins or other mollusc allergens. There is an ELISA test kit for the detection of crustacean tropomyosin on the market (Poms *et al.*, 2003). It is not known whether this will detect also mollusc tropomyosin. Cross-reactivity of allergens of some molluscs with those of crustaceans and sometimes other species, with simultaneous absence of cross-reactivity with other molluscs is a reason for concern. For example, an antibody raised against abalone tropomyosin recognised abalone, crustaceans and chicken, but no other molluscs than abalone (Lu *et al.*, 2004).

9. DOSES TRIGGERING CLINICAL REACTIONS

Specific data on doses of mollusc triggering food allergic reactions are rare in the literature. The reported respiratory reactions to vapours when cooking molluscs and to passive exposure via clothing suggest that triggering doses in airway exposure may be very low (Jeebhay *et al.*, 2000; Desjardins et *al.*, 1995; Carrillo *et al.*, 1992; Nava *et al.*, 1983; Clarke, 1979). Maulitz *et al.* (1979) report that 100 g of canned oysters triggered exercise-induced anaphylaxis. Wu and Williams (2004) report that fatal anaphylaxis occurred after ingestion of three snails. In DBPCFC (double blind placebo controlled food challenge), the cumulative dose of dried snail causing 20% decrease in FEV_1 (forced expiratory volume, a measure of lung function) was 120 mg and 400 mg, respectively, in two children (Pajno *et al.*, 2002).

CONCLUSIONS

Molluscs are a large and diverse group, which includes some 100,000 species living in salt water, in freshwater and on land. Some molluscs are an important food source. Molluscs are used as an added ingredient in some processed foods like soups and sauces and in products like surimi. Case reports and patient series indicate that several molluscs, e.g. snail, oyster, clams, mussels, squid, abalone and octopus, can cause food allergic reactions, some of them life-threatening. Prevalence of self-reported mollusc allergy ranges from about 0.15% (4/2716) in school children in France to about 0.4% (or 20% of all seafood allergic cases) in a household survey of 14,948 individuals in the US.

A major allergen of most molluscs is the abundant muscle protein tropomyosin, which has been well characterised in several mollusc species. Tropomyosin is an allergen also found in crustaceans, dust mites, and cockroach and other insects. Characterised tropomyosins in molluscs and different crustaceans show similarities but also significant differences in allergenic structures. In addition to tropomyosin, there is evidence that molluscs contain a number of other allergens, but these are poorly defined. Serological and clinical cross-reactivity between mollusc species, as well as between molluscs and crustaceans and house dust mite has been reported. Mollusc allergens do not cross-react with fish allergens. However, there is a possibility that patients reacting to fish infested with the parasite Anisakis might also react to molluscs due to cross-reactivity.

Mollusc allergenicity is not reliably reduced by food processing. Tropomyosin allergenicity is heat-resistant. Although the allergenicity of some other mollusc allergens appears to be destroyed by heat treatment, there are also reports of increased allergenicity after heating.

There is little information on the lowest dose of mollusc allergen that can cause a clinical reaction. In one double blind placebo controlled food challenge study with dried snail reactions were observed in the low hundred-mg range.

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ACKNOWLEDGEMENT

The Scientific Panel on Dietetic Products, Nutrition and Allergies wishes to thank Elide Pastorello, Martin Stern, and Jean-Michel Wal for their contributions to the draft opinion.