

**Opinion of the Scientific Panel on Dietetic Products, Nutrition and Allergies
on a request from the Commission related to a notification from DSM on
fish gelatine for use as a formulation aid (carrier) in vitamin and carotenoid
preparations pursuant to Article 6, paragraph 11 of Directive 2000/13/EC-
for permanent exemption from labelling**

(Request N° EFSA-Q-2006-161)

(Adopted on 15 October 2007 by written procedure)

SUMMARY

Fish is an important allergenic food. Fish allergy has been reported to affect 0.2%-2.2% of the population in European countries. Allergic reactions to fish can be severe. The major allergen of fish is the muscle protein parvalbumin. The present application concerns fish gelatine produced from fish skin for use as a formulation aid (carrier) in vitamin and carotenoid preparations. Gelatine is made by denaturation of collagen.

Allergens of concern are residual amounts of parvalbumin, and gelatine itself. The information provided by the applicant indicates that the production process of gelatine from fish skins for this particular purpose is well standardized. A monoclonal and a polyclonal ELISA assay for measuring parvalbumin in fish gelatine have been developed, with a limit of detection of 1µg/g and 0.04µg/g, respectively. None of the assays detected parvalbumin in ten commercial lots of gelatine. According to the applicant, daily fish gelatine intake from vitamin preparations intended for use in food supplements, colourings and beverages is in the low milligram range. Estimation of the highest concentration of fish gelatine in vitamin containing preparations available on the market, indicates a concentration of 30mg per litre, or 7.5mg per 250ml serving. Assuming a parvalbumin content in gelatine of 0.04µg/g, the estimated intake of parvalbumin with one serving will be 0.0003µg.

In a double blind placebo controlled food challenge study, none of 30 fish-allergic patients tested reacted to a cumulative dose of 3.6g of codfish gelatine, while a confirmed mild reaction was experienced by one individual at a cumulative dose of 7.6g.

Taking into account the information available, the Panel considers that it is unlikely that fish gelatine used as a formulation aid (carrier) in vitamin and carotenoid preparations will trigger an adverse allergic reaction in susceptible individuals under the conditions of production and use specified by the applicant.

KEY WORDS

Fish gelatine, carrier, vitamin preparations, carotenoid preparations, parvalbumin, food allergy.

BACKGROUND

In November 2003, the European Parliament and the Council adopted Directive 2003/89/EC¹ amending Directive 2000/13/EC, as regards indication of the ingredients present in foodstuffs.

Annex IIIa of the Directive specifies a list of food ingredients or substances that are known to trigger allergic reactions or intolerances in sensitive individuals for which no labelling exemptions are allowed. Whenever the listed ingredients/substances or their derivatives are used in the production of foodstuffs, they must be labelled.

Article 1, paragraph 11, subparagraph 2 of the Directive establishes a procedure allowing for temporary labelling exemption of derivatives from ingredients listed in Annex IIIa for which it has been scientifically established that it is not possible for them to cause adverse reactions. In accordance with this provision, submissions of request for temporary labelling exemption were notified to the Commission before 25 August 2004. The Commission, after consultation with the European Food Safety Authority, adopted a list (Directive 2005/26/EC²) of those ingredients which are temporarily excluded from Annex IIIa until 25 November 2007, pending the final results of the notified studies.

Consequently, applicants who submitted a dossier in 2004 on the basis of subparagraph 2, resulting in the inclusion of a product in the list of Directive 2005/26/EC, and who are seeking exclusion of that product from Annex IIIa beyond 25 November 2007 will have to submit a request enclosing the final results of the notified scientific studies. Therefore in the context of the permanent labelling exemption procedure, the European Food Safety Authority is asked to provide scientific opinions on the submissions in accordance with the present terms of reference.

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 evaluate the scientific data submitted by DSM Nutritional Products in the framework of the procedure laid down in Article 6, paragraph 11 of Directive 2000/13/EC. On the basis of that evaluation, EFSA is requested to issue an opinion on the information provided, and particularly to consider the likelihood of adverse reactions triggered in susceptible individuals by the consumption of the following ingredients/substances used under the conditions specified by the applicant: fish gelatine for use as a formulation aid (carrier) in vitamin and carotenoid preparations.

ASSESSMENT

The extent of the fish-allergic population is uncertain. A Danish population-based study with double blind placebo controlled food challenge (DBPCFC) verification of allergy (Osterballe *et al.*, 2005) found 0.2% of the adult population allergic to codfish. The total number of fish-

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. OJ L 308. 25.11.2003, p. 15.

² Commission Directive 2005/26/EC of 21 March 2005 establishing a list of food ingredients or substances provisionally excluded from Annex IIIa of Directive 2000/13/EC of the European Parliament and of the Council. OJ L 75, 22.03.2005, p. 33-34.

allergic individuals may be somewhat higher, because some fish-allergic individuals do not react to codfish. Two population-based American studies based on telephone surveys (Sicherer *et al.*, 2004; Vierk *et al.*, 2007) gave numbers in the same range, whereas in a European questionnaire-based study (Woods *et al.*, 2001) 2.2% reported (self-perceived) fish allergy or intolerance.

Taking into account the numerous and well documented reports of allergic individuals (NDA, 2004a) reacting to fish, it is appropriate for the Panel to assess the likelihood of adverse reactions in allergic individuals consuming products containing gelatine made from fish skins.

A dossier submitted by DSM Nutritional Products to the European Commission pursuant to Article 6, Paragraph 11 of Directive 2000/13/EC as amended by Directive 2003/89/EC on 23 August 2004 for temporary exemption from labelling was the basis for an earlier assessment of gelatine by the NDA panel (NDA, 2004b). The present opinion is based on an updated dossier from the same organization, with an application for permanent exemption from labelling. The updated dossier contains new data mainly with regard to analytical studies on residual amounts of parvalbumin in gelatine.

1. Manufacturing process

1.1 Procedures and quality control

Gelatine is produced by extraction and hydrolysis of fibrous, insoluble collagen from fish skin, as described in the opinion regarding temporary exemption (NDA, 2004b). The production process consists of several steps of extensive washing, acidification, heating and filtration. Thereafter, dry gelatine is produced by evaporation of water from a thin film of liquid concentrate. Representative samples are taken during the subsequent grinding process and sent to an independent laboratory for testing to comply with set specifications. The applicant follows a Quality Management Plan and refers to a standardised manufacturing process for the specified use of the product.

1.2 Removal of parvalbumin during production

Mostly parvalbumins have been isolated from fish muscle. However, it is uncertain whether parvalbumin has been looked for in fish skin and fish scales. The allergenicity of fish parvalbumin is not easily destroyed by heat, proteolytic activity or denaturation with chemicals. The applicant states that during washing of fish skins as part of the production process “the water-soluble allergens are substantially reduced”. Documentation is provided in the form of data on falling levels of parvalbumin in the washing water with increasing numbers of wash cycles. However, the data are derived from a model system measuring parvalbumin in washing water and not in skin extracts. Quantification in relation to washing was attempted only by SDS-PAGE and Western blotting using a monoclonal antibody to tuna parvalbumin. The Panel notes that the described procedures are not sensitive or specific enough to enable the determination of low amounts of parvalbumin in fish skin extracts.

2. Characterisation of the product and its use

2.1 Characterisation of fish gelatine

Fish gelatine is denatured collagen from fish skins. Only the skins of certain cold water fish are used, with cod, pollock, and haddock being the most common species.

Fish gelatine has an approximate molecular weight of 60,000Da. The CAS (Chemical Abstract Services) number is the same for all gelatines including fish gelatine: CAS # 9000-70-8. The applicant provides specifications of the product they use, and states that they are in compliance with EU specifications, European Pharmacopoeia, US Pharmacopoeia and Japanese Pharmacopoeia.

2.2 Conditions of use and exposure levels

2.2.1 Conditions of use

Under the present application, fish gelatine is used for the micro-encapsulation of oil soluble substances, specifically vitamins A, D, E and carotenoids (e.g. β -carotene, canthaxanthin, lutein, lycopene) to bring the substances into suitable form for incorporation into processed foods like fruit-based beverages (so-called ACE drinks) and multivitamin drinks. Carotenoids are also used as food colours. The encapsulation process is described by the applicant. The products are water-dispersible and the nutrients are bioavailable. The use of fish gelatine as a formulation aid (carrier) in vitamin/carotenoid preparations is protected by a patent owned by the applicant.

2.2.2 Exposure levels

The applicant claims that the amount of fish gelatine in the final products introduced by vitamin and carotenoid preparations is very low (see below). The amount of fish gelatine present in a number of fortified or coloured foods or beverages usually ranges from 2 to 20 mg/kg.

2.2.2.1 Fish gelatine intake calculated on the basis of typical reference values for vitamins

Reference values for nutrition labelling are currently laid down for vitamins and minerals (Council Directive 90/496/EEC). Relevant for this application are reference values for vitamins A (β -carotene), D and E. For a set of product forms listed by the applicant, estimated potential intakes of fish gelatine per day are as follows: 2.0-7.2mg fish gelatine for vitamin A/ β -carotene preparations, 6.0mg fish gelatine for vitamin E and 1.5mg fish gelatine for vitamin D preparations. Based on these calculations, the applicant states that the intake of fish gelatine per day will be below the tolerated dose of 3.6g reported by Hansen *et al.* (2004).

2.2.2.2 Fish gelatine intake calculated on the basis of existing products on the European market

German data are used, as this country has the highest consumption of the relevant products and is where the highest-dosed products can be found. According to the applicant, ACE and multivitamin beverages contain maximum 20mg β -carotene per litre (self-limitation of the German industry). The applicant estimates that the concentration of fish gelatine is 30mg per litre, or 7.5mg per 250ml serving. Consumption of 1.5 litres per day would result in 45 mg fish gelatine daily. Additional calculations based on seven different beverages on the German market give fish gelatine contents per 250ml serving over a range from 6.3 to 7.2mg, which are below the claimed lowest reported triggering dose. However, it must be taken into

consideration that a number of other sources (e.g. yogurt and cakes) may contribute to the total fish gelatine intake.

2.2.2.3 Parvalbumin intake with fish gelatine

Assuming a maximum parvalbumin content in gelatine of 0.04µg/g (LOD of the most sensitive ELISA, see below), and an intake of one serving with 7.5mg gelatine (see above) the estimated intake of parvalbumin with one serving will be 0.3 ng. Considering that the extractable amount of parvalbumin has been found to be 1.3µg/mg in cod (study in the dossier), 1.6µg/mg in mackerel (Kobayashi *et al.*, 2006), and 3.7-4.9µg/mg in carp (Vornanen *et al.*, 1999), the dose of parvalbumin in one serving appears to be lower than the lowest dose of parvalbumin till now reported to elicit a reaction in a DBPCFC study (low mg range of fish (Taylor *et al.*, 2002)). The Panel notes, however, the uncertainty with regard to the yield when extracting parvalbumin from fish meat, the uncertainties with regard to the accuracy of both the ELISA assays used, and the uncertainty with regard to establishing a triggering dose in allergic reactions to fish.

3. Evidence of non-allergenicity

3.1 History of non-allergenicity of the product

3.1.1 Literature search strategy

No formal literature search is described by the applicant, neither in the previous nor the present application. However, the applicant states that a literature search with a focus on new papers since 2003 was undertaken.

3.1.2 Historical evidence of safe use

The applicant claims that a history of safe use is established. Fish gelatine-containing vitamin and carotenoid preparations have been on the market since 1992. The applicants state their unawareness of any documented report about allergic reactions due to the presence of fish gelatine in the products in question.

It should, however, be mentioned that lack of clear labelling may be the reason for the absence of reports as consumers and health professionals would not be aware of the constituents of the products consumed, and therefore would not have suspected or reported fish gelatine as a cause for an allergic reaction.

3.2 Preclinical studies on allergenicity

3.2.1 Parvalbumin cross-reactivity

An important factor in allergenicity studies of fish parvalbumins is the degree of cross-reactivity between different fish species, and the species specificity of antibodies used. At least 31 parvalbumins have been described (Allergome database, 2007). Fish parvalbumins are assumed to be broadly cross-reactive (NDA, 2004a). However, clinical practice as well as laboratory experience indicate that cross-reactivity between fish species sometimes is limited or absent. An example was found in the present application, where an anti-carp monoclonal antibody employed in an ELISA showed binding to only five of seven fish species tested.

Bioinformatics analysis was undertaken to compare available amino acid sequences from commonly consumed fish parvalbumins, among them carp, cod and pollack. Homology studies of 18 fish parvalbumins from 10 species, based on publicly available sequences (NCBI, 2007), are reported. A given fish species contains usually 2-5 different parvalbumins. Two Atlantic and one Baltic cod parvalbumins were 63% to 74% identical, and parvalbumins from the other fish were from 53% to 92% identical to the three codfish parvalbumins. Carp parvalbumin shared 65%-85% identical sequences with cod parvalbumins. The three fish species that according to the applicant are most used for gelatine production (cod, haddock and pollack) all belong to the same family, *Gadidae*, and therefore are presumed to be more cross-reactive than more distantly related fish. Referring to the literature (Albersee, 2000), the applicants conclude that high levels of sequence identity were observed that indicate a high likelihood of IgE cross-reactivity. This suggests that antibodies to one or two of these parvalbumins could serve as reagents for detection of parvalbumins from a wide range of fish species. The Panels notes that the use of antibodies raised against parvalbumin from one species of fish entails a certain uncertainty, which may affect the detection and measurement of parvalbumin from other fish species.

No data have been presented regarding fish gelatine cross-reactivity.

3.2.3 *Analytical data on parvalbumin levels in fish gelatine*

A commercially available monoclonal antibody against frog parvalbumin was found to react in immunoblotting studies only with parvalbumin from some less relevant fishes and not codfish.

3.2.3.1 Monoclonal anti-carp parvalbumin

A commercially available monoclonal antibody against carp parvalbumin was used to develop a competitive ELISA. This ELISA was found to have an LOD of 0.05µg/ml, which according to the applicant is equivalent to 1µg/g in a solid sample. The sensitivity was verified by spiking experiments with known amounts of carp parvalbumin. Ten commercial batches of gelatine were analysed with this ELISA, none of them showing parvalbumin levels above the detection limit.

3.2.3.2 Polyclonal anti-cod parvalbumin ELISA

A rabbit polyclonal antibody made against specially purified cod parvalbumin was used as capture antibody. Due to technical difficulties an inhibition ELISA was not achieved, and a sandwich ELISA was developed. The LOD for cod parvalbumin with this ELISA is reported to be 0.04µg/g. Only cod parvalbumin was tested. Ten commercial batches of gelatine were analysed also with this ELISA, none of them showing parvalbumin levels above the LOD.

3.2.4 *IgE binding studies with fish gelatine*

A comprehensive overview over fish collagen and gelatine IgE reactivity was given with the opinion in relation to the application for temporary exemption (NDA, 2004b).

In brief, experimental studies showed that collagen's IgE binding capacity was thermostable and preserved also in peptide fragments. A number of studies, in particular from Japan, have reported human IgE binding to fish collagen and gelatine (NDA, 2004b), but in none of these studies were DBPCFC with collagen or gelatine performed. There are no reports of challenges

with fish collagen or gelatine apart from the two studies cited below. It must be emphasised that the presence of IgE-binding to gelatine does not equate with clinical allergy, and the clinical importance of fish gelatine as an allergen is uncertain.

3.2.5 Digestibility of fish gelatine

Fish gelatine was analysed for digestibility by pepsin. Peanut allergens were used as a reference. Compared to certain peanut allergens, fish gelatine digested quickly. The Panel notes that this information alone is of limited value when considering allergenicity.

3.3 Clinical studies

The two clinical studies outlined below are described and discussed in more detail in the opinion for temporary exemption (NDA, 2004b). Regarding challenge studies in fish allergic individuals with specific IgE to fish gelatine, the applicant states that it would in practice be impossible to find a sufficient number of such individuals available for testing, since the frequency of IgE seropositivity to fish gelatine in Europe appears to be in the order of 3% among fish allergic individuals.

3.3.1 Food challenge studies with fish gelatine

In a DBPCFC study by a Danish group (Hansen *et al.*, 2004), no adverse reactions were observed when 30 codfish allergic patients were given a cumulative dose of 3.6 g of codfish skin-derived fish gelatine. When given a cumulative dose of 7.6 g, two patients experienced a mild reaction, confirmed in repeated challenges in one of the two. There was no reaction to the next higher dose.

In a French single-blind study (André *et al.*, 2003) of 100 children and adults with IgE reactivity to various species of fish, fish (tuna) skin-derived collagen was used. It was found that 3 out of 100 sera showed IgE binding to tuna skin-derived gelatine. The three seropositive individuals were skin tested with fish gelatine, and the tests were negative. Similarly, a food challenge test using 5 g of tuna gelatine was also negative in these three subjects. The Panel notes that the allergenicity of tuna in several clinical and laboratory studies appears to be comparatively low.

CONCLUSIONS

Taking into account the information available, the Panel considers that it is unlikely that fish gelatine used as a formulation aid (carrier) in vitamin and carotenoid preparations will trigger an adverse allergic reaction in susceptible individuals under the conditions of production and use specified by the applicant.

DOCUMENTATION PROVIDED TO EFSA

Dossier submitted by DSM Nutritional Products to the European Commission pursuant to Article 6 Paragraph 11 of Directive 2000/13/EC as amended by Directive 2003/89/EC, on 29 September 2006.

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