Opinion of the Scientific Committee on Veterinary Measures relating to Public Health - Allergic reactions to ingested *Anisakis Simplex* antigens and evaluation of the possible risk to human health - 27 April 1998

Terms of reference:

The Committee was asked to provide an opinion concerning allergic reactions to ingested *Anisakis simplex* antigens, and to evaluate the possible risk to human health

1. Background: *Anisakis, anisakidosis*

Migrant larvae of several nematodes genera may reach humans by ingestion of infected fish, molluscs or crustaceans when they are consumed raw or not adequately salted, pickled smoked, or cooked. *Anisakis, Pseudoterranova,* and *Contracaecum spp* are the most relevant for public health (1) *Anisakis simplex,* belonging to the family heterocheilidae, live in the intestine of sea mammals and its larva infects small crustaceans (L1 stage), then cephalopods (squid) and fish (especially herring, mackerel, hake, blue whiting, cod...) (L3 stage). At fishing, when the fish dies, some larvae will migrate from the intestine to the abdominal cavity and the fish flesh. These migrated larvae may be ingested by the consumer. In most cases the larvae die in the lumen of the gastrointestinal tract when they are ingested by humans. Sometimes, they penetrate into the mucosa and elicit an inflammatory reaction which in a few cases lead to a severe eosinophilic granuloma associated with clinical symptoms. The disease "anisakiasis" (a better denomination should be "anisakidosis", according to the recent recommendations of Societies of Parasitology, at the international level), represents the clinical situation related to infection by living *Anisakis* larvae. Location of the larvae may be gastric, intestinal or ectopic. Anisakidosis may present as peptic ulcer disease, as a case of acute abdomen, as a bowel obstruction, or as abdominal pain, either vague or intense, with or without vomiting. It may or may not be associated with allergic symptoms. Due to the vagueness and/or the diversity of its symptoms, the disease is often misdiagnosed as appendicitis, peritonitis, gastric ulcer or tumor, ileitis, cholecystitis, diverticulitis, tuberculosis, peritonitis, cancer of the pancreas, or Crohn's disease (2, 3).

Although the first case was described by Leuckart in Groenland in 1876, the disease was more widely recognized and studied in the nineteen fifties and sixties when real "epidemics" of anisakidosis occurred in the Netherlands following ingestion of "green" (i.e. lightly salted) herring (154 proven cases between 1955 and 1968) (4, 5). The highest numbers of cases come from Japan (up to 1,000 cases per year) due to the very common and popular consumption of raw fish (sushi and sashimi) but were more usually related to *Pseudoterranova* infection which is able to give similar lesions and symptoms (6, 7). There was some controversy about the pathophysiology of peri- *Anisakis* granulomas and, thus, of the occurrence of clinical symptoms after *Anisakis* larvae ingestion. It was suggested that it represented a hypersensitivity reaction and, thus, that a natural or an acquired allergic predisposition was required. Some clinical observations were in keeping with an allergic nature of the illness. Experimental studies have given discording results: in one study, a second challenge 4 months after the primary infection in rabbits gave a stronger local reaction than the initial one; however another study with a prolonged follow-up of the primary infection, and challenge infections after oral administration or direct introduction in the appendix, demonstrated that one single larva, without any previous sensitization, was able to induce the granulomatous reaction, hence the disease, but that the resulting inflammatory reaction was very variable (8).

2. Scientific reasoning

2.1. Anisakis allergy

Besides anisakidosis, which has been successfully prevented in some countries by warning the public of the risk of eating raw fish and by freezing fish before consumption, the allergenic potency of *Anisakis* antigens, irrespective of *Anisakis* larvae ability to actively penetrate into the gastro-intestinal tract, was pointed out by Kasuya et al. in 1990 (9). A case of anaphylaxis after eating fish in a 52 year old woman otherwise non-allergic to fish was reported in Spain in
To prove the responsibility of Anisakis in the periodic anaphylactic episodes presented by the 1st patient diagnosed in Spain (10), larvae were collected from muscle tissue of hake obtained locally (identified by Dr D.I. Gibson, of the Natural History Museum, London) and an extract was prepared and stored at -40°C. A 1/100 dilution was used for skin prick tests. Samples of the extract heated at 40°C for 10 min, and at 100°C for 20 min were tested in parallel. A commercial test for the measurement of specific IgE to A. simplex is available. IgE-dependent sensitization was proven by in vivo and in vitro tests. Since this initial demonstration, additional in vitro studies have been conducted to define the antigens present in A. simplex, which could serve as allergens and induce IgE-dependent allergy in patients independently of active infection. Evidence has been given that these allergens are quite thermostable and that fresh, frozen and boiled extracts are equally able to elicit in vivo positive skin prick tests, in vitro histamine release by basophils, and to bind to serum specific IgE from allergic patients in vitro (12, 13, 14).

The profiles of bands found in sera of patients allergic to A. simplex after immunoblotting (13) seem to be different from those obtained when studying patients with anisakidosis (15): this could indicate that IgE responses are different in both cases, although technical problems cannot be excluded. On the other hand, among four patterns of immunoblotting, type I with a group of several bands of medium molecular weight and others of low molecular weight was significantly associated with proven allergy, while only one or more bands of medium molecular weight were present in subjects with "false positive" specific IgE detected using CAP-immunoassay (16).

A major problem in interpreting results of in vivo and in vitro testing of patients with a history of allergy after eating seafood (11, 17) and, moreover, of patients without any convincing fish-related history but only chronic recurrent urticaria (12, 18) or asthma (19), is represented by cross-reactivity with antigens from other helminths, especially nematodes, and even from non-related animals such as insects or crustaceans (20, 21). Many patients studied in the first Spanish reports on Anisakis allergy had concomitant specific IgE antibodies against Ascaris spp (10, 12, 13). A study using serum samples collected from 60 children with specific IgE to A. simplex and sensitization to arthropods showed a dose-dependent inhibition of Blatella germanica (German cockroach) and of Chironomus spp (red mosquito larvae) specific IgE detection after pre-incubation with the A. simplex extract. Immunoblot of Anisakis inhibited with Chironomus and B. germanica yielded a partial blot inhibition on bands below 41 KDa (20). From 60 selected sera with sensitization to Anisakis, 21 had specific IgE to cockroach; most of them had bands of 30-45 kDa on immunoblotting (20). A high cross-reactivity was observed in the serum of infected and/or immunized mice between A. simplex and
three other nematodes: Ascaris suum, Toxocara canis, and Hysterothylacium aduncum (21). Immunoblotting confirmed the high degree of cross-reactivity between the somatic antigen (SA) of A. simplex and that of other ascaridoids, although several A. simplex SA components in the 11-18 KDa range were recognized only by sera from mice infected, or immunized with the SA of A. simplex: two A. simplex pseudocoelomic fluid antigen components of 22 and 27 KDa were also only recognized by sera from A. simplex infected or immunized mice (21). Such cross-reactions are a major drawback for population studies which should be performed in order to assess the actual prevalence of A. simplex sensitization in different countries. Allergy to cockroach is more and more frequent in European countries (22), prevalence of Toxocara canis positive serology has also been reported to be very high in some areas (23). Moreover, cross-reactivity exists between shrimp, chironomids and German cockroach (22, 24) and between shrimp, snails and house dust mite (Dermatophagoides pteronyssinus), especially in those patients who have received mite immunotherapy (25-28). Finally, a periodate-sensitive band of medium-molecular weight in immunoblotting has been shown to be present in the serum of both patients with Anisakis allergy and controls, as well as in bakers with or without occupational allergy; it was not inhibited by a wheat extract nor by fungal amylase and was clinically irrelevant (29).

Determining the actual sensitizing agent may, therefore, be difficult in the absence of any clear history demonstrating the time-relationship between the ingestion of infected fish or other seafood and the clinical symptoms, if only skin tests and specific IgE measurements using commercially available methods are used for diagnosis. Immunoblotting could help in individual cases; its applicability to population studies is far less clear. A multicentric trial has been initiated by the Spanish Society of Allergy and Clinical Immunology, involving 50 allergy services throughout Spain, to study the epidemiological and clinical characteristics of Anisakis allergy in this country; results should be analyzed by July, 1998 (30; Fernandez de Corres, personal communication).

2.3. Prevention of Anisakis-related diseases

Cooking fish or seafood at 60°C for 10 minutes or freezing for 24 hrs can kill the parasite and prevent human infection. However, allergens are quite thermostable and neither cooking nor freezing can prevent allergic reactions in sensitized patients (14). Recent reports on larval anisakid infection of fish reveal that 39.4% of the fish from the fish market in Granada, Spain are contaminated, 26.1% being infected with 3rd-stage larvae (L3) of A. simplex (31). Fish from North Spain (Atlantic Ocean) had higher rates of infection than fish from South and South-east Spain (Mediterranean Sea) (31). Other unpublished studies show a similarly high prevalence of fish infected by Anisakidae in Madrid (31.7%) (report of the Laboratory of Hygiene of Madrid), with 17 out of 19 species infected in Galicia. Micromessistius poutassou (blue whiting) was the species with the highest infection rate (70-80%), with a mean intensity of 6 A. simplex L3 larvae per fish in Galicia, and 33.4 in Bilbao (11). Similar prevalence rates were observed in blue whiting caught in Scotland and in Italian fish (32), as well as in commercial marine fish-filets in the Nantes area (French west coast) (33). In this study (33), the most frequently infected fish were coal fish (Pollachius virens) and whiting (Merlangius merlangus). A previous study of commercial marine fish in the Paris area, in 1986-87, showed that A. simplex was present in 10 species, most frequently in herring (Clupea harengus): 82% of 682 fish, average 9 A. simplex L3 larvae per fish; red fish (Sebastes marinus), 86% of 36 fish, average 18 L3 per fish; and hake (Merluccius merluccius), 89% of 35 fish, average 31 L3 per fish (34). In the Bohai Sea, China, a total of 5,992 A. simplex larvae were collected from 121/290 marine fish of 19/25 species and in 8 squids among 108 cephalopods of 3 species (35). Among studies done in the USA and which revealed that salmon was one of the most parasitized fish, one examined 50 sockeye salmon (Oncorhynchus nerka) caught during spawning migration: all were found to be infected with A. simplex L3 (36).

Incidence of anisakid infected fish is, thus, fairly high worldwide.

Regulations in order to protect consumers against Anisakidosis have first been edicted in the Netherlands: blast-freezing of fish is an efficient measure to kill the larvae. Heavy salting, smoking above 50°C, or boiling/frying fish are safe measures to prevent Anisakidosis. Special regulations have been given concerning "lightly salted herring", and marinated or smoked herring so that additional freezing could be mandatory if suitable salting, marinating or smoking procedures could not be reached. The main points of this regulation are given in directive 91/493/EEC on sanitary measures for the production and the placing on the market of fishery products.

3. Conclusions
1. It is clear that the current regulation does not protect the consumers against allergic hazard due to ingestion of killed parasites.

2. The occurrence of *Anisakis* larvae in exviscerated fish is the result of a delay in the period between the catch and gutting in modern fishing. Whenever possible, this period of time should be reduced. New methods of "blast freezing" at sea might also improve the situation. However there will be a certain number of *Anisakis* larvae in the fish flesh and it is probable that no intervention will be able to destroy the allergenicity of the larval antigens.

3. Prevention should, thus, focus on information of the European consumers, the fishing industry and of physicians about this risk.

4. The real incidence of the allergic accidents after eating contaminated fish and, conversely of the prevalence of *A. simplex* sensitization in patients with clinical conditions as various as urticaria (11,12,17,18), anaphylaxis (11,12,17), asthma (9), and/or contact dermatitis (37) should be carefully assessed. Cross-reactivity with other nematodes, and insects well known to be involved in allergic diseases, raises some doubt on the accuracy of prevalence rates already published (39) and should be taken into account if any prospective epidemiological study is planned. Moreover scombroid fish poisoning, due to ingestion of large quantities of histamine produced by bacterial degradation of blue fish flesh, could be misleading in the clinical interpretation of symptoms and history (38).

5. Well conducted and coordinated studies should be encouraged, using a common methodology, which could give a comprehensive appraisal of allergic or allergy-like events related to fish consumption.

4. References

- 18. Del Pozo MD, Audicana M, Diez JM, Munoz D, Ansotegui IJ, Fernandez E, Garcia M, Etxenagusia M,


