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 crustraceans when they are consumed raw or not adequatelly 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

1995, in the Basque Country (10). Since the publication of this well documented case, the same team has reported 28 cases of IgE-dependent hypersensitivity caused by *Anisakis* (11). The symptoms were : urticaria/angioedema in 17 patients, facial angioedema in 28, gastrointestinal symptoms (vomiting, diarrhea and abdominal pain) in 12, respiratory symptoms in 7 and anaphylactic shock in 3. Fourteen patients had to be treated in an emergency department, 3 had to be hospitalized and one had a respiratory arrest.

The fish implicated were hake (*Merluccius merluccius* L) in 11 among 25 cases, anchovies (*Engraulis encrasicholis* L) in 6, cod (*Gadus morhua* L) in 4 and different others in 5. Three patients did not remember the ingested fish. Skin prick-tests performed to assess immediate-type hypersentivity were positive to 3 *Anisakis simplex* extracts and negative to fish and sea food in all patients. Specific IgE to *Anisakis* extracts were present in all patients' sera ; there were no specific IgE to fish (11). A systematic study of 25 patients diagnosed as having idiopathic recidivous urticaria and who usually ate fish or seafood was performed by another Spanish team, from Madrid (12). Nineteen patients had specific IgE to *A.simplex* in the serum, and 16 patients had positive skin prick tests to *Anisakis* extracts with a delayed reaction in 2 of them. Only one patient showed sensitization to fish.

Until now, no other reports from other regions of Spain, other European countries or elsewhere in the world (excepted the initial report from Japan in 1990) have been published. No cases, for instance, have been reported in Portugal, either in international or Portuguese-language journals or at national meetings (F. Inacio, Setubal ; personal communication). It must be noticed, however, that the parasitic origin of the sensitization may have been missed if not systematically looked for, and some cases misinterpreted as allergy to fish or seafood with so-called "negative" tests, and/or as "chronic urticaria" which is a condition where it is generally accepted that cause cannot be found in 40 to 60 % of cases.

2.2 *Anisakis* antigens and allergens

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°. 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 be 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 signicantly 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 interpretating 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 immunobotting 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 spawn-ing 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

- 1. Ruitenberg EJ, van Knapen F, Weiss JW. Food-borne parasitic infections - old stories and new facts. Vet Quarterly 1979;1:5-13.
- 2. Sakanary JA, McKerrow JH. Anisakiasis. Clin Microbiol Rev 1989;2:278-84.
- 3. Ishikura H, Kikuchi K, Nagasawa K, et al. Anisakidae and anisakidosis. In : Sun T, editor. Progress in clinical parasitology. Vol. III. New York: Springer-Verlarg, 1993:43-101.
- 4. Van Thiel PH, Anisakis. Parasitology 1960 ; 53 : 16.
- 5. Van Thiel PH, Kuipers FC, Roskam RT. A nematode parasitic to herring causing acute abdominal syndromes in man. Trop Geogr Med 1962 ; 2 : 97-113.
- 6. Oshima T. Anisakis and anisakiasis in Japan and adjacent area. Prog Med Parasitol Japan 1972; 4:301-93.
- 7. Sugimachi K, Inokuchi K, Ooiwa T, Fujino T, Ishii Y. Acute gastric anisakiasis. JAMA 1985;253:1012-3.
- 8. Ruitenberg EJ, Berkvens JM, Duyzings MJM. Experimental *Anisakis marina* infections in rabbits. J Comp Path 1971;81:157-63.
- 9. Kasuya S, Hamano H, Izumi S, Mackerel-induced urticaria and *Anisakis*. Lancet 1990;335:665.
- 10. Audicana MT, Fernández de Corres L, Muñoz D, Fernández E, Navarro JA, del Pozo MD. Recurrent anaphylaxis caused by *Anisakis simplex* parasitizing fish. J Allergy Clin Immunol 1995;96:558-60.
- 11. Fernández de Corres L, Audicana M, del Pozo M, Muñoz D, Fernández E, Navarro J, García M, Díez J. *Anisakis simplex* induces not only anisakiasis : Report on 28 cases of allergy caused by this nematode. J Invest Allergol Clin Immunol 1996;6:315-9.
- 12. Montoro A, Perteguer MJ, Chivato T, Laguna R, Cuéllar C. Recidivous acute urticaria caused by *Anisakis simplex*. Allergy 1997;52:985-91.
- 13. Del Pozo M, Moneo I, Fernández de Corres L, Audicana MT, Muñoz D, Fernández E, Navarro JA, García M. Laboratory determinations in *Anisakis simplex* allergy. J Allergy Clin Immunol 1996;97:977-84.
- 14. Audicana L, Audicana MT, Fernández de Corres L, Kennedy MW. Cooking and freezing may not protect against allergenic reactions to ingested *Anisakis simplex* antigens in humans. Vet Rec 1997;140:235.
- 15. Akao N, Ohyama T, Kondo K. Immunoblot analysis of serum IgG, IgA and IgE responses against larval excretory-secretory antigens of *Anisakis simplex* in patients with gastric anisakiasis. J Helminthol 1990;64:310-8.
- 16. Garcia M, Moneo I, Audicana MT, Del Pozo MD, Munoz D, Fernandez E, Diez J, Etxenagusia MA, Ansotegui IJ, Fernandez de Corres L. The use of IgE immunoblotting as a diagnostic tool in *Anisakis simplex* allergy. J Allergy Clin Immunol 1997;99:497-501.
- 17. Moreno-Ancillo A, Caballero MT, Cabanas R, Contreras J, Martin-Barrosso JA, Barranco P, Lopez-Serrano MC. Allergic reactions to *Anisakis simplex* parasitizing seafood. Ann Allergy Asthma Immunol 1997;79:246-50.
- 18. Del Pozo MD, Audicana M, Diez JM, Munoz D, Ansotegui IJ, Fernandez E, Garcia M, Etxenagusia M,

Moneo I, Fernandez de Corres L. *Anisakis simplex*, a relevant etiologic factor in acute urticaria. Allergy 1997;52:576-9.

- 19. Estrada Rodriguez JL, Gozalo Reques F. Sensitization to *Anisakis simplex* : an unusual presentation. Allergol Immunopathol 1997;25:95-7.
- 20. Pascual CY, Crespo JF, San Martin S, Ornia N, Ortega N, Caballero T, Munoz-pereira M, Martin-Esteban M. Cross-reactivity between IgE-binding proteins from *Anisakis*, German cockroach, and chironomids. Allergy 1997;52:514-20.
- 21. Iglesias R, Leiro J, Ubeira FM, Santamarina MT, Navarrete I, Sanmartin ML. Antigenic cross-reactivity in mice between third-stage larvae of *Anisakis simplex* and other nematodes. Parasitol Res 1996;82:378-81.
- 22. Crespo JF, Pascual C, Helm R, *et al.* Cross-reactivity of IgE-binding components between boiled Atlantic shrimp and German cockroach. Allergy 1995;50:918-24.
- 23. Margnaval JF, Carriere JP. *Toxocara* et toxocarose humaine. Paris : Flammarion Médecine Science 1994.
- 24. Eriksson NE, Ryden B, Jonsson P. Hypersensitivity to larvae of chironomids (non-bitting midges). Cross-sensitization with crustaceans. Allergy 1989;44:305-13.
- 25. Witterman AM, Akkerdaas JH, Leewen J, Zee JS, Aalberse RC. Identification of a cross-reactive allergen (presumably tropomyosin) in shrimp, mite and insects. Int Arch Allergy Immunol 1994;105:56-61.
- 26. Van Ree R, Antonicelli L, Akkerdaas JH, Garritani MS, Aalberse RC, Bonifazi F. Possible induction of food allergy during mite immunotherapy. Allergy 1996;51:108-13.
- 27. Vuitton DA, Rancé F, Paquin ML, Adessi B, Vigan M, Gomot A, Dutau G. Cross-reactivity between terrestrial snails (*Helix* sp) and house-dust mite (*Dermatophagoides pteronyssinus*) : 1 - *in vivo* study. Allergy 1998;53:144-150.
- 28. Guilloux L, Vuitton DA, Delbourg M, Lagier A, Adessi B, Marchand CR, Ville G. Cross-reactivity between terrestrial snails (*Helix* sp) and house-dust mite (*Dermatophagoides pteronyssinus*) : 2 - in vitro study. Allergy 1998;53:151-158.
- 29. Moneo I, Audicana MT, Alday E, Curiel G, del Pozo MD, Garcia M. Periodate treatment of *Anisakis simplex* allergens. Allergy 1997;52:565-9.
- 30. Buendia E. *Anisakis*, anisakidosis, and allergy to *Anisakis*. Allergy 1997;52:481-482.
- 31. Adroher FJ, Valero A, Ruiz-Valero J, Iglesias L. Larval anisakids (Nematoda : Ascaridoides) in horse mackerel (*Trachurus trachurus*) from the fish market in Granada, Spain. Parasitol Res 1996;82:319-22.
- 32. Orecchia P, Paggi L, Mattiucci D, Di Cave D, Catalini N. Infestazione da larve di *Anisakis simplex* e *Anisakis physeteris* in specie ittiche dei mari italiani. Parassitologia 1989;31:37-43.
- 33. Chord-Auger S, Miegeville M, Le Pape P. Anisakiasis in the Nantes area. From fishmongers' stalls to medical offices (in French). Parasite 1995;2:395-400.
- 34. Huang WY. Anisakis and human anisakiasis II. Investigation of the anisakids of commercial fish in the district of Paris (in French). Ann Parasitol Hum Comp 1988;63:197-208.
- 35. Ma HW, Jiang TJ, Quan FS, Chen XG, Wang HD, Zhang YS, Cui MS, Zhi WY, Jiang DC. The infection status of anisakid larvae in marine fish and cephalopods from the Bohai Sea, China and their taxonomical consideration. Korean J Parasitol 1997;35:19-24.
- 36. Deardorff TL, Kent ML. Prevalence of larval *Anisakis simplex* in pen-rared and wild-caught salmon (*Salmonidae*) from Puget Sound, Washington. J Wildl Dis 1989;25:416-19.
- 37. Carretero Anibarro P, Blanco Carmona J, Garcia Gonzales F, Marcos Durantez M, Alonso Gil L, Garces Sotillos M, Perez Gimenez R, Juste Picon S, Gutierrez Ortega MC. Protein contact dermatitis caused by Anisakis simples. Contact Dermatitis 1997;37:247.
- 38. Sánchez-Guerrero IM, Vidal JB, Escudero AI. Scombroid fish poisoning : A potentially life-threatening allergic-like reaction. J Allergy Clin Immunol 1997;100:433-4.
- 39. Garcia Palacios L, Gonzales ML, Esteban MI, Mirabent E, Perteguer MJ, Cuellar C. Enzyme-linked immunosorbent assay, immunoblot analysis and RAST fluoroimmunoassay analysis of serum responses against crude larval antigens of *Anisakis simplex* in a Spanish random population. J Helminthol 1996;70:281-9.