EUROPEAN COMMISSION
HEALTH & CONSUMER PROTECTION DIRECTORATE-GENERAL

Scientific Steering Committee

OPINION ON :

THE FEEDING OF WILD FISHMEAL TO FARMED FISH AND RECYCLING OF FISH WITH REGARD TO THE RISK OF TSE

ADOPTED BY THE SCIENTIFIC STEERING COMMITTEE AT ITS MEETING OF 6-7 MARCH 2003.

THE FEEDING OF WILD FISHMEAL TO FARMED FISH AND RECYCLING OF FISH WITH REGARD TO THE RISK OF TSE

OPINION

MANDATE:

Mammalian MBM and other mammalian products have historically been fed to farmed fish. Furthermore, intra-species and intra-order recycling via feed is common practice in fish farming. It is therefore important to address the question whether the latter practice could enable mammalian TSE agents to establish themselves in fish and for species adaptation of such agents to occur. This could lead to the development of a TSE in fish that might lead to a TSE epidemic in fish and/or create a health risk for the consumer. The outcome of the assessment would improve the scientific basis for the possible updating of the animal waste disposal legislation and other legislative texts in the field of veterinary public health. The Scientific Steering Committee (SSC) was therefore invited:

(1) to advise whether the feeding of wild fishmeal to farmed fish presents any risk to animal or human health vis-à-vis TSE's;

(2) if appropriate, to suggest examples of conditions under which intra-species or intra-order recycling of fish could be allowed.

The SSC asked the TSE/BSE *ad hoc* Group to prepare a scientific report to serve as basis for an opinion on the two questions. The report, finalized by the TSE/BSE *ad hoc* Group at its meeting of 5 September 2002, is attached. This report is largely based on various SSC opinions and reports of the TSE/BSE *ad hoc* group related to animal waste disposal and intra-species recycling, on elements from the (draft) report of the Scientific Committee on Animal Health and Welfare on "*The use of fish waste in aquaculture*" and on the interim results of the FAIR CT97 3308 project entitled "*Separation, identification and characterization of the normal and abnormal isoforms of prion protein from normal and experimentally infected fish*"

BACKGROUND:

1. Very little is known about the possible occurrence of TSEs in fish. No targeted (epidemiological) surveys have been conducted to detect pathological changes in fish consistent with TSEs. Limited research results currently available are inconclusive regarding whether or not TSE agents from other orders (e.g. mammals) can be transmitted to fish and lead to replication and disease, or whether or not (certain) fish species could generate or support TSE agent replication based upon the existence of a piscine prion-protein molecule.. However, these possibilities cannot be totally excluded as recently a homologue to prion-protein was identified in the pufferfish *Fugu rubripes*, showing high homology with mammalian PrP sequences and in another publication the normal isoform of amyloid protein (PrP) was identified in brains of spawning salmon.

   On the other hand, intra-species and intra-order "recycling" of fish materials occurs naturally in most if not all fish environments. It is likely that natural predation would offer limited scope for amplification of the agent and the "infectivity" could remain confined to a small number of the sea or freshwater fish or mammals. This principle may, however, not apply if the TSE agent were external to the fish environment/ecosystem and it is therefore justified to avoid the introduction of such agents to the fish environment, as this

could possibly result in fish presenting a risk to other animal or human health vis-à-vis TSE's. At this stage of knowledge the SSC, can only assume that the same biological rules that apply to mammalians might apply to fish. This is probably the best one can presently achieve, awaiting the results of current research and the realisation of the urgent requirement for further research to be carried out.

2. It is further appropriate to highlight the following *additional* uncertainties that result from such an approach:
   - Unknowns exist regarding the structures of putitive fish PrP's and how they might compare with the structures of mammalian PrP's. Homologies between them would influence the magnitude of the species/order barrier (e.g., transmission of BSE from cattle to fish).
   - Strictly speaking, intra-species recycling refers to the recycling of one given animal species to the same species, for example trout to trout. If fish-meals fed to a given species have been derived from a mixture of various / different fish species, it would be more appropriate to use the term "intra-order" recycling. In this case the level of the barrier is likely to be higher than in case of intra-species recycling, assuming that this is determined in fish by the PrP gene sequence and that there is a natural variation in the sequence between fish species. In practice there is the potential for a mixture of both types of recycling to occur.
   - If TSEs were naturally present in fish populations, they may not manifest themselves in the same way as the known TSEs of mammalian species or may even not be recognised as a disease entity.

OPINION:

1. The risks caused by recycling in general, are addressed in the SSC opinion of 17 September 1999 on *Intra-Species Recycling - the risk born by recycling animal by-products as feed with regard to propagating TSE in non-ruminant farmed animals.*

2. From the limited available research results, scientific literature on TSE's in fish and routine examinations of fish brain in the course of fish disease diagnosis, it can be concluded that there is no evidence that a natural TSE exists in fish and that there are no indications of replication of scrapie or BSE agent in experimental transmission studies.

   On the question *whether the feeding of wild fishmeal to farmed fish presents any risk to animal or human health vis-à-vis TSE's*, the SSC therefore concludes that there is currently no evidence of any such risk existing.

   The data from the transmission experiments in the above-mentioned FAIR project and from other sources are still very limited and incomplete. Only three species of fish (Trout, Turbot and Sea Bream) are included in the experiments and no marine mammals, which could be more susceptible to TSE's than fish, have been studied so far in this respect. Therefore, as always, ongoing research should be monitored closely to permit a possible update of this conclusion should research results call for such update.

3. Some theoretical risks could exist, linked to feeding possibly TSE-contaminated feeds to animals currently believed to be not susceptible, including fish. These risks include the possible build-up of a pool of infectivity in animals that do not develop disease but may potentially be able to harbour the agent as residual infectivity in the digestive system and/or replicate the agent. The latter risk is higher when intra-species recycling is

practised due to the absence of a species barrier. Also the risk of adaptation of the agent to hitherto non-susceptible hosts should be considered.

Regarding the request to, *if appropriate, suggest examples of conditions under which intra-species or intra-order recycling of fish could be allowed*, the SSC therefore considers in general that potentially TSE infected feed should not be fed to fish and that sourcing of fish by-products (including for their use in fish-derived feed) should not be performed from fish that have been exposed to potentially infected feed.

4. With regard to the appropriate treatment of fish materials, the SSC refers to its opinion of June 1999 on "Fallen stock"[1] and to the Report of the Scientific Committee on Animal Health and Animal Welfare on "The use of fish by-products in aquaculture" adopted on 26 February 2003.

[1] Scientific Opinion on The risks of non conventional transmissible agents, conventional infectious agents or other hazards such as toxic substances entering the human food or animal feed chains via raw material from fallen stock and dead animals (including also: ruminants, pigs, poultry, fish, wild/exotic/zoo animals, fur animals, cats, laboratory animals and fish) or via condemned materials. Adopted By the Scientific Steering Committee at its meeting of 24-25 June 1999.

REPORT ON THE FEEDING OF WILD FISHMEAL TO FARMED FISH AND RECYCLING OF FISH WITH REGARD TO THE RISK OF TSE.

Rapporteur: Dr E. Vanopdenbosch

I. MANDATE

Intra-species or intra-order recycling is common practice in fish and it is thus justified to address the theoretical risk that such recycling could lead, for example, to the adaptation of TSE agents to certain fish species and/or the building up of an infectivity pool which could create a health risk for the consumer and/or to a TSE epidemic in fish. The outcome of the assessment would improve the scientific basis for the possible updating of the animal waste disposal legislation and other legislative texts in the field of veterinary public health. The Scientific Steering Committee (SSC) was therefore invited:

(1) to advise whether the feeding of wild fishmeal to farmed fish presents any risk to animal or human health vis-à-vis TSE's;

(2) if appropriate, to suggest examples of conditions under which intra-species or intra-order recycling of fish could be allowed.

A scientific report to serve as basis for an opinion on the two questions was prepared under the rapporteurship of Dr. E. Vanopdenbosch and with inputs from Prof. C.L.Bolis, Prof.Em.B.Lahlou, Dr.P.Brown, Dr.R.Bradley, Dr.Ph.Poujeol, Prof.Dr.D.Dormont, Dr.C.Ducrot and Dr.G.Wells. The report was finalised by the TSE/BSE *ad hoc* Group at its meeting of 5 September 2002.

2. PRELIMINARY REMARK

The current report is largely based on the following documents:

- SSC Opinion (EC, 1999a) on the risks of non conventional transmissible agents, conventional infectious agents or other hazards such as toxic substances entering the human food or animal feed chains via raw material from fallen stock and dead animals (including also: ruminants, pigs, poultry, fish, wild/exotic/zoo animals, fur animals, cats, laboratory animals and fish) or via condemned materials.
- SCC Opinion (EC, 1999b) the risk born by recycling animal by-products as feed with regard to propagating TSE in non-ruminant farmed animals.
- SSC Opinion (EC, 2000) on the Scientific basis for import bans proposed by 3 member states with regard to BSE risks in France and the Republic of Ireland; on the Scientific basis for several measures proposed by France with regard to BSE risks and on the Scientific basis for banning animal protein from feed for all farmed animals, including pig, poultry, fish and pet animals.
- Interim results (2002) of the FAIR CT97 3308 project entitled "*Separation, identification and characterisation of the normal and abnormal isoforms of prion protein from normal and experimentally infected fish*"
- Scientific Committee on Animal Health and Animal Welfare (2002). Draft report on "The use of fish waste in aquaculture."

3. FEEDING OF FARMED FISH

(See also the Report of the Scientific Committee on Animal Health and Animal Welfare on "The use of fish by-products in aquaculture" adopted on 26 February 2003.)

Since the end of the Second World War, the rate of growth of marine fisheries has been consistently somewhat higher than the rate of growth of the world's human populations. It has therefore been much higher than the rate of growth of agricultural food production. In fact, since the 1950's, practically each year's world fish catch has set a new record.

Aquaculture is defined as the farming of aquatic organisms including fish, molluscs, crustaceans and aquatic plants. Farming implies some intervention in the rearing process to enhance production, such as regular stocking, feeding and protection from predators.

Artificial feeding of fish is one of the principal ways of increasing production in fish farming. In intensive fish farming artificial feeding is essential for growth and even in extensive farming, some artificial feeding is usually required. The majority of fish farmed in intensive aquaculture systems in the EU are carnivorous, having a high requirement for protein in their diets. Generally, fishmeal is used as the major source of protein in feeds formulated for cold-water fish rations.

Because many species of fish, which are farmed, are carnivorous by nature they feed on other species of fish and crustaceans. Consequently, the feed of farmed marine and freshwater fish is mainly composed of re-cycled dead fish in the form of fishmeal and fish oil. The fishmeal is predominantly produced from a variety of ocean-caught marine fish.

Farmed and wild fish also often have particular dietary requirements in relation to fats and amino acid requirements. The salmonids have a requirement for omega-3 (n-3) fatty acids of longer chain lengths and certain amino acids. Consequently, the most important ingredient in the diets of farmed fish is fishmeal.

Mammalian-derived materials have also been used, to some extent, as an ingredient for feeding farmed marine and freshwater fish. For example, up to recently, blood meal was used in fish feeds. However, because of EU legislation banning such ingredients, it is no longer used.

Fishmeal is obtained from whole dead wild caught fish or trimmings of such fish after filleting for human consumption The most widely used technique for fish meal processing is the wet reduction process, which is operated continuously and requires large amounts of raw material. The fish is steam cooked and pressed. The pressing of the cooked fish results in a protein fraction called press cake, and a mixed water and oil fraction with suspended and soluble protein. Oil and the water fraction with proteins are separated. The stick water is concentrated through evaporation. The temperature used, particularly at the drying stage, should be hot enough to kill any bacteria but not so hot that it denatures the protein. A drying temperature of 15-80°C is usually considered optimum.

The feeding with fishmeal raises the question of intra-species or intra-order recycling of fish tissues. Generally, although recycled fish in the form of fishmeal is the principle ingredient of food for farmed fish, recycled farmed fish tissues are not used as an ingredient of fishmeal produced for fish feeds. Even if intra-species recycling of

fish tissue did occur, the heat and drying treatment used to produce fishmeal should be sufficient to destroy any conventional fish or human pathogens, but not TSE agents if present.

4. RESEARCH ON TSEs IN FISH

4.1. THE EC FAIR CT97 3308 PROJECT: "SEPARATION, IDENTIFICATION AND CHARACTERISATION OF THE NORMAL AND ABNORMAL ISOFORMS OF PRION PROTEIN FROM NORMAL AND EXPERIMENTALLY INFECTED FISH"

The project, has four principle objectives with corresponding results summarized as follows:

1: Characterization of normal isoforms of fish PrP and its coding nucleotide sequence: The amphibian (X. Laevis) PrP was sequenced. Using probes designed for screening fish cDNA, some clones showed homology with the prion probe and were partially sequenced, but it is unclear from these data if a true PrP sequence was identified. A final conclusion will be drawn after complete sequence data of all the clones.

2: Attempted transmission of TSE to fish from ovines and bovines: several different species of fish were inoculated with scrapie and BSE infected material. Trout and turbot were inoculated simultaneously (intracerebrally, intra-peritoneally and intramuscularly) with scrapie infected material and trout and sea bream were inoculated with BSE infected material. Scrapie agent inoculated turbot had infectivity as demonstrated by mouse inoculation in brain and spleen (15 days post inoculation [pi]) and brain (90 days pi). Infectivity was also found to persist sporadically in the intestine of fish fed with high doses of scrapie infected material. Trout and sea bream which were inoculated with BSE material did not show evidence of infection up to four months pi.

The transmission experiments with tissues from fish infected with scrapie are still in progress. Otherwise the experiments with material from fish infected with BSE are completed. (Further detail of the outcome of the transmission studies is given in **APPENDIX**)

3: Establishing a diagnostic test for PrP detection in fish tissues. As this is dependent on the outcome of objective 1, no test has yet been developed.

4: Evaluation of the uptake and binding of normal fish PrP. It was not possible to draw conclusions.

Comment on experimental studies:

a) The transmission protocol maximises the chance of identifying residual inoculum and minimises the chances of identifying agent, which has infected the fish and is being amplified/replicated in the fish tissue because:

- The inoculum used is mouse adapted scrapie (139A)
- Mouse (unspecified strain/panel) bioassay is being used for detection of infectivity in fish tissues
- There is no evidence that any of the antibodies use on fish tissues for IHC or WB have any cross reactivity with "fish PrP".

- There has been no sub-passage of tissues from exposed fish in fish of the same species. This would be the only practical way of addressing the question of whether fish can be infected, the problems of adaptation through intra-specific passage etc.

b) The research project has, so far, not found any evidence for replication of TSE agents in fish. This is in line with negative results of searches in fish databases, which were unable to detect a sequence with similarities to known prions (Joly et al., 2001), from which it was concluded that a potential fish PrP gene is probably very different from those characterised in mammals and that it would be extremely unlikely to share common pathological properties. However, this is somewhat in contradiction with the data from Gibbs *et al* (1997) describing, for the first time, the presence of a normal isoform of amyloid protein (PrP) in brains of spawning salmon. Also, in contrast is a recent publication (Suzuki *et al*., 2002) identifying a PrP-like molecule in the pufferfish (*Fugu rubripes*), showing high homology with mammalian PrP sequences, but some structural inconsistency. These are the only available data at present, clearly demonstrating that a lot more needs to be known about piscine PrP genes, PrP and variation in sequences of each.

c) The final outcome of the project should contribute to the understanding as to whether fish are possible carriers of residual infectivity or whether there is direct evidence of transmission of TSE to fish. it should also inform on the potential risk connected to fish derived foods for human and animal, the establishment of analytical protocols for PrP detection in fresh fish food and the comparison of the molecular properties of normal and abnormal isoforms of PrP.

4.2. OTHER DATA ON TSES IN FISH

The availability of (recent) data and research results on TSEs in fish is quite limited. In its report[2] in support of its opinion of 24-25 June 1999 on "Fallen stock", the SSC concluded as follows:

> "*So far, no evidence for TSE in fish was found. Alderman (1996) reports that the Fish Diseases laboratory at Weymouth (UK) has for 25 years been involved in studying the diseases of marine and freshwater fish. During that time the laboratory has not observed any scientific evidence of any condition which might in any way be described as a spongiform encephalopathy in fish, whether of species used to produce fishmeal, or directly for human food, from the UK, other EU member states or from elsewhere in the world. .*
>
> *What precedes is confirmed by Professor Hugh Ferguson of the Institute of Aquaculture at Stirling University (SEAC, 1999, communication to the SSC secretariat). He reports that fish brains are examined quite frequently, and in young fish often as a result of investigations for gill infections. As there are recognized diseases of fish that could cause vacuolation, fish experts are conscious of concerns about TSEs. Nothing suggestive of a TSE has been found however."*

The TSE/BSE *ad hoc* Group considers that both from the literature and from limited observations on fish, there is no evidence that TSEs would naturally exist in fish but

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[2] Scientific Report on The risks of non conventional transmissible agents, conventional infectious agents or other hazards such as toxic substances entering the human food or animal feed chains via raw material from fallen stock and dead animals (including also: ruminants, pigs, poultry, fish, wild/exotic/zoo animals, fur animals, cats, laboratory animals and fish) or via condemned materials. Adopted By the Scientific Steering Committee at its meeting of 24-25 June 1999.

that the possibility cannot be totally excluded. More research is required to improve the confidence of this conclusion.

5. THE RISK OF RECYCLING OF FISH WITH REGARD TO TSEs

5.1. GENERAL

The epidemiological risk depends on the origin and properties of the raw material and the field of application of the product. Unfortunately, from an economic point of view, recycling as feed is the most profitable way, but also theoretically the most dangerous way, of dealing with animal by-products.

Intra-species recycling could be regarded as more dangerous than producing feed for phylogenetically less related species, because of possible species barrier effects. However, in the absence of any data on species barrier effect in fish, the potential importance of intra-species recycling versus intra-order recycling cannot be estimated at present and neither are indications available that recycling in fish can be considered in the same context as is done for the domestic animal situation. In this respect reference can be made to the natural and experimental transmission history of mammalian TSE's, suggesting a wide phylogenetic susceptibility within the Order. In the cases of BSE and CWD the species barrier, in terms of oral route, is probably negligible across several species of the respective phylogenetic families and sub-families of the host. The kudu may be even more susceptible for BSE than domestic cattle and BSE also affects Felidae under "recycling" conditions.

Nevertheless, as long as the TSE problem is not relevant for fish and meat and bone meal from other possibly TSE infected species is not used as feed in aquaculture, recycling would not create an increased risk in respect to TSE in fish. The assessment would have to be reviewed, in line with the general principles of intra-species or intra-order recycling, if evidence is found of replication of TSE agent in fish.

The use of resulting products as fertiliser further reduces the epidemiological risk of recycling of organic wastes with respect to direct transmission to susceptible hosts but it increases the risk of uncontrolled and indirect transmission to susceptible hosts or exposed materials with epidemiological importance as feed or food.

In addition, if TSE was to be shown to exist in fish, the process designed for treatment of fish material in order to produce a fertiliser must be designed in such a way that the TSE agent is maximally inactivated.

The safest way for treating organic wastes of animal origin is processing at 133 °C under 3 bar steam pressure for at least 20 min. If this causes technological problems which might be expected with fish material other time/temperature relationships may be applied but they have to be validated.

Fishmeal is obtained from drying, heating and pressing of whole dead wild caught fish or trimmings of such fish after filleting for human consumption. Generally, although recycled fish tissues in the form of fishmeal is the principle ingredient of food for farmed fish, recycled farmed fish are not used as an ingredient of fishmeal produced for fish feeds. Even if intra-species recycling of fish did occur, the heat and drying treatment used to produce fishmeal should be sufficient to destroy any conventional fish or human pathogens, but not totally TSE agent.

5.2. THE POSSIBILITIES OF TSE'S BEING RECYCLED IN FISH.

Wild fish

Many species of wild fish are carnivorous. There are two main scenarios that may result in a build-up of TSE's in wild fish.

Firstly, it is possible to hypothesise that a spontaneous TSE could develop in wild fish and that wild sea or river fish would have the capacity to recycle a TSE. In wild sea fish any pelagic fish (which move continuously in shoals and are the major source of fishmeal for farmed fish) a TSE might conceivably manifest in the early stages as an inability to swim properly, the individual fish would fall out of the shoal and become the prey of larger members of its own or other species eg demersal (ground level, solo feeders) or marine mammals. Such fish or mammals could then become "infected" and eventually fall prey to further carnivorous fish of the same or other species or marine mammals. A mature or semi-mature "infected" fish would most likely be eaten by a larger member of its own or another species. If the biological principles of infection with TSE in fish is similar to that in mammals, it may be difficult for adult fish to become infected by eating "infected" material. However, even in mammals, little is known about age related differences of susceptibility to TSE, but it is possible, as suspected for BSE in cattle that, also in fish, adults are less susceptible than the young of the species.

In the absence of information on the ID50 and mean incubation times for TSE's in any sea or freshwater fin fish only assumptions may be made. It is likely that natural predation would offer limited scope for amplification of the agent and the "infectivity" could remain confined to a small number of the sea or freshwater fish or mammals.

The second scenario involves direct exposure to TSE infected mammalian carcasses or their parts. Pelagic, demersal sea fish or freshwater fish could be directly exposed to mammalian TSE's through direct exposure to a dead TSE infected animal or its parts. Such an exposure could, as with the case of a spontaneous development of a fish TSE, initiate a cycle which could be propagated to other pelagic, demersal, freshwater (coarse or game) fish or marine of freshwater mammals. However, as for spontaneous development and under natural predation conditions, it is unlikely that significant amplification would occur among wild fish.

Dumping fish waste/offal at sea or in fresh water is likely to increase any theoretical possibility of recycling a TSE among wild fish as all ages, and sizes of fish could consume the waste.

Farmed fish

Farmed fish in general, need a protein source in their feed that originates from fish and is generally provided by a diet based on fishmeal. For this reason the possibility of recycling a TSE in farmed fish would be greater than is the case for wild fish.

To date, there is no evidence of a TSE in wild fish and therefore, no obvious possibility of "infected" wild fish being caught and processed into fishmeal. Likewise, although scavengers such as crustaceans or even marine mammals could also be infected, such fish or animals generally have a limited contribution to fishmeal. However, even a low-grade infection in the source fish could initiate a cycle in farmed

fish if entire, or parts of, "infected" farmed fish were recycled without measures being taken to inactivate TSE's.

It is possible that without treatment to inactivate infectious prions, fishmeal and fish oil could transmit "infectious" prions to farmed fish. The processing parameters for fishmeal (generally a temperature of 85°C is used with other physical processes) would not inactivate infectious prions. If materials from farmed fish were processed at these parameters only, and then fed back to farmed fish recycling of infectious prions to fish or to mammals could occur. Intra-species recycling, due to the absence of a species barrier could increase the risk that TSE cases occur or undetected pools of infectivity develop. However, although intra-species recycling could be regarded as more dangerous than producing feed for phylogenetically less related species, because of possible species barrier effects, in the absence of any data on species barrier effect in fish, the potential importance of intra-species recycling versus intra-order recycling cannot be estimated at present and neither are indications available that recycling in fish can be considered in the same context as is done for the domestic animal situation.

Farmed fish in Europe could have been exposed to feed containing meal derived from the blood of ruminants. However blood from ruminants is considered to be low risk by the oral route for transmission of ruminant TSE's, when taking into account the recommendations in the SSC opinion of 13-14 April 2000 on the "*Safety of ruminant blood with respect to TSE risks"*

Farmed fish could likewise be directly exposed to a mammalian TSE by direct exposure to an infected dead animal or its parts. This is an unlikely, but possible scenario.

Recycling farmed fish as feed for other farmed fish would greatly increase the risk of amplifying a TSE in fish and should be avoided.

5.3. SSC OPINIONS ON THE RISK OF RECYCLING OF FISH WITH REGARD TO TSE

From chapters 3 and 4, it can be concluded that to date there has been no evidence of TSE found in fish. Fish brain is examined quite routinely in fish disease diagnosis and to date no changes similar to those described for TSE have been reported. However, it should be taken into account that a prion infection in fish might not present as an obvious TSE.

In addition, the above mentioned FAIR CT97 3308 research project is looking at normal and abnormal prion proteins in fish and has, so far, not found any evidence for replication of TSE in fish[3].

However, the possibility cannot be totally excluded as in a recent publication (Suzuki et al., 2002) a homologue to prion-protein was identified in the pufferfish *Fugu rubripes*, showing high homology with mammalian PrP sequences and Gibbs *et al* (1997) described for the first time the presence of normal isoform of amyloid protein (PrP) in brains of spawning salmon. These are the only available data at present, clearly demonstrating that a lot more needs to be known about piscine PrP genes, PrP and variation in sequences of each.

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[3] The final outcome of that project should contribute to the assessment of the possibility of transmission of TSE to fish, the evaluation of the potential risk connected to fish derived foods for human and animal, the establishment of analytical protocols for PrP detection in fresh fish food and the comparison of the molecular properties of normal and abnormal isoforms of PrP.

Intra-species or intra-order recycling of fish should not present a risk with regard to TSEs, provided a number of conditions are satisfied. These conditions have already been listed in various SSC opinions and reports. The TSE/BSE *ad hoc* Group considers that they are still valid.

The opinions of interest can be listed as follows:

a. The opinion *on* "The risk born by recycling animal by-products as feed with regard to propagating TSE in non-ruminant farmed animals", adopted on 17 September 1999.

In general, this opinion recognises the recycling of animal by-products processed into basic biochemical substances as fat and protein this as an acceptable effective way of re-use of valuable materials. It accepts that intra-species recycling can be acceptable when the material of origin is from epidemiological point of view safely sourced with regard to TSE's and treated accordingly to prevent any spread of conventional diseases. It also notes that current disease monitoring systems are regarded to be unlikely to identify sporadic cases of TSE's in farmed fish. Monitoring of pathological changes wild fish over a period of 25 years for neurological disorders, on the other hand, has provided no anecdotal evidence leading to any indications of spongiform encephalopathies in fish**.**

b. The SSC opinion of 24-25 June 1999 on "Fallen stock"[4], which clarifies what can be considered as safe sourcing of fish materials and the processing conditions to be applied to fish waste.

c. Opinion of the Scientific Steering Committee (1) on the scientific basis for import bans proposed by 3 Member States with regard to BSE risks in France and the Republic of Ireland; (2) on the scientific basis for several measures proposed by France with regard to BSE risks; (3) and on the scientific basis for banning animal protein from the feed for all farmed animals, including pig, poultry, fish and pet animals. Adopted by the Scientific Steering Committee at its meeting of 27-28 November 2000

This opinion provides the possible scientific reasons for a general feed ban of meat-and-bone meal, applicable to all farmed animals including cattle, pigs, poultry, farmed fish and pet food.

6. REFERENCES

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4 Scientific Opinion on The risks of non conventional transmissible agents, conventional infectious agents or other hazards such as toxic substances entering the human food or animal feed chains via raw material from fallen stock and dead animals (including also: ruminants, pigs, poultry, fish, wild/exotic/zoo animals, fur animals, cats, laboratory animals and fish) or via condemned materials. Adopted By the Scientific Steering Committee at its meeting of 24-25 June 1999.

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APPENDIX: THE EC FAIR CT97 3308 PROJECT: "Separation, identification and characterisation of the normal and abnormal isoforms of prion protein from normal and experimentally infected fish"

Studies of the transmissibility of scrapie to fish

a. Experimental transmission:

- Groups of 30 Trout *(Onchorrhychus mykiss)* and Turbot *(Scophtalmus maximus)* were inoculated with mouse adapted scrapie agent (strain 139A) by simultaneous intracerebral (i.c.), intra peritoneal (i.p.) and intra muscular (i.m.) routes. There were 15 control animals per group.
- Brain, spleen, muscle, liver, intestine, kidney from 3 infected and one control fish were sampled at each of the following time points:. 15 days post inoculation (pi), 3 months pi, 6 months pi and every 6 months thereafter.
- All tissues were inoculated into mice and fixed for immunohistochemical studies.

The incomplete results are summarised in the following table, showing the number of mice positive/ number of mice inoculated (unconfirmed/pending result)

Post-inoculation	Turbot		Trout	
	Brain	Spleen	brain	Spleen
15 days	2/8 (1)	4/7 (0)	No results yet available, all mice still alive	
90 days	1/8 (7)	0/8 (5)		
180 days	-	-		
360 days	-	-		
Every 6 months thereafter				

No lesions were detected so far in infected fish tissues, IHC is being performed on those tissues which were positive on assay in mice.

b. Assessment of residual infectivity:

- Turbot and Trout force fed 139A scrapie infected or normal mouse brain homogenate and samples taken of brain, intestine, muscle at 1, 15, 30, 60 and 90 days.
- Residual infectivity was detected on mouse bioassay only in one of eight mice, which had been inoculated with Trout intestine, taken 1 day after oral inoculation. Results from scrapie transmissions to mice from fish more than 90 days post inoculation are awaited.
- Infectivity also found to persist sporadically in intestine of fish fed with high doses of scrapie.

Studies of the transmissibility of BSE to fish :

Experimental Transmission

- Groups of Trout and Sea Bream *(Spaurus aurata L.)* were fed or were inoculated i.c with BSE affected bovine material. Approx. 40 experimental and 15 control in each group.
- Abnormally swimming animals sacrificed and brains dissected. Samples (brain, muscle, spleen, liver, intestine, reproductive organs, eye, kidney) taken 1, 2, 15, 30, 60, 90, 120 days pi.
- No abnormal swimming between 1 to 120 days. No evidence of infection by histology, IHC or Western blot (prionics).
- Histological findings: No evidence of significant changes in the brains or other organs studied from fish sampled at 1, 2, 15, 30, 60, 90 and 120 days pi.
- Immunohistochemical findings: by using ABC-peroxidase technique with mAbs 2A11 and 6H4, no evidence of PrPres deposition has been detected in any sample. The effectiveness of McAb 2A11 on bovine and murine prion infected brains was previously verified with ABC-peroxidase technique, and immunohistochemistry with 6H4 was performed as described previously. However, in the absence of positive TSE infected fish controls and the uncertainty of the existence of a molecule in fish equivalent to mammalian PrP, the efficacy of these antibodies for detection of any surrogate marker for TSE infectivity in fish is unknown.
- Western blot technique: by using the "Prionics test" (mAb 6H4), every sample of all the groups were negative to the presence of proteinase-K resistant prion protein.
- It must be remembered that the present period of observation (4 months) is probably not sufficient to provide evidence that would make distinction between residual inoculum infectivity and pathogenetic amplification of agent.

In a further experimental step the project proposes to evaluate the possible transmission of prions (Scrapie and BSE) to different fish species (Sea Bream, Sea Bass *(Dicentrarchus labrax L.)* and Trout). The ultimate test would be to feed back/inoculate material from fish experimentally challenged into more fish of the same species. This should be considered.