EUROPEAN COMMISSION
HEALTH & CONSUMER PROTECTION DIRECTORATE-GENERAL

Directorate C - Scientific Opinions
C1 - Follow-up and dissemination of scientific opinions

PRIONS IN MUSCLE

STATEMENT ADOPTED BY THE SCIENTIFIC STEERING COMMITTEE

AT ITS MEETING OF 4-5 APRIL 2002

Statement:

Given the limited conditions of the research reported on and the consistent negative results of infectivity experiments with regard to the presence of TSE infectivity in muscles of cattle and sheep, there is currently no reason to revise the SSC opinions with regard to the safety of bovine and sheep muscles. Also, should infectivity be present at very low levels below current detection limits, the risk of exposure to BSE infectivity is reduced to negligible levels by risk reduction measures in place and by the fact that exposure would be via the relatively inefficient oral route.

Background:

1. Investigations published on 19 March 2002 in the Proceedings of the [USA] National Academy of Sciences (PNAS) by Stanley Prusiner's group indicate that skeletal muscles of scrapie-infected mice contain and may amplify the agent (Bosque *et al*, 2002). In this study, infectivity was found in hind-limb muscles of wild-type mice inoculated i.c. and i.p. with two mouse-adapted scrapie strains (RML and Me7). Infectivity titers were $10^{5.2}$ - $10^{6.4}$ ID_{50} units/g of muscle, which was lower by a factor of approximately 10^3 than in the brain. PrP^{Sc} was detectable in Western blots of muscles. Muscles showing the presence of PrP^{Sc} were described as hind limb muscles, whereas other muscle groups were negative, with the exception of one forelimb muscle. Hindlimb muscles were PrP^{Sc}-positive late in the incubation time, no information is given on other time points. Transgenic mice that were designed to express PrP^{Sc} only in skeletal muscles were used to show that prions do not only accumulate but are able to propagate in muscle.
2. There is no doubt, that the findings reported on are of great scientific interest, but their potential bearing on BSE and a possible transmission of BSE to humans must be weighed with care. Only limited data is available on the infectivity of skeletal muscle in TSE: efforts to infect non-human primates with muscle tissue from CJD patients have failed (Brown *et al,* 1994), out of 14 goats inoculated with muscle from scrapie-infected goats one developed clinical disease. (Pattison and Millison, 1962; Pattison, 1990). A low median lethal dose was also observed in

muscle of TME-infected mink (Marsh *et al*, 1969). Transgenic mice inoculated with scrapie have been reported to produce prions in muscle previously (Bosque *et al*, 1997).

3. Muscle tissue from field cases and experimentally BSE-infected cattle were used in earlier transmission studies. A series of experiments performed in the 1990s used 4 different muscles (masseter, semitendinosus, longissimus, diaphragm) from three infected clinically sick animals for intracerebral inoculation of wild-type mice (Fraser and Foster, 1993). None of the inoculated mice succumbed to BSE. The value of underestimation in this model resulting from titration across a species barrier was estimated at $10^{2.7}$. A detection of the mouse bioassay of approximately $10^{1.4}$ mouse [i.c./i.p.] LD_{50}/g is therefore equivalent to $10^{4.1}$ cattle [i.c.] LD_{50}/g. (EC, 2002).

   In order to obviate the species barrier in these experiments, groups of calves were inoculated intracerebrally with tissues from cattle killed sequentially after oral exposure to the BSE agent. Each group of calves was inoculated with a pool of skeletal muscles (masseter, semitendinosus, latissimus dorsi) pooled from the 2-3 cattle killed at each of the time points 6, 18, 26 and 32 months after exposure in the oral challenge study. As of March 31, 2002, these animals have survived 43, 66, 38 and 65 months respectively and remain healthy. From the survival data and dose/incubation curve in a titration of BSE infectivity in cattle, the survival times of the cattle inoculated with skeletal muscles, should they succumb to disease at this time, would represent approximately $10^1 – 10^2$ cattle i.c. LD_{50} / g of tissue.

   After 19 March 2002 the French Food Safety Agency (AFSSA, 2002) carried out tests on samples of lymphoid, nervous and various groups of muscle tissues (including posterior muscles) of various animal species including bovines (one with BSE, one healthy), 4 mice infected with BSE (2 in a pre-clinical stage, 2 in the terminal phase of disease), one sheep and one goat with scrapie (clinical stage) and 2 sheep experimentally infected with BSE (clinical stage). All samples tested negative for the presence of the pathological prion protein[1] in muscle tissues, both with ELISA and Western Blot tests. However, there is no direct comparison available between the sensitivity of the PrP^{Sc} detection used by Bosque *et al* (2002) and AFSSA (2002).

   In other words, the experiments performed with cattle muscle have not to date proven infectivity. But, they were performed on a small number of muscles from a small number of naturally or experimentally infected animals. In addition, the experiments addressing infectivity in cattle muscle have been performed in a model of limited sensitivity (wild-type mice) or, in the case of the cattle assays, are incomplete.

[1] This does not a priori imply that infectivity tests by bioassay would also necessarily give negative results.

4. The basic finding by Bosque *et al* (2002) of PrP^{Sc} and infectivity in skeletal muscles in scrapie-infected mice must be further investigated independently in other laboratories. Further experiments must include such essential parameters as a detailed anatomic description of the muscles investigated, the presence or not of peripheral nerves and a description of the time course indicating when muscles test positive in relation to the central nervous system and the lymphoreticular system. In addition, further experiments are required investigating the presence of PrP^{Sc} biochemically and the infectivity in skeletal muscles in BSE-infected cattle by inoculation; some of these can now be performed in transgenic animals expressing bovine PrP^{C} that have a very low if any species barrier and a relatively short incubation time (Buschmann *et al*, 2000). Such studies can be carried out more rapidly than bioassays using cattle, but it is unclear as to whether or not **available** Tg models would **necessarily** be the more sensitive assay.

5. Because of differences in experimental approaches, including methods of calculating/estimating infectivity in the different studies, any comparisons of titres relative to cattle tissues and the Bosque *et al* (2002) study mice cannot be made with any precision.

   Also, the Bosque *et al* (2002) findings must be considered in the context of the practicality of the occurrence of BSE in cattle and the corresponding risks of exposure of humans. These involve different species barriers (mice-to-mice as compared to cattle to humans), routes of administration (the much more efficient intracerebral infection as compared to oral consumption) and mandatory testing of slaughtered animals over the age of 30 months.

Acknowledgement

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