Evaluation of the "133°/20'/3 bars heat/pressure conditions" for the production of gelatine regarding its equivalency with commonly used industrial gelatine production processes in terms of its capacity of inactivating/eliminating possible TSE infectivity in the raw material. Report and Opinion adopted by the Scientific Steering Committee at its meeting of 21-22 January 1999

1. *The question*

On 28 October 1998, Directorate General VI - Agriculture of the European Commission invited the Scientific Steering Committee to evaluate an alternative process for the production of gelatine from bones regarding its equivalent efficacy in terms of eliminating TSE agents.

The exact formulation of the question is as follows:

"Is a treatment of all ruminant bone material which is not derived from animals born, reared and slaughtered in countries recognised BSE free or at negligible risk, to heating to at least 133°C throughout its substance for a minimum of 20 minutes at a pressure of three bars, with a particle size prior to processing of not more than 50 millimetres and complying with the updated report on MBM regarding the steam requirement without air trapped,

an acceptable alternative to the production conditions laid down in the opinion on the safety of gelatine, namely:

a process which ensures that all bone material is finely crushed and degreased with hot water and treated with dilute hydrochloric acid (at minimum concentration of 4% and pH<1.5) over a period of at least two days, followed by an alkaline treatment of saturated lime solution (pH>12.5) for a period of at least 20 days with a sterilisation step of 138-140°C during 4 seconds".

The evaluation was referred by the Scientific Steering Committee to the "Safety of Products" working group of the TSE/BSE ad hoc Group of the Scientific Steering Committee. Its report follows hereafter.

2. Background

The SSC (Scientific Steering Committee) adopted at its plenary meeting of 26-27 March 1998, an opinion on the safety of gelatine produced from ruminant bones manufactured following the classic acid-alkaline process.

This opinion elaborates on the sourcing of the raw material and provides the example of an "appropriate" production process for bone materials: bones finely crushed and degreased with hot water and treated with dilute hydrochloric acid (at a minimum concentration of 4% and pH <1.5) over a period of at least two days, followed by an alkaline treatment of saturated lime solution (pH >12.5) for a period of 20 to 50 days with a sterilisation step of 138-140°C during 4 seconds. Regarding the sterilisation step, the SSC further noted that the appropriate technique should be used, as its efficacy in contributing to the inactivation of a TSE agent will also depend upon the time needed to reach the temperature, the duration of the cooling and the atmospheric pressure during the process.

It was further stated: "Alternative methods with demonstrated equivalent efficacy in terms of eliminating BSE-agents may be acceptable. Such methods must be evaluated and acknowledged on a case by case basis, also against the BSE status of the source region or country and the type of material used. For bones coming from high or low risk countries, the alkaline step should be included".

It is in this context that the question was raised whether *"a treatment of all ruminant bone material which is not derived from animals born, reared and slaughtered in countries recognised BSE free or at negligible risk, to heating to at least*

133°C throughout its substance for a minimum of 20 minutes at a pressure of three bars, with a particle size prior to processing of not more than 50 millimetres and complying with the updated report on MBM regarding the steam requirement without air trapped, can be considered as an acceptable alternative to the production conditions laid down in the opinion on the safety of gelatine".

A process which is currently applied by the industry can be summarised as follows:

- Finely crushed bone chips are degreased with hot water (85-90°C, pH = approximately 5, during an average of 15 minutes);

- After centrifugation and pre-drying, the bone chips are dried (rotating drier) in a stream of hot air (over 400°C) and then calibrated (mean particle size 15-20 mm);

- The calibrated bone chips are first pre-heated with steam (115°C, 1.7 bars, 10 minutes) in an autoclave;

- The pre-heated bone chips are autoclaved and pressurised with steam at 133°C, 3 bars, 23 minutes and then after depressurisation the gelatine is extracted with water; (10°C in most steps, 20 minutes);

- The steam heating (133°C/3bars/20 minutes) and water extraction is repeated eight times on the residual bone chips;

The gelatine extraction yield is decreased after each step. To obtain sufficient concentration during the last 4 heatings, the extraction is realised with the gelatine liquid obtained in previous extraction steps.

- The extractions are finally purified by filtration, centrifugation and are sterilised during 5 seconds at least 148°C.

3. Report from the Working Group.

The bone material used for this particular preparation may potentially be cross-contaminated with (dried) brain, spinal cord and bone marrow [1].

It has been reported that it becomes more difficult to inactivate scrapie-infected brain-tissue by heat after it has been dried (Asher et al, 1986; 1987). However, it seems (Gelatine Delft, 1998) that the degreasing step, which precedes the drying of the bones, and carried out at a pilot scale which represent the commercial degreasing process under laboratory conditions [2], reduces the brain protein levels by a factor 300-800. It may be expected that, under operational conditions, this reduction is higher because the same laboratory experiments at pilot scale resulted in degreased bone with a fat content of 6%, compared with 3% in the commercial process.

The Working Group considers that the extrapolation of data on inactivation/reduction of the TSE agent during the process of rendering animal waste into meat-and-bone meal, to a similar heat treatment of bones can not be done automatically with a high degree of certainty. This means, that if the question has to be answered in detail, experiments using as exact conditions as possible should be performed. Otherwise one has to extrapolate several parameters, including the effects of water content [3], lipid content, particle size, heat penetration and so forth. These parameters may have both a stabilising and destabilising effect on the stability of the agent and may change the temperature or time settings of the treatment.

The experiments on which the reduction factor for 133C/20min/3bar is based, involves treatment for MBM production of raw material with a certain average composition and water content. If the raw material exclusively bone material (which may previously have been dried), heat penetration may be changed. (Thus a longer pre-heating phase or heating time may be required.) The water content may not be adequate for efficient inactivation.

During the common production process of meat-and-bone meal from fresh material, the steam is generated by the water naturally present in the tissues. The water content during such process is estimated at approximately 60%. The maximum water content of bones is about 25-30%.

Although dried bone chips have a water content of only around 10%, it is evident that they become hydrated during the manufacturing process. Gelatine could not be extracted unless the bone material has at this stage a sufficiently high water content. (According to GSB, 1998, the water content is approx.25% after the pre-heating step and approx. 50% after the first heat and pressure treatment). This means that there is little doubt that steam could be generated and that heat penetration into the 15-20 mm bone chips during the 133°C steam process does occur.

Therefore, whilst awaiting results from TSE inactivation experiments, the Working Group considers that the 133C/20min/3bar conditions, if applied withall relevant parameters listed in the Updated Scientific Report presented on 24-25 September to the Scientific Steering Committee on the safety of meat and bone meal derived from mammalian animals fed to non-ruminant food-producing farm animals (e.g., maximum particle size, enough water and saturated steam [4], core temperature reached in all parts of the material for at least 20 min [5], etc.), would result in a reduction of potential BSE infectivity which is close to or equivalent to the reduction realised during the production process of meat-and-bone meal from a fresh mixture of materials containing bones, meat and other animal offals. According to the SSC opinion of 26-27 March 1998, this process is accepted to result in an infectivity reduction of at least 3 $\log_{10}$, whereas the reduction of the acidulation + liming process of the typical gelatine production process is approx 2.84 $\log_{10}$. (INVERESK Research, 1998).

4. Opinion of the Scientific Steering Committee

a) The SSC wishes to propose that any future request for the evaluation of production processes in terms of their equivalency in TSE infectivity inactivation/elimination with other already documented and validated processes, should be accompanied with the results of a validation study and/or a supporting report on the TSE inactivation/elimination capacity of the process.

b) The SSC recognised that it becomes more difficult to inactivate scrapie-infected brain-tissue by heat after it has been dried, that the raw material used for the production of meat-and-bone meal and for gelatine has a different composition (e.g., water, fat and protein content) and different physical characteristics and that there may be different heat transfer and inactivation conditions during production. In general, there is an uncertain comparability of "133°/20'/3 bars" heat/pressure/time conditions during the processing of fresh animal waste into meat-and-bone and (fresh or dried) bone material into gelatine.

In the absence of a scientific and comprehensive report on or the results of a validation study on the TSE infectivity inactivating capacity of such processing conditions and the intended end-use of the produced gelatine being for human (and possibly animal) consumption, the uncertainties about the residual risk should therefore be reduced to the minimum possible.

The SSC is also concerned that an acceptance of the equivalent inactivation of TSE infectivity in the present process for the production of gelatine with the process described in the SSC opinion of 26-27 March 1998 may trigger the submission for approval of a number of other production processes for which no validation has been carried out.

Given these concerns, the SSC cannot conclude that "133°/20'/3 bars" heat/pressure/time conditions as described in the report of the Working Group would result in an equivalent safe product compared with the acid-alkaline industrial gelatine production process described in its opinion on the Safety of Gelatine of 26-27 March 1998.

Therefore, for gelatine derived from ruminant bones, the Scientific Steering Committee's Opinion on the Safety of Gelatine adopted on 26-27 March 1998 and updated on 3 April 1998, remains valid. At present, the only preliminary conclusion can be that ruminant bones from animals certified fit for human consumption, to be used for production of gelatine with the alternative system described in the above Section 2 (Background), will have to come from BSE-free or BSE-negligible risk countries.

c) The industry is invited to organise an independent experiment showing that the series of successive "133°C/20'/ 3 bars" steps for the production of gelatine, results in a BSE infectivity reduction which is at least equivalent to the reduction obtained during the "133°C/20'/3 bars" production process defined in the SSC opinion of 26-27 March 1998

and in the Updated Scientific Report of 24-25 September 1998 on the safety of meat-and-bone meal and which accept an infectivity reduction of at least 3 log $_{10}$. These experiments should be carried out under conditions similar to the ones in the real industrial processes. The inactivation should be assessed at least for the series as a whole of successive "133°C/20'/ 3 bars" steps and preferably also for the production process as a whole. The data should clearly show that also dry contaminated material can be reduced in infectivity.

5. Literature references

Asher,D.M. et al (1986) Practical inactivation of scrapie agent on surfaces. Abstracts of the IXth International Congress of Infectious and Parasitic Diseases, Munich, 20-26 July.

Asher, D.M. et al (1987) Attempts to disinfect surfaces contaminated with etiological agents of the spongiforrn encephalopathies. Abstracts of the VIIth International Congress of Virology, Edmonton, 9-14 August, p. 147.

E.C. (European Commission), 1998a. Opinion of the Scientific Steering Committee on the Safety of Gelatine. Adopted on 26-27 March 1998.

E.C. (European Commission), 1998b. Opinion of the Scientific Steering Committee on the safety of meat and bone meal from mammalian animals, naturally or experimentally susceptible to Transmissible Spongiform Encephalopathies. Adopted on 26-27 March 1998.

E.C. (European Commission), 1998c. Updated Scientific Report presented on 24-25 September to the Scientific Steering Committee on the safety of meat and bone meal derived from mammalian animals fed to non-ruminant food-producing farm animals. Adopted on 24-25 September 1998

Gelatine Delft Company, 1998. Information on the results of laboratory tests carried out at pilot-scale on the efficacy of the degreasing step with respect to the reduction of brain material possibly present in raw bone material. Provided in the letter of 4.12.1998 of Taylor, D., member of the Working Group, to the SSC secretariat.

GSB (Gelatine Smits Beheer BV, 1998). Gelatine heat pressure process. Letter of 9.12.98 to Prof.Dr.A.Osterhaus, member of the Scientific Steering Committee. 4 pp (Commercial - in confidence).

Inveresk Research, 1998. Validation of the clearance of scrapie from the manufacturing process of gelatine. Final report. Inverest Project N° 855028. Inveresk Report N° 14682. Tranent (Scotland), 41 pp.

Literature references listed in E.C., 1998a, 1998b and 1998c.

SEAC (Spongiform Encephalopathy Advisory Committee, 1998. November 1998 Reoprt tro the (UK) Ministers: A review of infectivity in bone marrow and dorsla root ganglia in cattle infected with BSE. 4 pp.

Wells, G.A.H., Hawkins, S.A.C., Green, R.B., Austin, A.R., Dexter, I., Spencer, Y.I., Chaplin, M.J., Stack, M.J., Dawson, M., 1998. Preliminary observations on the pathogenesis of experimental bovine spongiform encephalopathy (BSE): an update. Veterinary Record, Vol.142: pp 103-106.

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[1] Preliminary results of the still ongoing BSE pathogenesis experiment in cattle (Wells et al, 1998) are not fully conclusive: the (mice) tests for infectivity of bone marrow were only positive in the group killed at 38 months after infection with BSE, when clinical disease was evident in the cattle, and not at an earlier (2 to 36 months) or later (40 months) time after exposure to BSE. The current SEAC conclusion (SEAC, 1998) is that "the positive result at 38 months cannot be discounted and may indicate that infectivity in bone marrow occurs occasionally, when clinical signs are apparent and there are already very high levels of infectivity in the central nervous system." It is noted that BSE infectivity in bovine bone-marrow has been detected in only one still ongoing experiment, and only after the onset of clinical signs.

[2] Ten grams of pig-brain thoroughly mixed with 1 kg of bone-chips typically used by gelatine manufactures (average particle size: 12 mm, maximum: 20 mm).

[3] For example, for meat-and-bone meal the water content is approximately 60%.

[4] "Saturated steam" means that all air is evacuated and replaced by steam in the whole sterilisation chamber.

[5] The working group wishes to stress that this period of 20 minutes should be continuous, without interruptions.