EUROPEAN COMMISSION
HEALTH & CONSUMER PROTECTION DIRECTORATE-GENERAL

Scientific Steering Committee

UPDATED OPINION ON

THE SAFETY WITH REGARD TO TSE RISKS OF GELATINE DERIVED FROM RUMINANT BONES OR HIDES

ADOPTED BY THE SCIENTIFIC STEERING COMMITTEE

AT ITS MEETING OF 12-13 SEPTEMBER 2002

UPDATED OPINION ON THE SAFETY WITH RESPECT TO TSE RISKS, OF GELATINE PRODUCED FROM RUMINANT BONES OR HIDES [1]

A. MANDATE AND BACKGROUND

This opinion addresses the following questions regarding the safety of gelatine produced from ruminant material: *"Can gelatine produced from ruminant bones or hides be considered to be free of TSE infectivity? If not, under which condition of sourcing of the material (geographical origin, animal origin, type of tissue) and/or age of the animal and/or production process, can it be considered as safe?"*

Since its first opinion of 21-22 January 1999 on the safety of gelatine the Scientific Steering Committee (SSC) regularly received new research results and information regarding the TSE infectivity inactivation capacity of gelatine production processes or -steps. The SSC each time updated its at that moment standing opinion on the safety of gelatine. Early 2002, the final results of recent TSE inactivation studies already reported on in the SSC's latest update of 28-29 June 2001 became available. The TSE/BSE *ad hoc* Group evaluated this new information and prepared the attached report, which served as basis for the SSC opinion hereafter.

B. GENERAL ASPECTS

a. The experiments currently available to assess the TSE infectivity reduction capacity of a production process cannot demonstrate a complete destruction of all TSE infectivity in a test sample, but only a quantitative or semi-quantitative reduction in the amount of infectivity, the "clearance" factor, which is limited by the sensitivity of the assay system and the starting titre of the TSE spiked material. It is thus not possible in the current state of knowledge to conclude that any given process for the production of ruminant-derived products would result in an end-product that is completely free of TSE agent. However, it can be stated that the currently commonly used gelatine production processes, as listed in the attached report, have a considerable TSE infectivity reduction capacity *exceeding* a factor of 30.000 ("4.5 logs")[2]. For several processes the clearance factor is (much) higher.

   The Scientific Steering Committee still considers that careful sourcing of the raw materials, where needed in combination with appropriate processing, remains a key-factor for producing safe gelatine.

b. When ruminant ***hides*** are used for the production of gelatine, they are usually obtained from bovines[3]. On the basis of current knowledge it can be considered

---

[1] Little is known about TSEs in ruminants other than cattle, sheep and goats; the present opinion therefore does not necessarily cover possible risks associated with TSEs in other ruminant species.

[2] The actual most recent research results indicate clearance factors exceeding 4.8 logs. The SSC considers it justified to round this data downwards to the nearest 0.5 log.

[3] Should hides from small ruminants be used, the SSC's wishes to refer to its following opinions: (1) Pre-emptive risk assessment of 8-9 February 2001 should BSE in small ruminants be found under domestic conditions; (2) Opinion of 4-5 April 2002 on Safe sourcing of small ruminant materials ((Safe sourcing of small ruminant materials should BSE in small ruminants become probable: genotype, breeding, rapid TSE testing, flocks certification and Specified Risk Materials); (3) Opinion of 10-11 January 2002 on TSE Infectivity distribution in ruminant tissues (state of knowledge, December 2001)

that the parts of bovine hides used for the production of gelatine do not present a risk with regard to TSEs, provided contamination with potentially infected materials is avoided. The risk of contamination of the skin with TSE agent by spillage of blood and/or CNS tissues is small if slaughter and skinning are appropriately performed. The SSC considers that - regardless of type of production process, but assuming that any gelatine from hides production process would have some TSE infectivity reduction capacity at least equivalent to a collagen production process[4] - the respect of the recommendations on sourcing listed further on will result in a safe end-product.

c. The risk of contamination with TSE infectivity is much higher with ***bones***, as compared to hides. Moreover, it has been reported that it becomes more difficult to inactivate TSE-infected brain tissue by heat after it has been dried. It is therefore justified to recommend the use of appropriate production conditions for gelatine obtained from bones. The production processes (steps) reported on in the report of the TSE/BSE *ad hoc* Group have a TSE infectivity inactivation capacity exceeding 4 logs. This is considered to be sufficient for the production of safe gelatine, *provided* they are applied in combination with appropriate sourcing of animals and raw materials. The attached report of the TSE/BSE *ad hoc* Group lists a number of gelatine production processes or -steps that comply with this criterion.

   The SSC considers that the filtering, ion-exchange and sterilisation (at least 138°C during 4 seconds) steps at the end of the production chain do have a TSE infectivity reduction capacity. However, at this moment it is impossible to quantify the additional TSE infectivity reduction within the overall production process.

d. The SSC considers that the existence of (1) various production processes with different levels of TSE inactivation, (2) countries and regions with different levels of TSE risk, (3) various levels of human or animal exposure and (4) various types of raw materials with different levels of potential infectivity, justify different alternative applications or modulation of the "SRM, geographical source, production process and end-use" criteria for risk assessment, provided of course sufficient data to support such modulation are available. In order words, an equally safe end-product may result from, for example, severe and validated processing conditions in combination with less severe SRM rules, or vice versa.

e. The SSC further considers that appropriate storage, labelling and use of industrial gelatine is needed to avoid possible mixed uses or contamination with food or feed-grade gelatine, unless the industrial gelatine complies with the food or feed-standard criteria. Also, if the intended end use cannot be verified and controlled to exclude any human or animal consumption or use, then the conditions outlined for food-standard gelatine should apply also for gelatine for industrial or technical use.

These opinions imply that the question on the safety with regard to BSE infectivity of small ruminant hides would need to be re-addressed should it become probable or evident that BSE is present in small ruminants.

[4] See SSC opinion of 10-11 May 2001 on the Safety with respect to TSE risks of collagen produced from ruminant hides

C. SOURCING OF THE RAW MATERIALS FOR GELATINE USED IN FEED, FOOD OR COSMETIC PRODUCTS AND FOR NON-MEDICINAL AND NON-PHARMACEUTICAL TOPICAL USES ON UNDAMAGED SKIN.

For countries where the presence of one or more cattle clinically or pre-clinically infected with the BSE agent in a region or country is highly unlikely (geographical BSE risk level I) sourcing of raw materials from any animal is in principle safe with regard to BSE risks. Sourcing from animals that passed the ante-mortem inspection as fit for human consumption would add additional safety.

For other countries, the safest sourcing of the material would in principle be from animals that passed (for hides) the *ante-mortem* inspection as fit for human consumption or (for bones) both the *ante-* and *post-mortem* inspection[5]. The risk of cross contamination with specified risk materials or potentially contaminated materials should of course be minimal.

D. SOURCING OF THE RAW MATERIALS FOR GELATINE USED IN REGISTERED PHARMACEUTICAL PRODUCTS AND FOR PARENTERAL USE.

Gelatine in pharmaceuticals may be administered by the oral, topical or parenteral route or as implantable medical devices that may persist at the site of administration for longer periods of time. The products from one source may be administered to very large numbers of people and/or to special groups in terms of age, low body weight, longevity, preventive needs, etc.

The SSC therefore considers it justified that for these applications, higher standards are applied and that the safety of gelatine for parenteral or ophthalmic administration, in topical products where these are likely to be applied to large areas of skin or to open wounds, for vaccines or for use in implantable devices (including use as excipients in these groups of products), needs to be assessed on a case-by-case basis. The SSC recommends that in any case the use of a special grade[6] of gelatine should be considered. This would also require sourcing of the raw materials either from countries where the presence of one or more cattle clinically or pre-clinically infected with the BSE agent in a region or country is highly unlikely (GBR I) or, for other countries, from closed herds. (See the SSC opinion of 23 July 1999 on *The conditions related to "BSE Negligible risk (closed) bovine herds*.)

E. THE END USE OF THE GELATINE IS EXCLUSIVELY INDUSTRIAL OR AS A TECHNICAL PRODUCT.

The conditions for raw material for food or feed-standard gelatine should apply or if the animals from which the raw material is derived are not fit for human consumption, the recommendations in the SSC opinion of 25 June 1999 on "Fallen stock"[7] should be complied with. In addition, the specified risk materials should be removed and the gelatine should be submitted to an appropriate production process, as discussed in the attached report of the TSE/BSE *ad hoc* Group.

---

5 This implies the rapid *post mortem* testing for BSE, if and where appropriate.

6 To be defined.

7 Complete title: *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.*

If the intended end use cannot be verified and controlled to exclude any human or animal consumption or use, or if no dedicated production lines exist for technical and other uses, then the conditions outlined for food-standard gelatine should apply.

Note: The SSC questions whether it is realistic to consider separate sourcing, production and tracing of raw materials and gelatine for technical uses. Also, the risk is not theoretical that such product would become a way of disposing of certain specified risk materials and that eventually the overall risk may turn out to be higher because of the high proportion of these potentially contaminated SRMs in the raw materials.

F. The attached report provides additional data that can be used for the comparative quantitative assessment of different gelatine production methods. These data can be used for the assessment of public health effects of gelatines produced with different processes.

EUROPEAN COMMISSION
HEALTH & CONSUMER PROTECTION DIRECTORATE-GENERAL

Scientific Steering Committee

REPORT ON THE CURRENT STATE OF KNOWLEDGE ON THE TSE INFECTICITY CLEARANCE CAPACITY OF VARIOUS GELATINE PRODUCTION PROCESSES.

FINALISED BY THE TSE/BSE AD HOC GROUP AT ITS MEETING OF 5 SEPTEMBER 2002

TABLE OF CONTENTS

I. BACKGROUND.

On 21 January 2000, the Scientific Steering Committee adopted an update of its scientific opinion and report of 21-22 January 1999 on the Safety of Gelatine. The opinion provided the example of the (classic) acid-and-lime production process, which is considered to clear TSE infectivity that is possibly present by a factor of approximately 2.84 $\log_{10}$: "*bones finely crushed and degreased with hot water and treated with dilute hydrochloric acid (at a maximum 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*". As an alternative to the liming step, a 0.25 - 0.30 molar NaOH treatment during 5-7 days at 15 °C ± 2° was considered about 2 $\log_{10}$ more efficient. Finally, the opinion stated that for bones coming from high or low risk countries, the liming or (above) NaOH step should always be included.

The SSC, however, expressed its concern regarding the fact that the material used for certain TSE infectivity inactivation studies, the TSE agent did not consist of spiked bones but of scrapie infected brains, which are two different environments. The SSC considered that, for a final assessment to be made, inactivation experiments should be carried out on spiked bone material. It also recommended that research on the elimination and inactivation of TSE, including BSE, agents during the gelatine manufacturing process should also be carried out on raw material really used for gelatine production and for the production process as a whole.

Mid 2001, provisional results and information regarding the TSE infectivity inactivation capacity of various gelatine production processes or -steps resulting from current research became available. They have been reported on in the SSC's update of 28-29 June 2001. In August 2002, final results (Taylor et al, 2002a, Taylor et al, 2002b) became available and TSE/BSE *ad hoc* Group evaluated this new information and prepared the report hereafter. The bio-assays in a mouse model, on which the results are based, have been conducted for 600 days or more, which is beyond the longest period of survival after infection of the used types of test mice recorded so far. Some of the results have however not yet been published as peer-reviewed papers and the report hereafter may therefore need to be amended in the light of the final peer-reviewed version of the experimental set-ups and results, when they become available.

II. TSE INACTIVATION CAPACITY OF VARIOUS GELATINE PRODUCTION PROCESSES

II.1. NEW DATA ON TSE INFECTIVITY INACTIVATION CAPACITY OF THE ACID AND THE ALKALINE LIMED PRODUCTION PROCESSES.

The new validation studies presented in Grobben *et al* (2002), Taylor *et al* (2002a) and Taylor *et al* (2002b) show that the total TSE infectivity clearances that can be expected from these processes are higher than the ones previously given in the SSC's opinion of 21 January 2000. From these new results appears that the acid process after degreasing and demineralisation has a total clearance of approximately 2.6 $\log_{10}$. Filtration, ion-exchange and UHT sterilisation reduced further the infectivity below detection level and the estimated clearance level of the

process as a whole (gelatine production and purification) is estimated to be at least 4.8 $\log_{10}$

The long (classic) alkaline step after degreasing and demineralisation currently shows a total clearance of 3.7 $\log_{10}$ as compared to the reduction of infectivity of the acidulation + liming steps in classic typical gelatine production process estimated at approximately 2.84 $\log_{10}$ in the SSC opinion of 21 January 2001. The total alkaline process, including filtration, ion-exchange and UHT sterilisation reduced the infectivity below the detection level corresponding to an estimated clearance level of the process as a whole of at least 4.9 $\log_{10}$

II.2. A VARIANT OF THE ALKALINE PROCESS (SHEPHERD, 1999).

This process has already been reported on in the Report attached to the SSC opinion of 21 January 2000 on the Safety of gelatine (Shepherd, 1999). The replacement of the liming step, by a 0.25 - 0.30 molar NaOH treatment during 5-7 days at 15 °C ± 2° (at pH between 13.4 - 13.5) seems about 2 $\log_{10}$ more efficient. Currently, the total estimated infectivity reduction is approx. 4.82 logs after 5 days and 5.25 logs after 7 days (filtration, ion exchange and sterilisation steps not included).

II.3. A SHORT NAOH-TREATMENT INTRODUCED AFTER THE DEMINERALISATION STEP IN THE ACID MANUFACTURING PROCESS (GROBBEN *ET AL*, 2002).

The short NaOH-treatment is carried out with 0.3M NaOH during 2 hours at pH 13 at room temperature after the demineralisation step followed by washing and acidulation to pH 2 with diluted hydrochloric acid and subsequent extraction with hot water.

In the inactivation experiment (Grobben *et al,* 2002), spiking was carried out in such a way that it both simulated the situation in which the spinal cord could be infected, together with the possibility of adventitious contamination on bone surfaces: macerated mouse-brain infected with 301V was injected into bovine spinal cord. In addition, macerate was smeared onto the surfaces of the crushed bones. The infectivity of the 301V mouse brain is estimated at $10^{7,7}$ intracerebral ID_{50}/g. This spiked material was then submitted to the following scaled-down gelatin production process (Grobben *et al*, 2002):

1. A classic degreasing step of the finely crushed bone chips was carried out with hot water (at 70-90°C) Thereafter the bone chips were dried in hot air at 109-119°C for 40 minutes. The temperature of the chips is not higher than 85°C.
2. Secondly the classic demineralisation step was applied with increased concentrations of Hydrochloric acid (0.5-2.5-4%) during 4 days in order to dissolve the mineral part of the bone. The produced ossein is washed several times with water.
3. In the following step, the ossein obtained from demineralisation is immersed for 2 hours in a solution of 0.3 M sodium hydroxide at pH 13 at room temperature[8]. After draining of the hydroxide, the ossein is washed with water

[8] The pH is maintained at 13 by the addition, when needed, of 1M solution of NaOH.

and immersed overnight in dilute hydrochloric acid at pH 2 and thereafter intensively washed with water.

4. The gelatine is extracted from the ossein with hot water in 3 or 4 steps. The temperature during the first extraction is 60°C and increased by a further 10° for the subsequent extractions. The gelatine extract obtained is between 2 and 8%.
5. The gelatine is further purified by filtration and ion-exchange and finally sterilised at 138°C - 140°C during approx. 4 seconds and dried.

Results of the inactivation study: The inactivation study show that including an additional short NaOH treatment (0.3 M NaOH during 2 Hours at pH 13 at room temperature) after the demineralisation step in the acid process resulted in no BSE infectivity detectable in mice at 666 days after infection. (Note: Given that the infectivity levels in these bones has already been reduced by washing procedures and exposure to acid, the exact inactivation capacity of the single sodium hydroxide step cannot be determined.

Conclusion: The TSE/BSE *ad hoc* Group concludes that this novel acid manufacturing process (including degreasing, demineralisation and short NaOH treatment, but excluding the final processing steps of filtration and sterilisation) has an estimated total infectivity clearance capacity above 5.4 $\log_{10}$, which exceeds the one of the more currently used (long) limed process.

II.4. THE "133°/20'/3BARS" HEAT/PRESSURE/TIME SYSTEM (GROBBEN *ET AL*, 2002).

In the experiment to assess the inactivation of the "133°/20/3 bars" heat/pressure/time conditions for the production of gelatine fresh crushed bones material was artificially infected with macerated mouse brain, infected with the 301V strain of mouse passaged BSE agent. The BSE infected starting material is a simulation of the worst case scenario and imitates the case that the bone is cross-contaminated with infected tissue and also contains backbone from which infected spinal cord is not completely removed.

The spiked bone raw material, after degreasing and subsequent drying, was submitted to a scaled down model of autoclaving at 133°C during 20 minutes at 3 bars, followed by gelatine extraction by hot water. The raw product is then further processed (refined, filtered, sterilised) as in the other production procédées. A sample summarised description of the process is 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 always submitted to a first autoclaving step with steam at 133°C, 3 bars, 20 minutes and then after depressurisation the gelatine is extracted with water (10°C in most steps, 20 minutes). These conditions are not only necessary for the TSE inactivation but also for the gelatine manufacturing process.

- Autoclaving and extraction are repeated up to seven times, but are done at conditions with a somewhat lower temperature (for instance 125°C) or during a somewhat shorter time. The precise conditions depend on the specific properties of the sued bone and on the demands on the product to be obtained.

  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 4-5 seconds at least 138°C.

The clearance factor was determined by comparing the infectivity of the gelatine extract after the autoclaving and pressurising step with steam at 133°C, 3 bars, 20 minutes with the infectivity of the starting material. In the experiment the combined clearance factor of the degreasing step and autoclaving step was thus determined.

Results of the inactivation study. The titre of the mouse brain used as spiking material is (after 600 incubation days) estimated to be approximately $10^{8.7}$ ID_{50}/g. The total infective load of the staring material was estimated at $10^{9.3}$ ID_{50}. After an incubation time of 600 days, none of the inoculated mice have died. The process therefore appears to have reduced the infectivity titre below the level of detection in mice.

Conclusion. The TSE/BSE *ad hoc* Group concludes that the "133°/20'/3bars" heat/pressure/time conditions, as described, can be considered as having a higher inactivation capacity (reduction higher than 6.6 $\log_{10}$) then the (long) acid-limed bone gelatine process which is usually considered as the benchmark production process for what concerns TSE infectivity inactivation.

II.5. THE EFFICACY OF THE INDIVIDUAL PROCESS STEPS OF THE FINISHING UNIT OPERATIONS IN TERMS OF TSE INFECTIVITY REMOVAL AND/OR INACTIVATION (ROHWER *ET AL*, 2001).

In all gelatine manufacturing processes (from bovine bones, hides from cattle and pigs) the raw material is first submitted to a pre-treatment in order to isolate the ossein from the collagen, after which the gelatine is extracted with warm water. The water-gelatine extract is than purified by filtration, ion-exchange and finally sterilised and dried.

The Gelatine Manufacturers of Europe (GME) supported by the E.C. Food Nutrition and Health Programme conducted a study in which three individual steps at the end of the gelatine production process were tested singly or in tandem for their ability to remove or inactivate the Hamster adapted 263 K strain of scrapie agent[9] that is used to spike the gelatine extracts (Rohwer *et al*, 2001). In a scaled down model, the crude gelatine extracts obtained from bones (or hides) contain before purification 2 to 8% gelatine. The gelatine-water solutions were first filtered to remove coarse particles using cellulose cake[10]. After filtration, salts were removed by ion-exchange columns and concentration of the solution to about 20 % in a vacuum evaporator. U.H.T. sterilisation was then applied for at least 4 seconds

---

[9] An identical experiment employing mouse adapted BSE is currently under titration.

[10] The experiments with diatomaceous earth powder are still ongoing.

at 138-140 °C. Finally the gelatine solution was cooled to form a gel, which was dried in a stream of warm air.

Results of the inactivation study. The details of the results are provided in Rohwer *et al* (2001). They show:

- UHT sterilisation inactivates approximately 4 $\log_{10}ID_{50}$ in a four seconds exposure to 138°C - 140°C.
- Filtration and ion-exchange remove approximately 1.5 $\log_{10}ID_{50}$ infectivity. Both removals are by mechanical trapping.
- If it should be proven that the scrapie infectivity reduction capacity of the gelatine purification and sterilisation steps are additive, then the total inactivation would be more than 5 $\log_{10}ID_{50}$ of TSE infectivity.

Discussion and conclusion

The final production steps as reported in Rohwer *et al* (2001) certainly have a TSE significant infectivity reduction effect, and this is corroborated by the above reported results of Grobben et al (2002), Taylor et al (2002a; 2002b). But at this moment it is impossible to quantify their individual additional TSE infectivity reduction capacity within the overall production process, because the experiments have been carried out on gelatine extracts spiked *after* the acid and alkali steps.

Further experiments enclosing all the production steps as a whole are needed, to evaluate the additivity of the reductions realised in each step.

The sterilisation step provided the most significant reduction in scrapie infectivity. This result is in agreement with Rohwer (1984) but seems surprisingly high if compared with other studies on TSE inactivation by sterilisation (for example Schreuder *et al*, 1998; Grobben *et al*, 2002; Taylor *et al*, 2002a; 2002b). Regarding the efficacy in terms of TSE infectivity clearance of the sterilisation step, the TSE/BSE *ad hoc* Group therefore considers that the 4 log reduction after a 4 second exposure to 138-140°C must be confirmed before the result can be accepted.

III. OVERALL SUMMARY AND GENERAL CONCLUSIONS.

The following are considered to be gelatine production processes (steps) that have a TSE infectivity inactivation capacity exceeding 4.5 logs. Provided they are applied in combination with appropriate sourcing of the raw materials, they will result in an end product with a (TSE) risk level close to zero. Details of the processes are given in the appropriate sections of this report.

1. **The alkaline and acid processes, as described in the SSC opinion of 21.01.00:**

   In the alkaline process, the bones are finely crushed and degreased with hot water and demineralised with dilute hydrochloric acid (at a maximum concentration of 4% and pH <1.5) over a period of at least two days to dissolve the calcium phosphate of the dried degreased bone chips. The remaining organic matrix, called ossein, is washed with water. Follows then an alkaline treatment of saturated lime solution (pH >12.5) for a period of 20 to 50 days, a neutralisation step with dilute sulphuric acid, stepwise extraction with hot

water and the purification steps filtration and ion-exchange) and finally the sterilisation step of 138-140°C during 4 seconds.

In the acid process, the lime and neutralisation steps are replaced by an acid treatment during which the washed ossein is kept for 10-12 hours in dilute hydrochloric acid.

2. **A variant of the alkaline process given in the SSC opinion of 21.01.00**

   Instead of the liming step in the above alkaline process, a 0.25 - 0.30 molar NaOH treatment during 5-7 days at 15 °C ± 2° (at pH between 13.4 - 13.5) is introduced.

3. **A variant of the acid process:**

   In addition to the demineralisation step, the ossein is immersed for 2 hours in a solution of 0.3 M sodium hydroxide at pH 13 at room temperature. After draining of the hydroxide, the ossein is washed with water and immersed overnight in dilute hydrochloric acid at pH 2 and thereafter intensively washed with water.

4. **A gelatine-adapted heat/pressure/time process**

   In this process the bone raw material is always submitted to an autoclaving step at 133°C during 20 minutes at 3 bars (saturated pressure, all air removed) followed each time by hot water extraction of the gelatine.

According to the above, the gelatine production, including final the production steps of filtration and sterilisation, would result in an infectivity clearance of *at least* $10^{4.5}$ for the alkaline and acid production processes. For the heat/pressure process, the reduction may even be beyond 10^{6}.

Filtering and sterilisation (at at least 138°C during 4 seconds) steps at the end of the production chain do have an infectivity reduction capacity. However, it is still impossible to quantify their individual additional infectivity reduction within the overall production process as the available experiments have been carried out on gelatine extracts spiked after the acid and alkali steps.

Drying may stabilise the TSE agents against heat inactivation and the gelatine comes in contact with many hot surfaces during the manufacturing process. The TSE/BSE *ad hoc* Group considers that an important exception to the potential value of refinement would be if it came at the cost of contamination by dried gelatine or other dry particles carried forward from earlier steps in the process.

IV. ACKNOWLEDGEMENT

The TSE/BSE *ad hoc* Group gratefully acknowledges the rapporteurship of Prof.Dr.M.Vanbelle.

V. REFERENCES:

GME (Gelatine Manufactures Europe), 2002. Letter of 4 September 2002 to the SSC secretariat, providing comments on the draft Report on the Quantitative Risk Assessment for Gelatine, Tallow and DCP (version III)

Grobben A.H. ,Taylor D.M., Steele P.J., 2001. Enhancing the safety of gelatine with regard to the bovine spongiform encephalopathy agent. Veterinary Record (in press).

Grobben, A.H., 2001. Letter of 28 July 2001 to the secretariat of the Scientific Steering Committee providing further details on the autoclaving step.

Grobben, A.H., Schreuder, B.E.C., Somerville, R.A., Steele, P.J., Taylor, D.M., 2002. Report on the inactivation/removal of TSE infectivity by the manufacturing processes of gelatine. Status report on 13 June 2002 provided to the secretariat of the Scientific Steering Committee not for public release. 13 pp.

Grobben, A.H., Schreuder, B.E.C., Steele P.J., 2001. Preliminary report of the validation of the inactivation of TSE agent by the Heat and Pressure process for the manufacturing of gelatine. *(In press, June 2001).*

IAH (Institute of Animal Health, Edinburgh, UJ), 2002. 301V spike material used in GME validation studies. Report prepared for GME (Gelatine Manufacturers Europe). Confidential

Rohwer ,R.G., 1984. Virus-like sensitivity of the scrapie agent to heat inactivation. *Science* **223**, 600-602

Rohwer R.G., Grobben A.H., MacAuley C.M., 2001: Intermediate data on the removal and inactivation of TSE agents by the individual process steps of the finishing unit operations of the gelatine manufacturing process. (provided in confidence)

Schreuder,B.E.C. Geertsma R.E. ,van Keulen L.J.M. , van Asten J.A.A.M. , Enthoven P., Oberthür R.C. , de Koeijer A.A., Osterhaus A.D.M.E., 1998. Studies on the efficacy of hyperbaric rendering procedures in inactivating bovine spongiform encephalopathy (BSE) and scrapie agents. The Veterinary Record, 2 May, pp. 474-480.

Shepherd, A., 1999. Validation of the Manufacturing Process for gelatine to show Reduction of the Scrapie Agent. Inveresk report N° 16032 sponsored by Leiner Davis Gelatine (International) (Botany, Australia). Tranent (Scotland), 29 pp. (Confidential)

Taylor, D.M., Somerville, R.A., Steele, P.J., Grobben, A.H., 2002. Validation of the clearance of TSE agent by the acid bone gelatine manufacturing process. Final report of the Research experiment Ref.N° 0667/acid301V, sponsored by GME Sector Group of CEFIC and the Research Directorate general of the European Commission. Institute of animal health, Neuropathogenesis Unit, Edingburg (UK), 91 pp.

Taylor, D.M., Somerville, R.A., Steele, P.J., Grobben, A.H., 2002. Validation of the clearance of TSE agent by the alkaline gelatine manufacturing process. Final report of the Research experiment Ref.N° 0667/alkaline301V, sponsored by GME Sector Group of CEFIC and the Research Directorate general of the European Commission. Institute of animal health, Neuropathogenesis Unit, Edingburg (UK), 96 pp.