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UPDATED OPINION ON

THE SAFETY WITH REGARD TO TSE RISKS OF GELATINE DERIVED FROM RUMINANT BONES OR HIDES FROM CATTLE, SHEEP OR GOATS

(INCLUDING AMENDMENTS TO THE SCIENTIFIC REPORT ATTACHED TO THE OPINION OF 21 JANUARY 2000)

ADOPTED BY THE SCIENTIFIC STEERING COMMITTEE

AT ITS MEETING OF 28-29 JUNE 2001

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

A. MANDATE AND BACKGROUND

The present opinion addresses the following questions regarding the safety of gelatine produced from ruminant bones: *"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?"*

During the first half of 2001, the Scientific Steering Committee (SSC) received results and information regarding the TSE infectivity inactivation capacity of gelatine production processes or -steps resulting from current research. The SSC took this opportunity to update and complete its opinion on the safety of gelatine of 21 January 2000 and to bring it also in line with the recently adopted opinions on the geographical BSE-risk, on specified risk materials and on the safety of other products such as tallow, collagen and fertilisers. The update results from the attached scientific report prepared by the TSE/BSE *ad hoc* Group. The attachment completes the report accompanying the opinion of 21 January 2000. Both opinions and reports will eventually be merged into one single document.

It is mentioned that the available new information is based on research that is in a far-advanced but not yet fully final stage. To date, the bio-assays on mice, on which the results are based, have been conducted beyond the longest period of survival after infection of the used types of test mice recorded so far. The protocols foresee nevertheless an additional period of observation of approx. 6 months and the opinion hereafter may need to be amended in the light of additional results should they become available.

B. GENERAL

The Scientific Steering Committee considers that careful sourcing of the raw materials, where needed in combination with appropriate processing, will result in safe gelatine.

When ruminant ***hides*** are used for the production of gelatine, they are obtained from bovines[2]. On the basis of current knowledge it can be considered 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

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[1] Little is known about TSEs in other ruminants and the present opinion therefore does not nececessarily cover possible risks associated with TSEs in other ruminant species.

[2] Note: should hides from small ruminants be used, the SSC's wishes to refer to its *Pre-emptive risk assessment* of 8-9 February 2001 *should BSE in small ruminants be found under domestic conditions.* It provides a state of affairs regarding tissue infectivity distribution on the basis of the most recent results of the ongoing experiments on BSE in small ruminants. They seem to indicate that BSE, experimentally transmitted to sheep, is likely to show a disease development comparable to scrapie in sheep and that the BSE agent may therefore be present in peripheral nervous tissues of small ruminants. This would 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.

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 production process would have some TSE infectivity reduction capacity at least equivalent to a collagen production process - the respect of the recommendations on sourcing listed further on will result in a safe end-product.

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, *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 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.

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.

C. SOURCING OF THE RAW MATERAILS 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 (GBR 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 (1) 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[3] and (2) if the risk of cross contamination with specified risk materials or potentially contaminated materials is minimal.

(*) **Note**: The SSC accepts 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 exposure and (4) various types of raw materials with different levels of potential infectivity, would scientifically justify a modulated

[3] This implies if and where appropriate the rapid *post mortem* testing for BSE.

application of the "SRM, geographical source, production process and end-use" criteria, provided of course sufficient data to support such modulation are available.

D. SOURCING OF THE RAW MATERAILS 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 safety of gelatine for parenteral or ophthalmic administration, in topical products where these are likely to be applied to large areas of ~~damaged~~ 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[4] 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 herds that are certified BSE-free. (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 food or feed-standard tallow 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"[5] 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.

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.

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[4] To be defined.

[5] 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.*

AMENDMENTS TO THE REPORT OF 21 JANUARY 2000 ON THE SAFETY WITH RESPECT TO TSE RISKS, OF GELATINE PRODUCED FROM RUMINANT BONES

I. BACKGROUND.

1. On 21 January 2000, the Scientific Steering Committee adopted an updated scientific report and opinion on the Safety of Gelatine.

   The opinion provides the following example of an appropriate production process in terms of eliminating TSE agents is: 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° is considered about 2 $\log_{10}$ more efficient. Finally, it was stated that for bones coming from high or low risk countries, the liming or (above) NaOH step should always be included.

   The Scientific Steering Committee called for the results of the research on the TSE agent inactivation during the manufacturing of gelatine to be made urgently available, in order to possibly revise or broaden the above definition of appropriate production processes.

2. The opinion also invited the industry 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 $\log_{10}$. The SSC considered that 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.

3. The SSC finally 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.

4. During the first half of 2001, the Scientific Steering Committee (SSC) received results and information regarding the TSE infectivity inactivation capacity of gelatine production processes or -steps resulting from current research. It is mentioned that the available new information is based on research that is in a far-advanced stage but not yet fully final: to date, the bio-assays in a mouse model, on which the results are based, have been conducted for 400 days or more, which is

beyond the longest period of survival after infection of the used types of test mice recorded so far. The protocols foresee an additional period of observation of approx. 6 months and the opinion hereafter may need to be amended in the light of additional results should they become available.

II. AMENDMENTS TO THE REPORT OF 21 JANUARY 2000.

II.1. NEW DATA ON TSE INFECTIVITY INACTIVATION CAPACITY OF THE "CLASSIC" ACID AND LIMED PRODUCTION PROCESSES (GROBBEN *ET AL*, .2001).

Although the results of the new validation studies presented in Grobben *et al*, 2001 are not yet fully final, they show that the total TSE infectivity clearances that can be expected from these processes is higher then 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 3.7 $\log_{10}$. The long (classic) alkaline step after degreasing and demineralisation currently shows a total clearance of 4.2 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.

II.2. A VARIANT OF THE ACID-ALKALI PROCESS DESCRIBED IN THE SSC OPINION OF 20-21 JANUARY 2000.

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) In stead of the liming step, 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).

Reference:

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)

II.3. A SHORT NAOH-TREATMENT INTRODUCED AFTER THE DEMINERALISATION STEP IN THE ACID MANUFACTURING PROCESS OF BONE GELATINE (GROBBEN *ET A*, 2001).

a. The question

The TSE/BSE *ad hoc* Group addressed the question whether a treatment with 0.3 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, was an acceptable alternative to the production conditions laid down in the SSC opinion of 21 January 2000 on the safety of gelatine.

b. Summary description of the inactivation experiment.

Spiking was carried out in such a fashion 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. This spiked material was then submitted to the following scaled-down gelatin production process (Grobben *et al*, 2001):

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[6]. 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. 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.

c. <u>Results of the inactivation study</u>.

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 585 days[7] after infection. The infectivity of the 301V mouse brain is currently[8] estimated at $10^{8,2}$ intracerebral ID_{50}/g. The total load of spiked test material was $10^{9,2}$ intracerebral ID_{50}.

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.

e. <u>Conclusion</u>

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 reduction capacity which exceeds the one of the more currently used (long) limed process.

<u>Reference</u>:

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

7 The experiment will be ended at 600 days.

8 As the observations on mice are still ongoing, the titre determination is not final.

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).

II.4. ON THE EQUIVALENCY OF THE "133°/20'/3BARS" HEAT/PRESSURE/TIME CONDITIONS WITH THE LONG ACID-ALKALI GELATINE PRODUCTION PROCESS.

a. The question.

The TSE/BSE *ad hoc* Group addressed the question whether the "133°/20/3 bars" heat/pressure/time conditions 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 21 January 2000.

b. Summary description of the inactivation experiment

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. (This process can be repeated up to eight times, after which no gelatine can be extracted anymore.) 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 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 up to 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 4-5 seconds at least 138°C.

The clearance factor was determined by comparing the infectivity of the gelatine extract with the infectivity of the starting material. In the experiment the combined clearance factor of the degreasing step and autoclaving step was thus determined.

c. Results of the inactivation study.

The titre of the mouse brain used as spiking material is currently (after approx. 400 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}. To date, after an incubation time of more then 400 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. It must be noted however, that the titration is not yet complete or past the maximum incubation period of approx.400 days for which cases of 301V infections in mice have been previously reported in literature.

d. 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 then the (long) acid-limed bone gelatine process which is usually considered as the benchmark production process for what concerns TSE infectivity inactivation.

Reference:

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).*

II.5. ON THE EFFICACY OF THE INDIVIDUAL PROCESS STEPS OF THE FINISHING UNIT OPERATIONS OF THE GELATINE MANUFACTURING PROCESS IN TERMS OF TSE INFECTIVITY REMOVAL AND/OR INACTIVATION.

a. The question.

The TSE/BSE *ad hoc* Group evaluated the outcome of a validation study on the TSE infectivity reduction capacity of the filtration, ion-exchange and sterilisation steps, as it is expected that some of these final steps have potential to reduce further any residual TSE infectivity with the raw material.

b. Summary description of the inactivation experiment

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].

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

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

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

c. <u>Results of the inactivation study</u>.

The details of the results are provided in Grobben *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.

e. <u>Discussion en Conclusion</u>

The final production steps certainly have a TSE infectivity reduction effect, but at this moment it is impossible to quantify their additional TSE infectivity reduction capacity within the overall production process, because the available experiments have been carried out on gelatine extracts spiked *after* the acid and alkali steps. 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). Regarding the efficacy in terms of TSE infectivity clearance of the sterilisation step, the TSE/BSE *ad hoc* Group therefore considers that the the 4 log reduction after a 4 second exposure to 138-140°C must be confirmed before the result can be accepted.

<u>References</u>:

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.

II.6. OVERALL SUMMARY AND GENERAL CONCLUSIONS.

The following are considered to be gelatine production processes (steps) that have a TSE infectivity inactivation capacity exceeding 4 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.

1. The acid-alkali process, as described in the SSC opinion of 21.01.00:

In this process bones are 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.

2. **A variant of the acid-alkali process given in the SSC opinion of 21.01.00**

   In stead of the above liming step, a 0.25 - 0.30 molar NaOH treatment during 5-7 days at 15 °C ± 2° (at pH between 13.4 - 13.5).

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 repeatedly autoclaved at 133°C during 20 minutes at 3 bars (saturated pressure, all air removed) followed each time by hot water extraction of the gelatine.

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 the 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.

The TSE/BSE ad hoc *Group calls for the results of the research on the TSE agent inactivation during the manufacturing of gelatine to be made urgently available, in order to possibly revise or broaden the list of appropriate production processes presented in the attached report.*
