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 5-6 DECEMBER 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. There are three major factors that influence the risk of exposure from animal by-products in relation to BSE:

   (1) The titre of infectivity likely to be found in the tissue used in its manufacture. This is directly linked to the source of tissue and animal.

   (2) The effectiveness of the process used for the inactivation (or the elimination) of the agent.

   (3) The kind of application (i.e., food, feed, cosmetics, medicinal products and devices, technical uses).

   Processes which are guaranteed to eliminate all infectivity have so far not been described for products such as gelatine, tallow, meat-and-bone-meal and dicalcium phosphate. TSE infectivity clearance experiments are likely to be announced in the near future for certain tallow derivatives. In addition there is no evidence that experimental spiking of tissues with high BSE titers results in similar conditions as material from naturally infected animals or fallen stock. The SSC therefore considers that careful sourcing of the raw materials as specified in Sections C, D and E, where needed in combination with appropriate processing, remains a key-factor for producing safe gelatine.

b. Based on today's scientific knowledge, the safety assessment of all kinds of products and processes by which those products are manufactured can only be based on certain types of tests of which the mouse bio-assay with intra-cerebral injection is the most sensitive one. These experiments are also used to assess possible infectivity levels in materials such as meat, milk and other parts of the animal body.

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[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.

The experiments currently available to assess the TSE infectivity reduction capacity of a production process do not 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. Risk assessments should thus not focus only on the question whether the infectivity is completely destroyed, but rather whether a product, made under the worst case scenario conditions would be harmful to the consumer.

For gelatine, experiments are available showing that a product made from a starting product with an experimental high starting TSE agent titre can reduce the infectivity to such an extent that, after intracerebral injection, the product will not contain infectivity at detectable levels. Recent experiments show 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.

c. 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 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 contact with infected 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.

d. The risk of contamination with TSE infectivity is much higher with ***bones***, as compared to hides. In particular, the dorsal root ganglia and the spinal cord pose a higher risk in the second half of the incubation period,. Moreover, it has been reported that it becomes more difficult to inactivate TSE-infected brain tissue by

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[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)

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

heat after it has been dried. It is therefore justified to recommend, in addition to the removal of specified risk materials (for gelatine: skull and vertebrae), the use of appropriate production conditions for gelatine obtained from bones. The production processes (steps) reported on in the attached report of the TSE/BSE *ad hoc* Group have a TSE infectivity inactivation capacity exceeding 4.5 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 UHT 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, even though it has been calculated to be higher than 10^2 for the acid manufacturing process and 10^1 for alkaline process.

e. In its opinion of 13-14 April 2000[5], the SSC considers that the additional safety gained from the removal of vertebral column in addition to the skull for the production of [tallow and] gelatine is limited [but not quantified] in countries with a lower BSE risk, but should be considered as sufficiently important to exclude the vertebral column in higher risk countries. The SSC is currently quantifying the difference of BSE risk reduction in products such as tallow and gelatine, with and without removal of the bovine vertebrae. Pending the outcome of this assessment, and in accordance with its opinion of 12 January 2001[6], the SSC considers that skull and vertebrae from bovines above 12 months should not be used for the production of gelatine if the risk that the animals carry BSE is not low (GBR I countries or if the conditions listed in the SSC opinion of 12 January 2001 are complied with for animals born after the total feed ban or under other specific conditions.

   In the light of its forthcoming quantitative assessment of the residual BSE risk in products such as gelatine, tallow and dicalcium phosphate, the SSC also expects to justify a modulation of the "SRM, geographical source, production process and end-use" criteria for risk assessment by 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.

f. 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

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[5] **EC (European Commission), 2000.** Opinion on Quantitative Risk Assessment on the Use of the Vertebral Column for the production of Gelatine and Tallow. Adopted by the Scientific Steering Committee at its meeting of 13-14 April 2000.

[6] **EC (European Commission), 2001.** Opinion on the questions submitted by EC services following a request of 4 December 2000 by the EU Council of Agricultural Ministers regarding the safety with regard to BSE of certain bovine tissues and certain animal-derived products. Opinion of the Scientific Steering Committee adopted on 12 January 2001.

food-standard gelatine should apply also for gelatine for industrial or technical use.

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 should not present a safety problem with regard to BSE risks. Sourcing from animals that passed the ante-mortem inspection as fit for human consumption would add additional reassurance.

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[7]. The risk of cross contamination with specified risk materials or potentially contaminated materials should be assured.

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[8] 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.

There is no risk associated with gelatine for exclusive industrial or technical applications if the conditions for raw material for food or feed-standard gelatine apply. However, if the animals from which the raw material is derived are not fit for human consumption[9], the recommendations in the SSC opinion of 25 June 1999 on

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

8 To be defined.

9 The association of Gelatine Manufactures of Europe states that its industry has stopped voluntarily several years ago to use raw materials from high risk countries. Based on historic procedures and

"Fallen stock"[10] 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 quite possible 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.

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current legislation, their gelatine operations are sourcing raw material for all applications exclusively from animals found fit for human consumption.

[10] 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.*

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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

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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 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. This research was carried out at specialised institutes in Edinburgh (UK), Delft (The Netherlands) and the USA (See literature list). 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. This allows for cases to be detected after the usual endpoint which can occur after such treatments. The experimental protocol and results have been reviewed by the TSE/BSE *ad hoc* Group.

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.

In recent validation studies presented in Grobben *et al* (2002), Taylor *et al* (2002a) and Taylor *et al* (2002b) fresh crushed bones material was artificially infected with macerated mouse brain, infected with the 301V strain of mouse-passaged BSE agent. 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 calculated at $10^{7,7}$ - $10^{7,8}$ intracerebral ID_{50}/g.

Drying may stabilise the TSE agents against heat inactivation. 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. In the experimental design the spiked bones have been dried after degreasing to simulate real conditions where the drying process might stabilise TSE agents against inactivation.

Spiking by injection into the bovine spinal cord plus contamination of the surface was designed to represent a worst case scenario with regard to the infectivity level of the starting raw material level. It was thought to be equivalent to all animals being clinically infected and without the removal of brain and spinal.

These experiments 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 it 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 further reduced the infectivity below detection level and the clearance level of the process as a whole (gelatine production and purification) is calculated 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 of approximately 2.8 $\log_{10}$ 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 calculated 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.

The spiked material was 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[11]. 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.

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, 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);

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

- 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 performed (?) 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. After an incubation time of 600 days, none of the inoculated mice have died. The process appears to have reduced the infectivity by a factor higher than 6.6 $\log_{10}$ to a 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 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[12] 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

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

to remove coarse particles using cellulose cake[13]. 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.

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

Experiments enclosing all the production steps as a whole have been done for the alkaline and acid processes (Grobben *et al*, 2002; Taylor *et al*, 2002a;b) (see Sections II.1. and II.3.) However, the inactivation capacity of the finishing operations alone can only be evaluated if there is sufficient infectivity available or left over from the previous production steps. This was not the case in these experiments.

The study design of Rohwer *et al* (2001) used a re-spike of the gelatine solution to assess which removal and inactivation possibilities are included in the purification process. The results show that the final production steps as reported by them 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 remains impossible to *exactly* quantify their individual additional TSE infectivity reduction capacity within the overall production process, because the Rohwer experiments have been carried out on gelatine extracts spiked *after* the acid and alkali steps.

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 it has been calculated to be higher than $10^{2.2}$ for the acid manufacturing process and $10^{1.2}$ for alkaline. However the TSE/BSE *ad hoc* Group 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. SKULL AND VERTEBRAE AS POSSIBLE RISK MATERIALS

The brain and the spinal cord have an estimated weight of approx. 500 g and 170-260g. Most of it would be washed away or removed (e.g., with the waste water), or

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

could not pose a risk (e.g. if the vertebral column is removed without splitting and/or if skull and spinal cord are removed without risk of contamination of bones that in fact are protected by the meat and other tissues). But under a worst case scenario a fraction may be spread over the carcass, vertebral column bones, etc.:

a. For brain and trigeminal ganglia, it may be assumed that a relatively small amount[14] is spilled during stunning, slaughter or removal of heads/skulls. The amount would be smaller under conditions where brain remains in the skull, SRMs are removed as a dedicated action and knives are cleaned and heat treated in hot water after this use. (Knives are not sterilised in regard to TSE agents). Only a fraction of it may eventually contaminate bone material, because other tissues protect the bones.

b. In the case of spinal cord, it has initially been estimated (LFRA, 1997, data for the UK in 1997 and prior to the EU-wide obligation to remove the spinal cord in countries where BSE cannot be excluded), that 10% or 20 grams on average can remain attached to vertebral column bones. According to a Press Release of 18 July 2002 by AFP, residues of spinal cord were found for 10% out of 10.000 bovine carcasses inspected by the French Veterinary Services, with a quantity of spinal cord residues found in all failures being below 2% (below 1% for 90% of the failures). This survey was carried out before 1 January 2002, when in France the removal of spinal cord by aspiration became obligatory.

   In Great-Britain the State Veterinary Service reported that between 1996 and 2001 no spinal cord was found in any carcass of an animal slaughtered in the UK. However, spinal cord has been found in a proportion of carcasses imported into the UK from some other EU Member States.

   It can, however, be accepted that the above risks of contamination are, for current slaughterhouse practices in the EU, an overestimate: SRM removal is under supervision and subject to possible controls, the evacuation techniques for the removal of the spinal cord have improved, the spinal canal may be cleaned before further processing, etc. Only a fraction of it may eventually contaminate (other, non vertebral column) bone material, because the bones are protected by other tissues. Possible contamination with spinal cord material would at the same time account for possible failures in the sorting out of vertebrae.

c. Possible contamination with Dorsal Root Ganglia (DRG) can be assumed to be proportional to the contamination of vertebrae with spinal cord and the possible failure of sorting out vertebrae.

d. Possible contamination is possible with fluid offals such as cerebro-spinal fluid (CSF) and liquids. It could be pointed out that in the standard slaughter process the major leak of CSF occurs below the carcass on to the floor as the head is severed with head downwards. Thus any risk is more or less confined to the head and even this is protected by the angle achieved as the severance cut is

[14] Quantified estimates will be provided in the forthcoming Report on the Quantitative assessment of the Residual BSE Risk in bovine-derived products: Gelatine, tallow and dicalcium phosphate from bones Tallow from fat tissues Tallow from rendered mixtures of tissues.

made. Within the frame of the current exercise, it may be assumed that this possible source of contamination, if any[15], would be largely accounted for by the above listed possible contamination with brain and spinal cord.

The TSE/BSE *ad hoc* Group is currently quantifying (amongst others) the difference of BSE risk reduction in products such as tallow and gelatine, with and without removal of the bovine vertebrae. Pending the outcome of this assessment, in the light of the above and in accordance with the SSC opinion of 12 January 2001[16], the TSE/BSE *ad hoc* Group considers that skull and vertebrae from bovines above 12 months may pose a risk if the risk unless it is low that the animals carry BSE (GBR I countries or if the conditions listed in the SSC opinion of 12 January 2001 are complied with for animals born after the total feed ban or under other specific conditions such as the ones linked to the UK-Date Based Export Scheme).

IV. 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.

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[15] It is not known if the CSF is infected in BSE and if so at what titre.

[16] **EC (European Commission), 2001.** Opinion on the questions submitted by EC services following a request of 4 December 2000 by the EU Council of Agricultural Ministers regarding the safety with regard to BSE of certain bovine tissues and certain animal-derived products. Opinion of the Scientific Steering Committee adopted on 12 January 2001.

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, but it has been calculated to be higher than $10^{2.2}$ for the acid manufacturing process and $10^{1.2}$ for alkaline.process.

Note: in the experimental design the spiked bones have been dried after degreasing to simulate reality in order to avoid that a stabilisation of TSE agents against inactivation might be missed and not recognised. The results thus reflect also the conditions of dried TSE particles carried forward from earlier steps in the process. However, the value of the refinement might be lower should, for example under certain field conditions, TSE infectivity be present in bones that were dried before transport and degreasing.

V. ACKNOWLEDGEMENT

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