

# BSE Safety of Gelatin

## Study Report on Overseas and Domestic Situations regarding the Validation Study on the Capacity of Gelatin Manufacturing Process to Inactivate TSE Infectivity

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The gelatin produced by member companies of Gelatin Manufacturers Association of Japan (GMJ) is a safe product for which safety against BSE is fully ensured.

Even before the advent of BSE in Japan, GMJ members have complied with relevant food and pharmaceutical regulations and committed to securing safe bovine material fitting for gelatin production through such measures as clarification of the country of origin of raw material, removal of BSE risk materials, and contamination control under close partnerships with their raw material suppliers.

In addition to this, BSE safety of gelatin is further enhanced by the fact that gelatin is a high-purity protein manufactured through various refining processes. It has been reported that different steps of gelatin production including chemical/physical process and heat treatment is capable to significantly inactivate BSE infectivity, and even if there is any chance of raw material containing BSE infectious agent due to cross-contamination, the infectious agent would not remain in the final product.

Gelatin Manufacturers Associations of Europe (GME) has continued conducting a variety of validation studies <sup>1), 2), 3), 4)</sup> for ten years in order to verify that the production process of gelatin is capable to inactivate potential BSE infectivity. As reported earlier, the study report of GME has been already introduced at "The meeting for the study report on BSE safety of gelatin" <sup>5), 6)</sup>, which was organized by GMJ in June 2002.

To further promote the understanding of BSE safety of gelatin manufactured in Japan, domestic specialists, who are involved in the study of BSE and/or the manufacturing of gelatin, conducted the above captioned study. The study verified the effectiveness of BSE measures implemented by GMJ member gelatin manufacturers and the capability of gelatin production process to inactivate BSE infectivity.

The following is a summary of the study report <sup>7)</sup>, which we would like to present to further emphasize the BSE safety of Japanese gelatin.

Please contact GMJ office or member companies for the details of the study report (Japanese only).

## **I. Investigation Outline**

This investigation was conducted as the Ministry of Economy, Trade and Industry's support project for the development of leather industry for 2004 referred to as "the overseas information research and domestic PR operations regarding the capability of gelatin production process to inactivate potential BSE infectivity". The following study was conducted by the working party composed of Dr. Naotaka Ishiguro, Doctor of Veterinary Medicine and Professor of Gifu University, who leads the study of BSE in Japan, and Dr. Suguru Sumita (formerly worked for Hyogo Prefectural Institute of Technology), Doctor of Engineering and Instructor of technical development in Hyogo Prefecture, who is familiar with gelatin manufacturing.

- A) Evaluate the validity of the validation study carried out with the laboratory models based on the manufacturing condition adopted by GME was evaluated.
- B) Consider whether the production activities of Japanese gelatin manufacturers are equivalent to those of GME members and review the actual situation of the assurance of BSE safety of Japanese bovine bone gelatin.

The working party visited the Institute for Animal Health in Edinburgh, U.K. on October 1, 2004 and was able to directly interview Dr. Grobbs, who conducted a laboratory scale experiment with a miniature model of the GME study, and Dr. Somerville and Dr. Taylor, who conducted a bioassay experiment, to get the details of the study. They also visited various regions in Japan, Germany and Belgium to conduct the research on the actual situation of production activities, targeting bovine bone gelatin plants of the three GMJ member companies in Japan (Nippi Inc., Jellice Co, Ltd., and Nitta Gelatin Inc.) and the three establishments of the two GME member companies in Europe (DGF Stoess AG in Eberbach and Memmingen, Germany and PB Gelatins in Vilvoorde, Belgium).

As a result, Japanese third-party experts confirmed the following.

- They confirmed the validity of the GME validation study <sup>2), 3), 4)</sup> with the test model based on the manufacturing condition adopted by GME.
- All the process conditions adopted by Japanese bovine bone gelatin manufactures are equivalent to those adopted by GME. The manufacturing is conducted properly so that it effectively contribute to the inactivation of BSE infectivity.
- Quality records of the production process and certificates for the country of origin, animal health, materials to be used, and SRM free are properly controlled and utilized so that the traceability of raw material is ensured.

Accordingly, it was further confirmed that Japanese gelatin manufacturers have properly implemented measures against potential risk of BSE, and therefore, Japanese bovine bone gelatin is a safe product for which BSE safety is ensured.

## **II. Abstract of the Study Report**

Since gelatin is a product used in a wide variety of food and pharmaceutical products, major challenges for gelatin manufacturers are to ensure sufficient safety and to verify it. The requirements of BSE measures for gelatin manufacturing are as follows: 1) Procurement of safe raw material. 2) Standardization and assurance of the production process. 3) Inactivation of potential TSE infectivity during the production process and verification of it.

## 1. Inactivation of potential TSE infectivity during the production process and verification of it

The purpose of the validation is whether the production process of gelatin is capable of removing or inactivating TSE infectivity if raw material contaminated by TSE infectious agent enters into the production process. The verification test was conducted with laboratory scale gelatin manufacturing equipment in order to confirm the degree of the capacity of each manufacturing process to inactivate high TSE infectivity with which raw material was artificially contaminated <sup>2), 3)</sup>.

### A. Starting Material

Raw material derived from pig or fish has no risk of BSE. Even raw material of bovine origin, if it originates from bone or hide, has no BSE infectivity, either. The potential risk of BSE in bovine gelatin is that highly infectious bovine tissues such as brain and spinal cord could contaminate bones. Such cross-contamination in bovine hide is highly unlikely. Accordingly, a starting material was bovine bone that was artificially contaminated with TSE infectivity (spiking) to set the infectivity level of the material at 1,000 times worse than the actual situation. This cross-contamination reflect a hypothetical “worst case scenario” which has never and could never occur under realistic conditions.

### B. Test with downscaled model of gelatin manufacturing process

Since each manufacturer has different manufacturing conditions and equipment, the test was designed to be conducted according to the minimum conditions that all the manufacturers have in common, considering the following points <sup>2), 3)</sup>.

1. Downscale basic conditions of all the process to an accurate laboratory scale model.
2. Also, maintain other conditions than basic ones as much as possible in the laboratory scale test.
3. Give consideration to those conditions that cannot be maintained in the laboratory test and that are not the requirements of the test so as not to have any effect on inactivation.

The gelatins produced through alkali and acid processes, both of which are traditional methods of gelatin manufacturing, were used for the validation. Bovine bone, on which spiking was performed, was crushed, degreased, and demineralized with diluted hydrochloric acid to produce ossein. Lime and hydrochloric acid were used for “alkali process” and “acid process”, respectively. As for “acid process”, short-time NaOH pre-treatment, which was a new method, was also examined. After washing the ossein, gelatin was extracted from it with hot water. The unpurified gelatin went through filtration, ion-exchange, concentrating, and UHT sterilization processes in order, and the final product was then produced.

The unpurified gelatin and the final product, refined gelatin, produced by the laboratory had the equivalent composition and characteristics to those of the gelatin produced by the actual gelatin production process. It was, therefore, confirmed that the technical aspect of the process was well modeled. Although those manufacturing conditions were equivalent to the minimum requirements of GME, manufacturing conditions adopted by each of the GMJ member manufactures are beyond this level.

### C. Evaluation of the Infectivity

The 301V strain of mouse-passaged BSE agent and the 263K strain of hamster-passaged Scrapie agent were used for spiking. The both 301V and 263K have constant and high infectivity, and a short incubation period after which an infection was developed in the rodents that received intracerebral injections. The two strains also cause apparent pathologic changes after an infection was developed. The 301V BSE strain has extremely strong thermal resistance. Those are the features of the strains used for spiking <sup>8)</sup>.

The infectivity present in the samples and remaining infectivity after processing was determined by intracerebral inoculation in VM mice for 301V (20 microliter / head) and hamsters for 263K (50 microliter / head), with the samples diluted ten times. After the animals were observed for maximum 600 days following the inoculation, they were killed to measure whether their tissue slices show spongy lesion. It can be said that the combination of the strains and the rodents used for this bioassay is the most sensitive one among TSE test methods.

### D. Results

The infectivity was detected in neither limed bone gelatin nor acid bone gelatin, which went through all the production process <sup>3), 4), 10)</sup>. Although there are some differences in the capacity of each process to remove the infectivity, each process has such capacity and the effects were cumulative. The clearance factors for acid and alkali treatments in pretreatment process were  $10^{2.6}$ - $10^{4.6}$ . The following refining processes (filtration, ion-exchange, and high-temperature sterilization) showed higher clearance factors <sup>9)</sup>. Especially, the clearance factor for the high-temperature sterilization stood at very high level of  $10^{3.0}$  for the 301, and  $10^{4.1}$  for the 263 <sup>9)</sup>, and no TSE infectivity was remained in the acid bone gelatin with short-time NaOH pre-treatment <sup>10), 11)</sup>. Accordingly, it was shown that high-temperature and strong alkali treatments contribute to the removal and inactivation of the TSE infectivity.

The contamination level of the strains used for spiking is at least 1000 times higher than the industrial situation. Furthermore, the bio-assay was conducted using rodents so that there was no species barrier effect. It means that the capacity of gelatin production process to remove/inactivate the infectivity is extremely high. It is, therefore, conceivable that the risk of BSE infection to human though gelatin consumption is close to zero <sup>12)</sup>.

## 2. Research on the Japanese gelatin production

### A. Control of the specified risk material (SRM) and traceability system of bovine bone

All the GMJ members depend completely on overseas for their bovine bone. GMJ members receive bovine bone originating from fresh bone as GME members do and also obtain from their suppliers such documents as certificates for the country of origin, animal health, animal tissues to be used, and SRM free. It was confirmed that management conditions of quality records and other documents at the three Japanese manufacturers are appropriate. GME has advised that the traceability of fresh bone after slaughter house is completely established. It is also reported that the traceability of CB (Crushed Bone) from outside EU is reportedly established. All the GMJ members

ensure traceability for their raw material as GME members do.

EMA's "Note for guidance on minimizing the risk of transmitting animal spongiform encephalopathy agents via human and veterinary medicinal products"<sup>13)</sup> requires bovine bone gelatin manufacturers to comply the following: 1) Clarify the country of origin and establish traceability. 2) Skulls and spinal cord as SRM should be removed from the starting material. The removal of vertebra is also recommended for bovine bone from high BSE risk countries. 3) Monitor production process based on HACCP and ISO9000. Japanese manufactures follow the requirements of EMA as they have been certified according to ISO 9000 and/or obtained certificates from EDQM (European Directorate for the Quality of Medicine). Even though gelatin manufacturers are not required to remove vertebra from bovine bone originating from BSE free countries including Australia, New Zealand, and India for edible use, GMJ members are increasingly removing vertebra at their own initiative to further ensure the safety.

#### B. Production process that contributes to the inactivation

The study showed that the manufacturing conditions of acid demineralization (osseification), alkali treatment (liming) of raw material, filtrate, ion-exchange, and sterilization at each GMJ member manufacture<sup>3), 4)</sup> were equivalent to those of GME model, which was verified by their inactivation study. Although the degreasing system of bovine bone of Indian origin<sup>14)</sup> is different from that of GME, it would not cause major difference in the inactivation, considering the effects of such processes as those which raw materials go through after degreasing and high-temperature sterilization in the gelatin refining process. Further to this, the introduction of hot water washing for Indian CB would enable the manufacturing conditions of Japanese manufactures to close to those of EU<sup>15)</sup>.

The minimum requirements presented in the GME study report for the process of raw material (demineralization and liming) and gelatin production process (filtration, ion-exchange, and sterilization) are less strict than the typical manufacturing conditions adopted by gelatin manufacturers. It means that the manufacturing conditions adopted by Japanese gelatin manufacturers fully meet the minimum requirements. Accordingly, the manufacturing conditions of Japanese manufacturers have an expected capability of inactivating the BSE infectivity.

#### C. Comparison of the conditions of domestic and overseas gelatin plants.

Comparing the conditions of GME's laboratory model and the process records and research results of production plants of Japanese manufacturers, it turned out, as a whole, that manufacturing conditions of Japanese manufacturers are equivalent to those of GME. It is rather natural to think that there would not be much difference in the manufacturing conditions adopted by gelatin manufactures if they pursue producing high quality gelatin from the same raw material at the lowest possible cost.

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