

## Hypro-Oss Sterilization & BSE safety

### BSE prevention regulations

The manufacturer has documents at its disposal confirming that the country has established compulsory notification of BSE and that all slaughtered animals older than 30 months are subject to obligatory examination based on the Notices of the Department of Agriculture no. 286/1999, Coll. of Laws, DOA no. 399/2001, Coll. of Laws, and DOA no. 400/2001, Coll. of Laws. The manufacturer monitors and reacts to current requirements or directives issued by the European Union, including the OIE notice Terrestrial Animal Health Code.

### Control of animal feeding

The country of origin and countries, from which the importation of cattle is allowed, must implement strict procedures in order to minimize the possibility of BSE transmission. The country of origin has prohibited the feeding of meat/bone powder obtained from ruminants. This material has been banned for feeding for 19 years, thus complying with the requirements of the norm C SN EN 12442-2. Furthermore, pursuant to laws that have been in effect since 1991, it is also prohibited to use animal protein from mammals for feeding cattle in the Czech Republic. Moreover, it is prohibited to import cattle that were fed with animal protein.

### Historical documentation and traceability

The raw material is obtained exclusively from healthy cattle, younger than 3 years. Each individual animal must be tested for BSE, its breeding documented and its descent must be traceable. It is not allowed to obtain the raw material from high risk animals, such as perished or compulsorily destroyed animals or animals with suspected TSE (Annex no. 12 NV no. 336/2004). The occurrence rate of BSE is continuously monitored using the latest information from OIE, taking into account the latest information from the Czech State Veterinary Service. The tissues are obtained from contractual slaughter factories in the Czech Republic and are predominantly of domestic origin. In cases where slaughtered animals are imported it is always necessary that these be animals suitable for human nutrition, which includes all requirements imposed by state and European legislation regarding safety in terms of the risk of BSE similar to domestic breeding.

### Risk of contamination with viruses or infectious agents, prions, BSE

The current state of knowledge on transmissible spongiform encephalopathies, particularly the sporadic occurrence rate of the BSE in the Czech Republic, warrant more detailed consideration of this risk, and consequently (the health ministry) recommended strict measurements for better controlling of the BSE disease;

1. The manufacturer makes use of bovine bone, gained from cattle younger than 3 years, under veterinary control, appropriate for human nutrition and tested for BSE with negative result;
2. The slaughter factories located within the territory of the Czech Republic are subject to legal norms that comply with European Union standards and are included in the list of approved subjects of the Czech State Veterinary Service.
3. It is obligatory to conduct clinical and laboratory verification of suspected cases. All currently slaughtered animals older than 30 months undergo compulsory

examination employing Western blot and Enfer tests, the confirmation method used in cases of positive results is immunohistological testing.

### Principles of elimination organisms causing BSE

The raw material source of Hypro-Oss is the bovine tibia. The selected raw materials are stored in frozen state in plastic package by temperatures of at least minus 18°C. The risk of contamination by microorganisms is controlled by storage at minus 18°C, which is regularly checked and documented. In the course of production, the raw materials are treated with bactericidal solutions of sodium chloride, peracetic acid and saturated solution of calcium hydroxide in order to control the risk of contamination with bacteria, viruses and yeasts.

### Methods used for inactivation of viruses and infectious agents

During the last years, important progress has been achieved in the methodology of prion inactivation. These procedures depend on synergic effect of tensides and alkaline liquids, or on glycans in alkaline medium and probably on catalyzed photolysis. These procedures induce structural changes such as decrystallization of prion protein (sensitive to proteolysis), which results in irreversible, non-toxic conformation of the prion protein. This procedure is similar to the non-reversible structural transformation of collagen to gelatine. Collagen treated this way and used in the production of Hypro-Oss consists of prion-free tissues, which are fully safe as TSE is concerned.

### Deactivation or removal of infectious agents

The method of collagen treatment includes several repeated extractions that are employed to remove non-collagen globular proteins from the connective tissue, since prion (the infectious agent of BSE) belongs to the category of globular proteins. The extraction process is an additional safeguarding procedure that results in reduced prion content, in case there is any in the tissue. Another technological process that is appropriate in view of deactivation of prions is the action of a saturated calcium hydroxide solution with pH-value of 12.5. The prion protein (globular protein) contains an elongated conformation of the beta-pleated sheet type that changes to a physiologically normal conformation (non-infectious) through the action of hydrotropic substances (saturated calcium hydroxides with PH 12.5). The action of hydroxides is recommended in the WHO Document CPMPaCVMP EMEA/410/01, Rev. 2 as an effective measure to reduce the risk of TSE transfer through medical devices.

### Observance of procedures in respect of crucial elimination parameters

The manufacturer must be able to provide documentation (Production Protocol) for each batch of the produced medical device Hypro-Oss, demonstrating that all decisive parameters identified in the general principles for elimination have been monitored and controlled during production. The manufacturer, however, is bound to continue monitoring all new changes and new information, so that any possible previously undetected risk is discovered.

### Gamma Irradiation

After final packaging of Hypro-Oss's glass bottles in a double blister box, Hypro-Oss undergoes Gamma Irradiation as final sterilization step. Gamma radiation sterilization is a process that effectively kills or eliminates almost all microorganisms like fungi, bacteria, viruses and spore forms. Gamma irradiation is

a physical means of decontamination, because it kills bacteria by breaking down bacterial DNA, thus inhibiting bacterial division.