# *Updated* Report and Scientific Opinion on the safety of hydrolysed proteins produced from bovine hides. Initially adopted by the Scientific Steering Committee at its meeting of 22-23 October 1998 and updated at its meeting of 25-26 May 2000

## *Executive Summary*

The SSC was asked to prepare a scientific opinion on the following question: *"Can hydrolysed protein (peptides and amino acids), derived from bovine hides, be considered to be free of BSE infectivity, independent from the source of the raw material? If not, under which conditions of sourcing of the material (geographical and animal) and/or of type of material used (e.g. specified risk materials) and/or age of animal and/or production process can it be considered as safe?"*

## Opinion of the SSC:

The SSC is of the opinion that hydrolysed proteins can be considered to be safe as long as the raw material ("fleshing") entering the hydrolisation process does not, for example through contamination, carry a high infective load and an appropriate transformation process is applied. Therefore, in order to prevent the risk of propagation of BSE, no material from animals suspected or known to carry the BSE agent, should be processed and the raw material should only be obtained from healthy animals. The following conditions should be fulfilled for arriving at a safe product:

(a) If the material comes from a source that is classified as BSE free or at negligible risk, the production process should result in a safe products with respect to all infectious agents other than TSE, but no *additional* conditions related to BSE are necessary.

(b) If the raw material comes from a source with a lower BSE risk, the hides have to be carefully prepared (brining, liming and intensive washing) and a transformation process must be applied. This must include a heat treatment with a proven capacity to reduce BSE-infectivity. The heat/pressure/time conditions currently used by the industry and which have been brought to the attention of the SSC ( ³ 140°C/ ³ 3.6bar/ ³ 30minutes), and on which this opinion is based, are regarded to be sufficient. These conditions are considered to have a significant reduction potential: they are more severe than those which have shown a reduction potential of $10^3$ (drying excluded) in the case of rendering [1] . Moreover, the heat treatment is preceded by a careful preparation. An additional alkaline treatment (pH ³ 11, ³ 3h at T ³ 80°C) would enhance the safety.

(c) The product should not be fed to ruminants nor be used as fertiliser when the hides are sourced from high risk countries, unless the following production conditions are met. Measures must be in place to minimise contamination of the hides with CNS tissue and the hides have to undergo careful preparation (brining, liming and intensive washing). A transformation process has to be applied which includes a heat treatment and an alkaline treatment. These treatments must have a capacity to reduce BSE-infectivity. The heat/pressure/time conditions currently used by the industry and which have been brought to the attention of the SSC (heat treatment: ³ 140°C/ ³ 3.6bar/ ³ 30minutes and an alkaline treatment at: pH ³ 11, ³ 3h at T ³ 80°C) are regarded to be sufficient.

(d) Processes applying less severe conditions would require a separate evaluation and could probably not be regarded to be similarly safe.

## *Full Opinion of the SSC*

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*infectivity, independent from the source of the raw material? If not, under which conditions of sourcing of the material (geographical and animal) and/or of type of material used (e.g. specified risk materials) and/or age of animal and/or production process can it be considered as safe?"*

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(a) If the material comes from a source that is classified as BSE free or at negligible risk, the production process should result in a safe products with respect to all infectious agents other than TSE, but no *additional* conditions related to BSE are necessary.

(b) If the raw material comes from a source with a lower BSE risk, the hides have to be carefully prepared (brining, liming and intensive washing) and a transformation process must be applied. This must include a heat treatment with a proven capacity to reduce BSE-infectivity. The heat/pressure/time conditions currently used by the industry and which have been brought to the attention of the SSC ( ³ 140°C/ ³ 3.6bar/ ³ 30minutes), and on which this opinion is based, are regarded to be sufficient. These conditions are considered to have a significant reduction potential: they are more severe than those which have shown a reduction potential of $10^3$ (drying excluded) in the case of rendering [3] . Moreover, the heat treatment is preceded by a careful preparation. An additional alkaline treatment (pH ³ 11, ³ 3h at T ³ 80°C) would enhance the safety.

(c) The product should not be fed to ruminants nor be used as fertiliser when the hides are sourced from high risk countries, unless the following production conditions are met. Measures must be in place to minimise contamination of the hides with CNS tissue and the hides have to undergo careful preparation (brining, liming and intensive washing). A transformation process has to be applied which includes a heat treatment and an alkaline treatment. These treatments must have a capacity to reduce BSE-infectivity. The heat/pressure/time conditions currently used by the industry and which have been brought to the attention of the SSC (heat treatment: ³ 140°C/ ³ 3.6bar/ ³ 30minutes *and* an alkaline treatment at: pH ³ 11, ³ 3h at T ³ 80°C) are regarded to be sufficient.

However, if hides are coming from animals certified as BSE-free which are processed on dedicated lines, the conditions for lower risk countries apply.

(d) Processes applying less severe conditions would require a separate evaluation and could probably not be regarded to be similarly safe.

The SSC considers that, as a general principle which it applied also when considering the issues of the safety of other products such as gelatine, meat-and-bone meal, tallow and dicalcium phosphate, an experimental verification of the capacity of the overall process to reduce or eliminate BSE infectivity is needed. The SSC is aware that presently a validation study on the safety of hydrolysed proteins with respect to BSE infectivity is ongoing. The above opinion may be amended according to the results of this study.

**Summary table: the safety of hydrolysed proteins derived from hides from bovines and intended as animal feed or fertiliser**

| **Source**<br><br>**(classification as to SSC)** | **Minimum conditions** |
|---|---|
| BSE FREE or<br><br>NEGLIGIBLE RISK | • Raw material to be obtained from healthy animals.<br>• The production process should result in a safe products with respect |

| | |
|---|---|
| | to all infectious agents other than TSE, but no *additional* conditions related to BSE are necessary. |
| LOWER RISK | • Raw material to be obtained from healthy animals.<br>• An appropriate production process, including (as brought to the attention of the SSC) careful pre-treatment (including brining, liming and intensive washing) and at least one heat treatment at ³ 140°C for ³ 30 min ³ 3.6bar [4]<br>• *Regarding the molecular weight of the end product: see section "Other considerations"* |
| HIGH RISK<br><br>(If hides are coming from animals certified as BSE-free, e.g. from certified animals [5] or certified herds or closed herds [6] , the conditions for lower risk apply.) | No application as a ruminant feed nor as a fertiliser, unless:<br><br>• Raw material to be obtained from healthy animals.<br>• Measures in place to minimise contamination of hides.<br>• Appropriate production process, including (as brought forward to the SSC): a careful pre-treatment (including brining, liming and intensive washing), a heat treatment ( ³ 140°C/ ³ 3.6bar/ ³ 30minutes) [12] and an alkaline treatment (pH ³ 11, ³ 3h at T ³ 80°C) [12].<br>• *Regarding the molecular weight of the end product: see section "Other considerations"* |
| Status unknown | Consider as high risk until otherwise proven. |

: This Classification of geographical risk does not prejudge the opinion of the SSC on the TSE/BSE status of any country nor the OIE classification.

## Main elements of the scientific justification of the answer

The infective load of a hide of an infected bovine animal is estimated to be low because hides itself have not been found to be infective and the main source of infectivity could be contamination in the slaughterhouse. It can be assumed that this load is further reduced by the preparatory treatment in the tannery (brining, liming and intensive washing). Accordingly it is regarded to be unlikely that the fleshing entering into the hydrolisation process carries a high infective load.

The industrial production processes which have been brought to the attention of the SSC include at least two steps, for which it can be assumed that they have a capacity to reduce the infective titre of the input material. A heat treatment at T ³ 140°C for at least 30 min at a pressure of 3.6 bar is more severe than the heat treatment applied in rendering (133°C/20'/3 bars) and it can hence be assumed that at least the same infectivity reduction could be realised. This assumption is further supported by the fact that the material heated here is in a fluid phase and hence much better penetrable than the particles entering a rendering process. An alkaline treatment at pH ³ 11 where the material is kept for 3h at T ³ 80°C is potentially similarly effective as the alkaline treatment in the gelatine process which operates at much lower temperatures but for much longer time. Even if it is not known if the two processes are fully additive, the hydrolisation process seems to have the capacity to reduce an infective titre significantly.

## Other considerations:

Given the complexity of the production process the Scientific Steering Committee strongly recommends that manufacturers implement and respect HACCP [7] procedures. It is essential to identify and describe hazards and critical points for the production process. Two of these points are the traceability and the treatment at origin of the raw material (e.g. minimising the contamination with specified risk materials, in particular CNS-tissues), and the preparatory

treatment of the hides before fleshing (brining, liming, washing. No recycling of treatment waters). It can also be assumed that the heat treatments (T>=140°C, >=3.6bar, >=30min) and the alkaline treatments are critical stages of the transformation process and should be carefully respected and controlled. Controlling the molecular weight would provide a good verification of the appropriateness of an applied transformation process. A value of a maximum molecular weight of the hydrolysed proteins below 10.000 Daltons could be used as an indicator

*Having regard to considerations provided by the TSE/BSE ad-hoc group at its meeting of 11 May 2000 and which are detailed in the annexes 1, 2 and 3, it may be concluded that a molecular weight of <10.000D cannot be seen as an absolute guarantee for safety, per se. The criterion is indicative, and not exclusive for the quality of the hydrolysing process and of the safety regarding possible residual TSE infectivity of the final product. It seems theoretically possible that the infectious fraction (segment, part) of the BSE-agent could be smaller. However, a product with most of the molecules having a MW of < 10.000D has most likely been produced by means of production processes which, together with appropriate sourcing and respecting the other safety conditions given in the above cited SSC-opinion, guarantee a safe product. A limited range of molecular weights above the target value of less than 10.000D is therefore unlikely to affect the safety of the final product, provided, of course, all the other criteria of the opinion are complied with. Thus, a molecular weight below 10.000D may be used as an indicator but not as a safety guarantee per se.*

: The above opinion of the SSC is based on the report of the working group of the TSE/BSE ad hoc Group, which was accepted by the TSE/BSE ad-hoc group and then by the SSC, following critical discussion and review.

## Report from the working group

### *1. Terms of Reference*

The SSC was asked to deliver a scientific opinion on the following question:

*"Can hydrolysed protein (peptides and amino acids), derived from bovine hides, be considered to be free of BSE infectivity, independent of the source of the raw material?*

*If not, under which conditions of sourcing of the material (geographical and animal) and/or of type of material used (e.g. specified risk materials) and/or age of animal and/or production process can it be considered as safe?"*

### *2. Context*

#### 2.1 Legislative/policy/scientific aspects

Commission Decision 94/381/EC [8] concerning certain protection measures with regard to BSE and the feeding of mammalian derived protein, prohibits the feeding of proteins derived from mammalian tissues to ruminant species.

The above Decision was amended by Commission Decision 95/60/EC [9] to exempt some animal products and by-products from the ban given that they present no health risk.

Among these products, there are amino acids obtained from hides by a process which involves exposure of the material to a pH of 1 to 2 followed by a pH of >11 followed by a heat treatment at 140°C for 30 minutes at 3.6 bar.

In Decision 95/60, the above derogation is limited to amino acids and it is not extended to peptides as well. In fact, the advice of the Scientific Veterinary Committee [10] to the Commission on this issue recommended only amino acids to be excluded from the feed ban. This advice was used as scientific background for the adoption of Decision 95/60.

#### 2.2 References to previous opinions of the Committee or other Commission Scientific Committees/international bodies.

The SSC is not aware of any scientific opinion on the safety of hydrolysed proteins from bovine hides except the

opinion of the Scientific Veterinary Committee of 12.12.1994 on different proteins derived from bovines, including amino acids (but not peptides). However, given the similarities of the gelatine production process when based on hides, reference is made to the different opinions issued on this subject by various Scientific Committees of the European Commission, the OIE, the WHO and of the US-FDA [11].

**2.3 Definition of terms.**

1. For the purpose of this opinion, *hydrolysed proteins* (HPRO) are defined as mixtures of polypeptides, peptides and amino acids obtained from the hydrolysis of collagen contained in the fleshing derived from bovine hides. Their production process includes successive treatments: degreasing, acid treatment [12] , alkaline treatment (liming), concentration, sterilisation, and filtration. Hydrolysed proteins are used as feed for monogastric and ruminant animals and as fertilisers, mainly for horticulture. It is not used in pharmaceutical preparations or in foods.

*2. Collagen* is a family of fibrous proteins, with a high tensile strength which are found in connective tissues such as the organic matrices of hides, bones, tendons, cartilage, cornea of the eye, blood vessels and teeth. The structural unit of collagen is tropocollagen. This protein is formed of three helical units wrapped around one another with a right twist. Each of these helices contains about 1,000 amino acids. The amino acid sequence of collagen is highly distinctive with every third residue as glycine (35%). Other important amino acids are alanine (11%), and proline (12%). The unusual hydroxyproline also occurs (9%) and there are a few % of hydroxylysine.

*3. Healthy animals* are defined as animals which have undergone an ante mortem inspection by an official veterinarian where it was determined that the animals were not suffering from a disease which is communicable to man and animals and that they do not show symptoms or are in a general condition such as to indicate that such disease may occur and they show no symptoms of disease or of a disorder of their general conditions which is likely to make their meat unfit for human consumption. (Definition as given in Directive 64/433/EEC, laying down the rules for ante mortem inspection)

***3. Assessment***

**3.1 Strategy adopted for the evaluation and risk assessment**

As for Gelatine the safety of hydrolysed proteins depends

(a) on the risk, that the raw material entering the production process carries the BSE agent, and

(b) on the ability of the production process to reduce or eliminate any residual infectivity, and

(c) on the final use of the product (as feed or fertiliser).

The assessment will discuss these three risk-components separately before a final conclusion is drawn.

**3.2 Assessment of the risk, that the raw material entering the production process for Hydrolysed Proteins (HPORs) carries the BSE agent.**

The typical hydrolysed proteins manufacturing process uses "fleshing" (which may contain residues from hides) as raw material (ASSALZOO, 28 May 1998).

The "fleshing" is made up of collagen, elastic fibres, fat and muscular traces. As a by-product from tanning, it is derived from hides which have been brined for 2 or more days, treated with sodium sulphide and lime at pH $^3$ 11 for at least 24 hours (liming process). The hides are then washed with regular shaking. After washing, the "subcutaneous layer" is mechanically separated to obtain the "fleshing". Small parts of the hides may remain in the fleshing.

***The hazard***

The hazard is here defined as the event that the raw material for the hydrolysis process carries the BSE infective agent. It depends on the event that the hides, the basic raw material, are carrying infectivity and the efficiency of the tanning process, precursor to the hydrolysis, to reduce that infectivity.

**Infectivity of hides**

The infectivity of hides with regard to BSE has been assessed by the SSC in its opinion on SRM of 9/12/97. In line with other scientific committees and international bodies (WHO [13] ) the SSC confirmed that no infectivity was detected in connective tissue and hides. This material has therefore not been classified as specified risk material.

However, the SSC underlined in the same opinion its view that contamination of non-infective tissues with highly infective tissues (e.g. brain, spinal cord) could pose a risk, particular if hide from the head is used. Also in its opinion on the safety of Gelatine (March, 1998) the SSC concluded that hides are safe, as long as contamination can be avoided.

The Scientific Steering Committee has also stated [14] that in cattle, sheep and goats TSE infectivity is not limited to nervous (brain) proteins but is also present in the lympho-reticular system of sheep. So far this has not been found for BSE infected bovines, even after spleen and lymph nodes were injected intercerebrally into cattle. The same holds true for infectivity in peripheral nerves, which has been shown for SCRAPIE in sheep but never for BSE in cattle.

**Contamination**

Contamination of hides with CNS (Central Nervous System) may result (a) from brain tissue spilt over the outside of the hides when stunning or pithing the animal, and (b) from spinal cord tissue spilt over the *outside of the* hide when removing the head. No data are available on the amount of CNS material that can be attached to a hide by this way.

**Reduction of the infectivity of hides by the tanning process:**

Manzke et al., 1996, have shown that during the degreasing step in the gelatine process (largely washing of crushed bones with hot water), 98-99% of the protein of nervous origin (e.g. S100, GFAP and others) are removed. The detection method used (ELISA test) was very sensitive with a detection threshold from 30 pg for S100 and 7 pg for GFAP.

Hides are not only washed but first brined for 2 or more days, then depilated and subsequently exposed to an alkaline treatment. Only then the hides are washed with hot water in order to clean them from the brining residues. This series of processes is likely to reduce any contamination beyond the level that can be reached by washing alone.

However, the SSC notes [15] that the above conclusion may be valid for the reduction in protein levels, but not necessarily for the reduction of BSE infectivity to the same extend. Prions, or any other yet unidentified BSE-agent, are not necessarily removed in the same way as nervous proteins.

**Infective load of fleshing resulting from a hide of a BSE-infected cattle**

If hides from BSE-infective cattle are processed in a tannery, a part of the initial infectivity will be eliminated/washed away, but another part may survive that treatment. The resulting infective load of a single hide, which has been contaminated by infected CNS tissue, is depending on the total amount of CNS spilt on the hides and the capacity of the tanning process to reduce this load. Possible mechanisms for such a reduction are either of a physical or a chemical nature. The physical impact is quite severe (depilation and washing) and could be supported by the chemical impact of the liming. As a result is it likely that the infective load of an originally contaminated hide is significantly lower at the end of the tanning process than before.

***The SSC is of the opinion that it is unlikely that the fleshing obtained from bovine hides contains high loads of infectivity*** .

***BSE infectivity of the raw material which enters the hydrolysis process.***

The production of hydrolysed proteins starts with the so-called fleshing, the subcutaneous layer of bovine hides. This fleshing is processed in batches of 6 to 280 tons, equivalent to at least 350 hides per batch. As the fleshing is more or less liquid (amorphous), it can be assumed that any infectivity entering the batch is evenly distributed in the batch, i.e. a good dilution can be expected.

The probability that a batch contains BSE infective is proportional to the risk that infected animals are slaughtered and their hides are contaminated with CNS from the infective animals. This risk is known as the 'geographical risk' or the 'sourcing risk' because it depends on the origin of the animal.

The infective titre of the fleshing entering the hydrolysis process is depending on the geographical risk: If a higher proportion of hides is contaminated with BSE, the input titre increases . The theoretically possible infective load of the raw material entering the hydrolysis process is therefore proportional to the geographical risk of the raw material source.

Given the fact that the infective load of the fleshing from an infected animal is not likely to be high, the maximum infective load of the batch of the fleshing entering the hydrolysis process is equivalent to the level of one contaminated hide. It would only be reached if all hides entering a batch would come from infective cattle or would be contaminated with CNS from infective cattle, a rather unlikely assumption.

**Given the fact that hides are regarded to be free of BSE infectivity, even if the animal is infected; given the fact that the maximum infective load of a contaminated hide is not likely to be high; given the fact that it is unlikely that a high proportion of the hides used for a batch of fleshing which enters the hydrolysis process, could be contaminated;**

**the SSC is assuming that the infective load of the batch of fleshing entering the hydrolysis process is unlikely to be significant in countries which do not have a high incidence of BSE.**

*on geographical risk assessment:*

*The SSC has issued an opinion on the information needed to assess the epidemiological status of a country or region with regard to TSE (23/01/98 and 20/2/98). In its opinions on the safety of Gelatine, Tallow and MBM (March, 1998), it has used three preliminary risk categories for categorising the geographical origin of animals: BSE free or of negligible risk; lower BSE-risk; and higher BSE-risk. It is currently preparing a methodology for assessing the geographical risk on the basis of the information requested.*

*The OIE, at its 66th Annual General Session (29 May 1998), has discussed a new version of the OIE International Animal Health Code on Bovine Spongiform Encephalopathy (BSE). It identifies four categories or zones with regard to BSE:*

*1. BSE- free country or zone (conditions defined).*

*2. Country (or zone) that has not demonstrated a BSE free status and has not declared any indigenous cases of the disease (definition under study)*

*3. Country or zone with a low incidence of BSE (definition under study)*

*4. Country or zone with a high incidence of BSE (definition under study).*

*For consistency reason this opinion will use the same classification as the previous opinions on gelatine, tallow and MBM. It is, however, evident that this classification may have to be revised, once a final classification scheme is defined. This may make a revision of the opinions on gelatine, tallow, MBM and other bovine-derived products necessary.*

**3.3 Assessment of the ability of the production process to reduce or eliminate any residual infectivity.**

The second element, which is essential for the safety of the final product, is the ability of the hydrolysis process to reduce or eliminate the BSE-agent.

**3.31 Description of the typical manufacturing process**

Two main schemes have been found to be applied by industry but deviations are possible. They are distinguished by a different sequence. Scheme (I) includes two separate filtration and two heating steps while scheme (II) has only one filtration and one heat treatment but adds a deodorification to the process.

***Scheme I.***

**I-1. Homogenisation**: The raw material is heated, ground and homogenised.

**I-2. Acid hydrolysis** [16] : By heating to 80-100°C and mixing with sulphuric acid ($H_2SO_4$). This phase lasts 6 hours. The pH is between 0 and 2.

**I-3. Degreasing in acid phase**: At the end of the acid hydrolysis phase, the fat is separated and processed in a stripping plant, finally stored and utilised for industrial applications.

**I-4. Alkaline treatment**: The proteinic degreased phase is mixed with lime ($Ca(OH)_2$) in order to have a pH above 11. It is heated for 2 h to 80-90°C and for 1h to 90-100°C.

**I-5. Filtration I**: The suspension of the precipitated calcium sulphate and the solution of polypeptide mixtures, peptides and amino acids are fed to a filter where the calcium sulphate is completely separated from the solution.

**I-6. Heat treatment (first sterilisation step)**: The alkaline solution of polypeptide mixtures, peptides and amino acids (pH $^3$ 11) is treated in a specific thermal plant. This operation lasts about 6 hours: 2.5 hours for heating to 140°C, 30 minutes at 140° (3.6 bars), and 2.5 hours for the cooling off.

**I-7. Filtration II:** By adding ammonium bicarbonate Ca is separated from the solution.

**I-8. Sterilisation:** Heating to 132°C for 22 seconds by direct steam injection.

**I-9. Concentration**: The solution is concentrated to reach a final product with up to 60% of dry matter.

**I- 10. Drying:** If required a drying may be added at an air temperature of 220°C.

***Scheme II***

**II-1 Homogenisation with alkaline treatment:** The raw material is ground, homogenised, heated (T 80 °C for 2 to 3 h) and treated with alkaline (pH >12).

**II-2 Degreasing:** The fat is separated from the mass at the end of the homogenisation phase at pH 11.5 -12.0. It is subsequently processed in a stripping plant, finally stored and used for industrial applications.

**II-3 Filtration:** different techniques applied.

**II-4 Acid hydrolysis [9]:** The degreased and filtered raw material is mixed with sulphuric acid ($H_2SO_4$) and heated to 70°C for 30 minutes at pH 1-2.

**II-5 Calcium separation**: The solution of hydrolysed proteins (mixture of polypeptides, peptides and amino acids) is treated with ammonium bicarbonate in order to remove the calcium linked to the mixtures of polypeptides, peptides and amino acids as calcium bicarbonate, which precipitates. The calcium carbonate is separated from the ammonium

solution of the polypeptide mixtures, peptides and amino acids.

**II-6 Deodorification** by oxygen gurgling.

**II-7 Concentration:** The solution is filtered to reach a concentrated product with 58-60% of dry matter (concentration at T° 53°C - 92°C).

**II-8 Heat treatment (sterilisation):** At pH ³ 11 the alkaline solution is heated over 7h to reach 140°C, kept at 140°C for 35 minutes (3.6bar), and cooled down over 7h.

**II-9 Drying**: The product can be dried by spray drying at air temperatures of 220°C.

**3.32 Discussion of the potential of the different production steps to contribute to a reduction of any residual BSE infectivity.**

The SSC is not aware of any completed study specifically addressing the ability of the manufacturing process of hydrolysed proteins to reduce or eliminate BSE infectivity. The only ongoing study which was brought to the attention of the SSC has not yet produced any results. For the time being, and in view of the apparent similarities, the following discussion is therefore based on work carried out with regard to gelatine production. It also extrapolates from other scientific knowledge. This implies that a truly quantitative risk assessment can not be carried out. As long as the appropriate quantitative information is not available, the risk assessment remains largely qualitative.

- The homogenisation, degreasing, filtration and calcium-separation steps are unlikely to reduce significantly a residual BSE infectivity because they do not impact on the protein fraction.

- For the alkaline treatment it is assumed that it has a significant capacity to reduce the BSE-infectivity. This assumption is based on the only available research results on this issue, which are relating to the gelatine production process. Given the higher temperature, at which this treatment is carried out here, it can be assumed that at least a similar reduction of any eventual BSE infectivity can be realised as for gelatine. The fact that for the production of gelatine the duration of the treatment is much longer is not regarded to be relevant in this context because the (limited) experimental results do not support the hypothesis that the BSE-titre decreases with prolonged treatment . It is therefore possible to assume that this step will provide a significant reduction (See also the SSC opinion on the Safety of Gelatine, adopted on 26-27 March 1998).

- There are no data upon which an assessment of the inactivation effect of the acid hydrolysis stage can be made. Therefore the SSC has not currently assumed any reduction potential for this part of the process.

- The heat treatment is considered to have a significant reduction potential. The applied conditions (140°C, 3.6bar, 30min) are more severe than those which have shown a reduction potential of $10^3$ (drying excluded) in the case of rendering [17].

The transformation process is not only conducted at 140°C, 3.6Bar, 30 minutes, but also under extremely alkaline conditions. According to the final report of the *Validation Study of the Clearance of Scrapie from the Manufacturing Process of Gelatine* (Inveresk, 1998a, 1998b), the reduction factors indicate that the liming treatments give a partial reduction of potential infectivity. However, the level of reduction achieved (reduction factor 2.33 $\log_{10}$) is not increased as the length of the incubation is extended. Moreover, combinations of autoclaving and hydroxide have shown to be extremely effective (even at 121°C) with rodent-passaged strains of CJD and scrapie agent where the infectivity titres in the brain-tissue were up to $10^{10}$ $ID_{50}$/g (Prusiner et al, 1984; Taguchi et al, 1991; Ernst & Race, 1993; Taylor et al, 1997). In addition, unpublished data are showing inactivation after boiling for a brief spell in alkali (D.Taylor, 1998, personal communication).

As long as no other data are available the SSC therefore assumes a reduction potential of at least a similar order of magnitude for the heat treatment. It is indeed not unlikely that the reduction capacity would be even higher. Reasons are the more severe conditions and the fact that the material is fluid and hence a better heating kinetic can be assumed.

- The concentration and sterilisation process, albeit also operating at high temperatures, are not regarded to add to the infectivity reduction beyond the level reached with the heat treatment.

**Based on this discussion the SSC concludes that the hydrolisation process, carried out as described above, has a significant potential to reduce any possible BSE infectivity in the initial fleshing. The severe heat treatment and the alkaline treatment are regarded to be the most relevant elements.**

**In view of the low titre of the input material, which normally can be expected, the SSC regards it highly likely that the final product, the hydrolysed proteins, are BSE free. However, no experiments have been made so far for the hydrolysis process as such [18] and all estimations have to be regarded as preliminary until appropriate studies have been carried out. It is also not certain that the infectivity reduction achieved at the different steps is fully additive. Further it is also not known if the resistance of any surviving infectivity would be enhanced, the so-called tailing effect has not yet been excluded to apply to the BSE-agent.**

**An experimental verification of the capacity of the overall process to reduce or eliminate BSE infectivity is needed.**

**3.4 The role of the final use of the product as regard to the transfer of BSE to animal or man.**

**3.41 Use in animal feed**

Current use: Hydrolysed proteins are fed to animals, including bovines. For example: for dairy cows, the daily intake rate may reach about 100g dry mass of hydrolysed proteins.

Importance of such an use:

- Allowable daily intake, oral: The oral minimum infective dose of BSE-contaminated material is not known, even for bovines. On the basis of currently available information the bovine threshold for a single oral dose of BSE infective material can be assumed to be below 1g of infected brain. Current estimates assume 0.1g to be sufficient to trigger infectivity in bovines (UK, MAFF-CVL, personal communication). There is evidence that the incubation time increases with smaller doses and hence this lower threshold level could not yet be verified by experiments. For other species no estimation of the threshold dose is available. However, it can be assumed that small ruminants may be equally sensitive.

- No information is available as to the impact of repeated small doses on cattle. The only experiment (Diringer *et al*, 1998) was carried out with scrapie in Hamster. It points, however, to a certain risk that small doses, given at short intervals, could accumulate to some extend.

- As long as the threshold value is not known it must be assumed that also a small dose would finally lead to a BSE case if a sufficiently large number of animals would be exposed to it.

**3.42 Use as fertiliser**

Hydrolysed proteins are used as fertiliser, mainly in horticulture, for example included in culture media for propagation of seedlings, cuttings, etc.

**The working group that its use as fertiliser can be regarded as safe if the hydrolysed protein is regarded to be safe. The conditions for reaching this degree of safety are defined in this report. Whenever this degree of safety is not guaranteed, hydrolysed proteins should not be used as fertiliser because a residual BSE-infectivity could not be fully excluded. Besides the risk of accidental exposure of man or ruminants to the agent, the unclear fate of the agent in the environment requires this precautionary approach.**

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***Annex 1*** : **Extract from the SSC minutes of 25-26 May 2000, on the basis of the TSE/BSE *ad hoc* Group's discussions at its meeting of 11 May 2000**

1. At its meeting of 22-23 October 1998, the Scientific Steering Committee Adopted a Report and Scientific Opinion on the safety of hydrolysed proteins produced from bovine hides. This opinion recommends a number of conditions with respect to sourcing and transformation process of the raw material. Regarding the transformation process, the opinion considers that " *controlling the molecular weight would provide a good verification of the appropriateness of an applied transformation process. A value of a maximum molecular weight of the hydrolysed proteins below 10.000 Daltons could be used as an indicator*."

2. At its meeting of 22-23 April 1999, the SSC decided that its Opinion of 22-23 October 1998 on the Safety of Hydrolysed Proteins would need to be updated in view of the following considerations:

"On the basis of the reasoning given in annex 2, the TSE/BSE *ad hoc* Group concluded at its meeting of 15 April 1999, that declaring hydrolysed proteins safe if the resulting peptides are less than 10.000 Daltons, could lead to a false sense of security. The ad hoc Group further concluded that the sourcing and (severe) processing conditions recommended in its opinion of 22-23 October 1998, together with the fact that bovine hides as such are not a specified risk material, are sufficient conditions for the final product to be safe. It further reiterated that any approval of other production processes should be done on a case by case basis and on the basis of the results of a report with research results with respect to the inactivation of TSE/BSE infectivity by the process."

3. The question can further be raised whether the figure of 10.000 Daltons is to be considered as an absolute threshold, i.e., that the product is unsafe if any molecules with a MW above 10.000D are found, or as a qualitative indicator of an average MW reflecting that after processing, the size of the molecules has a statistical spread and that a certain fraction of the molecules may have a MW above 10.000D. This would imply that the product after the hydrolysation process described in the opinion, is safe also if a small fraction [say 1-5%, to be defined on the basis of laboratory analyses] of hydrolysed proteins has a MW above 10.000.

The second option can be accepted only if it would not jeopardise the safety of the final product. Taking into account the following elements:

- the sourcing and (severe) processing conditions recommended in its opinion of 22-23 October 1998, together with the fact that bovine hides as such are not a specified risk material, are sufficient conditions for the final product to be safe.

- As can be derived from Annex 2, the minimum size of the fraction of a $PrP^{Sc}$ prion that could be infectious is not known. It may also be below 10.000.

- No standardised and generally acknowledged methods for the determination of the MW of hydrolysed peptides exist,

having regard to considerations provided by the TSE/BSE ad-hoc group at its meeting of 11 May 2000 and which are detailed in the annexes 1, 2 and 3, it may be concluded that a molecular weight of <10.000D cannot be seen as an absolute guarantee for safety, per se. The criterion is indicative, and not exclusive for the quality of the hydrolysing process and of the safety regarding possible residual TSE infectivity of the final product. It seems theoretically possible that the infectious fraction (segment, part) of the BSE-agent could be smaller. However, a product with most of the molecules having a MW of < 10.000D has most likely been produced by means of production processes which, together with appropriate sourcing and respecting the other safety conditions given in the above cited SSC-opinion, guarantee a safe product. A limited range of molecular weights above the target value of less than 10.000D is therefore unlikely to affect the safety of the final product, provided, of course, all the other criteria of the opinion are complied with. Thus, a molecular weight below 10.000D may be used as an indicator but not as a safety guarantee per se.

***Annex 2*****: Short report to the SSCon the basis of a Working Group report drafted by Dr.D.M.Taylor.**

***Subject*: Hydrolysed proteins: are they more safe if their molecular weight is less than 10kD?**

It has been argued that these products might be considered to be safe if the hydrolysed proteins have a MW of <10 kD. The basis for such a suggestion would be the knowledge that $PrP^{sc}$ has a MW of 27-30kD.

The TSE/BSE ad hoc Group is of the opinion that the use of such a criterion could lead to a false sense of security because it is not known whether protein sub-components or peptides of low MW derived from $PrP^{Sc}$ can trigger the conversion of $PrP^{C}$ to $PrP^{Sc}$. However, one needs to bear in mind that the 27-30 kD $PrP^{Sc}$ is itself a sub-component. It is the resistant 'core' that remains after proteolytic digestion of the full-length $PrP^{Sc}$ protein that has a MW of 33-35kDa. Therefore, there is existing formal proof that a somewhat truncated form of $PrP^{Sc}$ can convert $PrP^{C}$ into $PrP^{Sc}$. It is known that more severely truncated forms of $PrP^{C}$ can be converted to $PrP^{Sc}$. For example, a $PrP^{C}$ peptide consisting of only 21 residues had properties akin to $PrP^{Sc}$. [6] It was neurotoxic, and had a tendency to form amyloid fibrils analogous to the scrapie-associated fibrils (SAF) found in brain extracts from TSE-infected individuals. It must be assumed that this truncated form of $PrP^{Sc}$ might convert non-truncated $PrP^{C}$ to $PrP^{Sc}$. Also, a form of $PrP^{C}$ containing only 106 residues (MW approximately 10 kDa) was converted to $PrP^{Sc}$ *in vitro*. [7] In addition, when this truncated PrP was expressed in transgenic mice that were deficient for wild-type PrP, these mice developed scrapie when challenged with the RML strain of mouse-passaged scrapie agent, demonstrating the convertibility of this 106 residue $PrP^{C}$ to $PrP^{Sc}$. [8] More importantly, when brain-tissue from the scrapie-infected PrP106 mice was passaged into mice of the same genotype, they developed scrapie. [8] These data confirm that $PrP^{C}$ peptides with a MW of approximately 10 kDa can not only be converted to $PrP^{Sc}$, but that they can also convert $PrP^{C}$ to $PrP^{Sc}$. The above data tend to confirm the opinion that declaring hydrolysed proteins safe if the resulting peptides are <10 kDa could lead to a false sense of security.

The TSE/BSE *ad hoc* Group considers that only low levels of BSE infectivity could be present in the raw materials. The two typical manufacturing processes described in the SSC opinion of 22-23 October 1998 are likely to ***reduce very significantly*** even considerably higher levels of BSE infectivity than could ever be present under worst-case conditions. This is because of the combinations of heat (especially steam under pressure) and alkali that are used. A variety of data are available that show the effectiveness of heat combined with alkali on BSE and scrapie agents. [1-5]

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***Annex 3*:**

**s on the size of non-conventional transmissible agents causative of TSE**

**Prof.Dr.D.Dormont, submitted to the TSE/BSE ad hoc Group at its meeting of 9 September 1999**

**Preliminary comments:**

1) The linear relationship between the quantity of PrP partly resistant to proteinase K and infective titre has been verified in experimental models using a stabilised strain of NCTA. It is more difficult to demonstrate this relationship in the case of primary transmission.

2) To date trials involving injecting animals with the fully purified pathological prion protein (PrP-res) (extraction of the electrophoresis gel followed by renaturation) have never led to transmission of the disease; the same goes for recombinant PrP protein and for peptides that have neurotoxic properties *in vitro* (PrP 106-126).

3) Bearing in mind the uncertainties that still persist as to the nature of NCTAs, it is impossible to measure the exact size of the agent. Only an approximation of the size of the infective unit is possible (result of inoculation of the test animal).

4) The size of the PrP-c molecule measured in RMN is 38 Angströms for the corpuscular part and 300 Angströms for the flexible N-terminal part; no data of this kind are available for pathological PrP (10, 11).

**Methods used:**

There are two methods:

1) estimation of the size on the basis of gamma ray inactivation data;

2) estimation of the size on the basis of data obtained from zonal centrifugation, ultrafiltration and exclusion chromatography.

**Main results:**

- The radiobiology data indicate that the NCTAs are very small, between 64000 and 150000Da (1, 3, 4); however, these data do not make allowances for aggregation, a property which has been demonstrated for NCTAs by many teams. Besides, most of these data were obtained by irradiating brain homogenates, where the profusion of complex lipids may bias the results and lead to an overestimate of the radioresistance of the infective agent.

- The zonal centrification data indicate that the agent's sedimentation coefficient lies between 40S and 500S (7-9) (compare with parvoviruses, whose sedimentation coefficient is 100/110S (12)).

- The ultrafiltration trials with variable porosity filters followed by titration of the filtrate point to a size of 15 to 40nm. By way of example, an ultrafiltration with an exclusion threshold of 100 kD reduces the infective titre of a scrapies strain by 2.2 logs, whereas a membrane with a threshold of 30 kD reduces it by 4.9 logs and a membrane with a threshold of 10 kD reduces it by more than 6.5 logs (no infectivity detectable) (5) (6) (2).

The exclusion chromatography data show that the size of the agent lies between 30 and 50nm.

However, these measurements should be interpreted bearing in mind that the NCTAs can very readily aggregate; hence

these methods may overestimate the size.

- Certain nanofiltration trials have been carried out; a 25 nm filtration can reduce infectivity by approximately 2 logs; nanofiltration at 35 nm is relatively ineffective. By contrast, nanofiltration at 15 nm seems to be capable of eliminating all infectivity, even when the agent has first been treated with detergents to compensate for aggregation as much as possible (results obtained by a Japanese team).

**To sum up:** two factors must be considered in the scientific discussion: 1) the agent is small, of the order of 15 to 40 nm; 2) pursuant to the prion hypothesis, according to S.B.Prusiner, the infective unit contains $10^5$ molecules of pathological proteinase K-resistant PrP.

**Références bibliographiques :**

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[1] See also the opinion of the SSC on the safety of MMBM for non-ruminant food-producing animals, September 1998

[2] Healthy animals are defined as animals which have undergone an ante mortem inspection by an official veterinarian where it was determined that the animals were not suffering from a disease which is communicable to man and animals and that they do not show symptoms or are in a general condition such as to indicate that such disease may occur and they show no symptoms of disease or of a disorder of their general conditions which is likely to make their meat unfit for human consumption.

[3] See also the opinion of the SSC on the safety of MMBM for non-ruminant food-producing animals, 26-27 March 1998

[4] See the report of the Working Group attached to this opinion.

[5] Opinion of the SSC on the date based export scheme (9/12/97 and 20/2/98) and of the Scientific Veterinary Committee on the revised UK certified herds scheme (17/9/97)

[6] An opinion of the SSC on the criteria for closed herds guaranteeing that animals from these herds are BSE-free is forthcoming.

[7] Hazard Analysis and Critical Control Points

[8] OJ L 172, 7.7.94, p.23

[9] OJ L 55, 11.3.95, p.43

[10] Report from the Scientific Veterinary Committee on the risk from BSE of some products derived from ruminants. Adopted on 12 December 1994

[11] See reference list.

[12] Not in all cases

[13] Consultation on Medicinal and other Products in relation to Human and Animal Transmissible Spongiform Encephalopathies, Switzerland, 24-26 March, 1997

[14] SSC "Safety of bi-calcium phosphate". Preliminary opinion adopted on 15/5/98

[15] SSC opinion on safety of Gelatine, March 98

[16] Not in all cases applied.

[17] See also the opinion of the SSC on the safety of MMBM for non-ruminant food-producing animals, September 1998

[18] A study of the reduction potential is currently underway but no results are yet available.