THE SAFETY OF DICALCIUM PHOSPHATE PRECIPITATED FROM RUMINANT BONES AND USED AS AN ANIMAL FEED ADDITIVE

Report and opinion adopted at the meeting of the Scientific Steering Committee of 25-26 June 1998,

Following a public consultation on the preliminary opinion adopted on 14-15 May 1998

Remark: the present document contains opinions adopted by the Scientific Steering Committee of the European Commission, which is a neutral and independent scientific body.

I. EXECUTIVE SUMMARY

Note: Readers should keep in mind that the report and opinion only address the scientific aspects of the risk *assessment* of the issue (e.g., identification of hazards, levels of infectivity in the starting materials and final products, etc.). The risk *management and policing* aspects related to the implementation of an opinion, are not dealt with.

Products derived from raw materials from ruminant animals potentially infected with the BSE agent may contain residual infectivity, for example because the agent was not completely eliminated or inactivated during the successive production steps, including the sourcing of the material and the production itself. Therefore the Scientific Steering Committee (SSC) was requested to assess the safety regarding BSE risk, of products such as tallow, gelatine, meat and bone meal, peptides, amino acids, certain organic fertilisers, dicalcium phosphate precipitated from ruminant bones, etc.

The present report and opinion address the safety in relation to BSE risk, of dicalcium phosphate precipitated from ruminant bones and used as an animal feed additive.

Regarding dicalcium phosphate, the SSC addressed the following question:

"Can dicalcium phosphate derived from ruminant bones, be considered to be free of BSE infectivity?

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 the animal and/or production process can it be considered as safe?"

For the purpose of the present opinion, dicalcium phosphate is defined as a chemical ($CaHPO_4.2H_2O$) obtained from degreased bones which are demineralised by hydrochloric acid treatment and precipitated by a saturated lime solution.

Without prejudice to the full text of the report and opinion, which are given in chapters II, III and IV and which are the reference, some of the essential parts of the opinion can be summarised as follows:

- *Because of existing evidence of the possible presence of remaining impurities of a proteinaceous nature (approx. 0.5%, obtained as Nitrogen content multiplied by 6.25), as long as not more details on the composition and molecular weights of the residual protein fraction are available, and given the fact that the number of critical points in the whole production chain is quite large and that their monitoring may not always be easy and evident, the Scientific Steering Committee is of the opinion that the raw material should be obtained from appropriate sources and tissues. In any case, the raw materials should be submitted to an appropriate production process, as indicated in the report.*
- *For countries considered to be 'BSE free or classified as at negligible risk', raw material (bovine bones) can be used free without removal of specified risk materials when coming from animals certified as fit for human consumption. For lower risk countries, specified risk materials should first be removed to minimise the risks of possible contamination of the bovine bones. The origin of the bovine raw materials should be certified to be exclusively from animals that are fit for human consumption. For high risk countries the SSC recommends*

that no sourcing of ruminant raw materials from high risk countries is allowed. Countries with an unknown BSE status should be evaluated individually on the basis of a detailed evaluation using appropriate criteria. If no judgement on the basis of available evidence or because of a lack of information is possible, they should be considered as high risk countries. (However, the latter statement does not prejudge the opinion of the SSC on the TSE/BSE status of any country. Work on geographical risk assessment is ongoing.)

- *What precedes covers the approach to be followed if the risk of infectivity in the remaining impurities is to be reduced to the lowest possible level. In order to provide the Commission also with an alternative choice, the Scientific Steering Committee will eventually complete this approach with a quantitative risk analysis, taking into account the geographical origin of the raw material, the type of raw material, including the age of the animals, the removal or not of specified risk materials, the incidence, propagation and exposure risk components of the BSE borne risk and the reduction of infectivity during the production process itself. This requires results of experiments on and justified estimates of reduction factors during the various steps of the production process, from sourcing to marketing. However, these data are not always available, as some experiments are still ongoing or only in a planning phase.*
- *The SSC finally would further welcome that research laboratories and the industry make more results available of such infectivity reduction research as well as on the concentration and composition and molecular weights of the residual protein fraction in dicalcium phosphate.*

II. REPORT ON THE SAFETY OF DI-CALCIUM PHOSPHATE PRECIPITATED FROM RUMINANT BONES AND USED AS AN ANIMAL FEED ADDITIVE

1. DEFINITION

Dicalciumphosphate ($CaHPO_4.2H_2O$) is a chemical produced through precipitation from bones or inorganic material.

For the purpose of the present opinion dicalcium phosphate is defined as a chemical ($CaHPO_4.2H_2O$) obtained from degreased bones which are demineralised by hydrochloric acid treatment and precipitated by a saturated lime solution.

2. BACKGROUND

2.1. Introduction

The share of dicalcium phosphate precipitated from bones (GME, 1998) and used as animal feed, is estimated in the order of 10-15% of the total amount of 720.000-750.000 tons in Western Europe. (GME, 1998a)

The mandate of the Scientific Steering Committee is to advise the Commission on the risk exposure of animals to BSE from dicalcium phosphate as co-product of gelatine produced from ruminant bones, and used exclusively as an animal feed additive[1].

The typical dicalcium phosphate manufacturing process includes first a degreasing step of fine crushed bones in hot water (80° to 85°C). Regularly shaking removes a high percentage of proteins. The dried bone chips then undergo a demineralisation process: they are submitted over a total period of 4-5 days, to a sequence of solutions with an increasing hydrochloric acid concentration. The highest concentration being 4% of HCl during 2 days. This demineralisation of the fine bone chips produces a phosphoric liquor and osseine chips. In the typical dicalcium production process, the liquor, after treatment with lime, will give a precipitate of dicalcium phosphate. This precipitate is essored and air dried with inlet temperatures of 270°-325 °C during 15 minutes and outlet temperatures of 60-65°C.

(The osseine obtained is further used for the production of gelatine. Regarding the safety of gelatine, a separate report and opinion were adopted by the Scientific Steering Committee during its meeting of 26-27 March 1998).

The successive production steps which can reduce the potential infectivity of the raw material are thus, apart from the degreasing and demineralisation steps, different for gelatine and dicalcium phosphate.

On the safety of dicalcium phosphate precipitated from bones in particular, or on by-products in general, several opinions have been issued by international organisations or national institutions.

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[1] According to COLIPA (1997), dicalcium phosphate in cosmetic products comes from mineral sources.

According to the OIE (1997), by-products, such as gelatine and collagen, are considered to be safe if produced by processes (under study) which inactivate any residual BSE infectivity. According to OIE (1998), Veterinary Administrations can authorise without restriction and regardless of the BSE status of the exporting country, the import or transit through their country of dicalcium phosphate with no trace of protein or fat.

As stated in the opinion of 9 April 1996 of the Scientific Veterinary Committee, 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.

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

(3) The kind of application (e.g. food, cosmetics and medicinal products).

The Scientific Veterinary Committee stressed that the full data on all gelatine manufacturing processes have not been published, hence a full risk analysis cannot be carried out for gelatine. But by-products, such as gelatine aminoacids and dicalciumphosphate were recognised as giving the best possible guarantees of safety if produced in a process which ensures that all material is subjected to degreasing, followed by (for gelatine): acid and/or alkaline treatment followed by heating to 120° and these up to 138-140°C for 4 seconds. The product should be labelled to show the process to which it has been subjected. The Scientific Veterinary Committee emphasised also that: *"the specified bovine offals from UK cattle (brain, spinal cord, thymus, spleen, intestine and tonsils) as well as vertebral column and any tissues resulting from trimming carried out in accordance with EC and UK legislation on BSE, should not be used for any purpose (food, feed, medical, pharmaceutical or cosmetic use), whatever the process to which they are subjected."* A similar procedure should also be carried out for material originating from other countries with native cases of BSE.

In its opinion of 15 April 1996 on products derived from bovine tissues, especially gelatine, tallow and dicalcium-phosphate in relation with Bovine Spongiform Encephalopathy, the Scientific Committee Food concluded: *"Based upon current incomplete knowledge regarding BSE and its possible transmission to humans and the uncertainty about the inactivation of the infective agent, the Committee at present is only able to advise that bovine source materials for these products are to be taken only from geographical areas where BSE does not occur in epidemic conditions. The Committee urges that data required for a scientifically based risk assessment be generated by relevant bodies. Further research is needed especially to develop specific, sensitive and rapid methods for detection of the causative agent in biological materials."*

On the 3rd of April, 1997, the Multidisciplinary Scientific Committee (MDSC) expressed a similar opinion as that of the Scientific veterinary Committee on 9 April, 1996, stressing especially: *"That at the moment no production method can be considered as safe for gelatine and related products if the base material used is potentially infectious.*" The opinion further states: "The control of the nature, the geographical origin and the quality of the starting material is currently the only means

to assure the protection of public health. The control applied to the starting materials must be subjected to intensive monitoring." The MDSC also confirmed its view that *"the following tissues should not be used as starting materials: skull, vertebral column, brain, spinal cord, eye, tonsil, thymus, intestine and spleen. (See Commission decision of 11th June, 1996, 96/362/EC)."*

So far, bones, a raw material for the production of gelatine and dicalcium phosphate, have been considered as a material with no detectable infectivity. Bovine bone marrow, by analogy with bone marrow from sheep with scrapie, was classified as belonging to the category of low potential infectivity materials. However, in its opinion adopted on 8-9 December 1997, the Scientific Steering Committee states:

> *(on) **dorsal root ganglia**. New (unpublished)[2] evidence shows that the dorsal root ganglia - located within the general structure of the vertebral column - should be considered as having an infectivity for BSE equivalent to that of the spinal cord. The dorsal root ganglia proved infective at the same time after infection as the spinal cord, i.e. 32 months. The trigeminal ganglia were also infective, but so far no autonomic nervous system tissue has been found to be infective. The dorsal root ganglia cannot be removed without extreme difficulty. This therefore means that as a precautionary proposal the removal of the whole vertebral column (other than the coccyx) is now appropriate. Care needs to be taken to ensure that the removal of the vertebral column incorporates the lateral aspect of the vertebral bodies. This dissection may sometimes be difficult in practice unless the musculature is selectively removed from the vertebral bones for selling as bone-free meat.*
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> *(on) **Bone marrow** :*
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> 1. *Early studies with mice intracerebrally injected with bone marrow from cattle with spontaneous clinical BSE has not demonstrated infectivity (SEAC, 1994). However, studies on calves, experimentally infected by feeding 100g of BSE infected brain tissue, have now shown bone marrow infectivity in cattle studied at 38 months after feeding the BSE infected brain. These animals were clinically affected by BSE. (MAFF, unpublished evidence 5.12.1997). This has wide-ranging implications because it implies that long bones as well as vertebral columns must be considered potentially infective. The concerns on contamination and the dorsal ganglia mean that on these grounds alone the vertebral columns of older animals should be included in the category of specified risk material.*
> 2. *Several issues now emerge from the new report on bone marrow infectivity. First the apparent infectivity of bone marrow might need to be redefined. Bone marrow (on the basis of scrapie studies) was placed in Category III, i.e. as showing low infectivity. In previous bone marrow studies on clinical cases of BSE infected cattle, no infectivity was detected which might have suggested that the WHO classification was inappropriate in persisting with a Category III, rather than a Category IV, rating, i.e. no demonstrable infectivity. However, new evidence shows*

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2 Presently published as Wells et al., Veterinary Record, January 1998.

2 of 18 mice developing late clinical disease after having been injected with marrow from cattle of 38 months post infection. Another 3 mice also show immunocytological evidence of the presence of PrP^{Sc}, having been injected with the same bone marrow extract. Given the late development of this demonstrable infectivity in cattle bone marrow despite the substantial infective dose (100 g untreated BSE infective brain) it now seems appropriate to maintain the WHO classification for BSE as well as for scrapie. This signifies that BSE is increasingly being revealed as having a tissue based infectivity which seems similar to that of scrapie.

3. *This conclusion reinforces the concepts [...] that the different levels of infectivity do reflect a graded phenomenon and that it is unwise to consider the BSE agent as either present or absent in particular tissues.*
4. *The bone marrow findings also raise the issue of whether bones from older animals, e.g. >30 months, should be removed from the human food chain."*

As far as infectivity of bone marrow is concerned, the working group on gelatine of the Scientific Steering Committee already noted that the above statements referred to infectivity resulting from a single group of experimentally challenged cattle. However, infectivity of the bone marrow of naturally infected bovines has, to present knowledge, not been detected. According to Hadlow et al. (1982), infectivity has been reported in bone marrow of Suffolk sheep with natural, clinical scrapie but not in goats with natural scrapie(Hadlow et al., 1980).

The opinion on the safety of di –calcium phosphate prepared by the French Comité Interministériel sur le Encéphalopathies Subaigues Spongiformes Transmissibles (République Française 1996) and the report to the Scientific Steering Committee by Dormont (1997) already underlined the importance of clear sourcing of the bones, the production process and the quality control of the finished product. The degree of potential infectivity of the starting material and therefore its traceability and certification of origin seem essential. The Dormont (1997) report states (translated from French): *"The capacities of the production process of dicalcium phosphate to eliminate or inactivate Transmissible Non Conventional Agents* [in French: ATNC or Agents Transmissibles non Conventionnels] *is relatively weak. This should motivate a precise control of the raw material. This control must take into account not only the geographical origin of the bovine animals – which should originate from countries with a low incidence of BSE or from "BSE free" countries (as defined by the OIE) – but also the nature of the organs that enter into the initial biological material, be is as main component or as contaminant. It is thus indicated to eliminate from the production process of dic-calcium phosphate the organs susceptible to carry high titres of the infectious agent. The elimination of the central nervous system (encephalon and spinal cord) is thus critical*."

The SSC secretariat therefore started compiling additional material needed for assessing the safety of dicalcium phosphate, in particular with regards to the currently used industrial processes and on the TSE infectivity reduction capacity of the individual production steps and the production process as a whole.

2.2. The production of dicalcium phosphate.

In order to express an opinion on the safety of dicalcium phosphate it is important to take into account a number of aspects of the production methodologies and conditions. A sample typical production process description is given in the figure on next page (after GME, 1997).

Di-calcium phosphate is a co-product from the gelatine manufacture. (see: EC, 1998. Opinion on the safety of gelatine adopted by the Scientific Steering Committee of 26-27 March 1998).

A typical composition from bones serving for gelatine and dicalciumphosphate comprises: 64% hydroxyapatite (with 32% orthophosphate, 24% calcium, 7% carbonate and 1% magnesium) aside 28% proteins (23% collagen, 5% other nitrogen compounds). The water content from these bones averages 8%.

The typical gelatine manufacturing process from bones includes first a degreasing step of fine crushed bones in hot water (80° to 85°C). Regularly shaking removes a high percentage of proteins. The dried bone chips are then submitted, over a total period of 4-5 days, to a sequence of solutions with an increasing hydrochloric acid concentration. The highest concentration being 4% of HCl during 2 days. This demineralisation of the fine bone chips produces a phosphoric liquor that after treatment with lime, will give a precipitate of dicalcium phosphate at pH 4 to 7. The solid fraction containing the osseine is further processed for the production of gelatine. The wet precipitated dicalcium phosphate is than essored and finally air dried during 15 minutes with inlet temperatures of 270-325°C and end temperatures between 60-65 °C.

This $CaHPO_4.2H_2O$ contains 20.3% bound water aside less then 5% free water. Mineral impurities are less than 0.8%, organic impurities are less than 0.6% (with less than 0.15% lipids and less than 0.5% protein obtained as Nitrogen content multiplied by 6.25). (CEFIC, 1997; Piva, 1997)

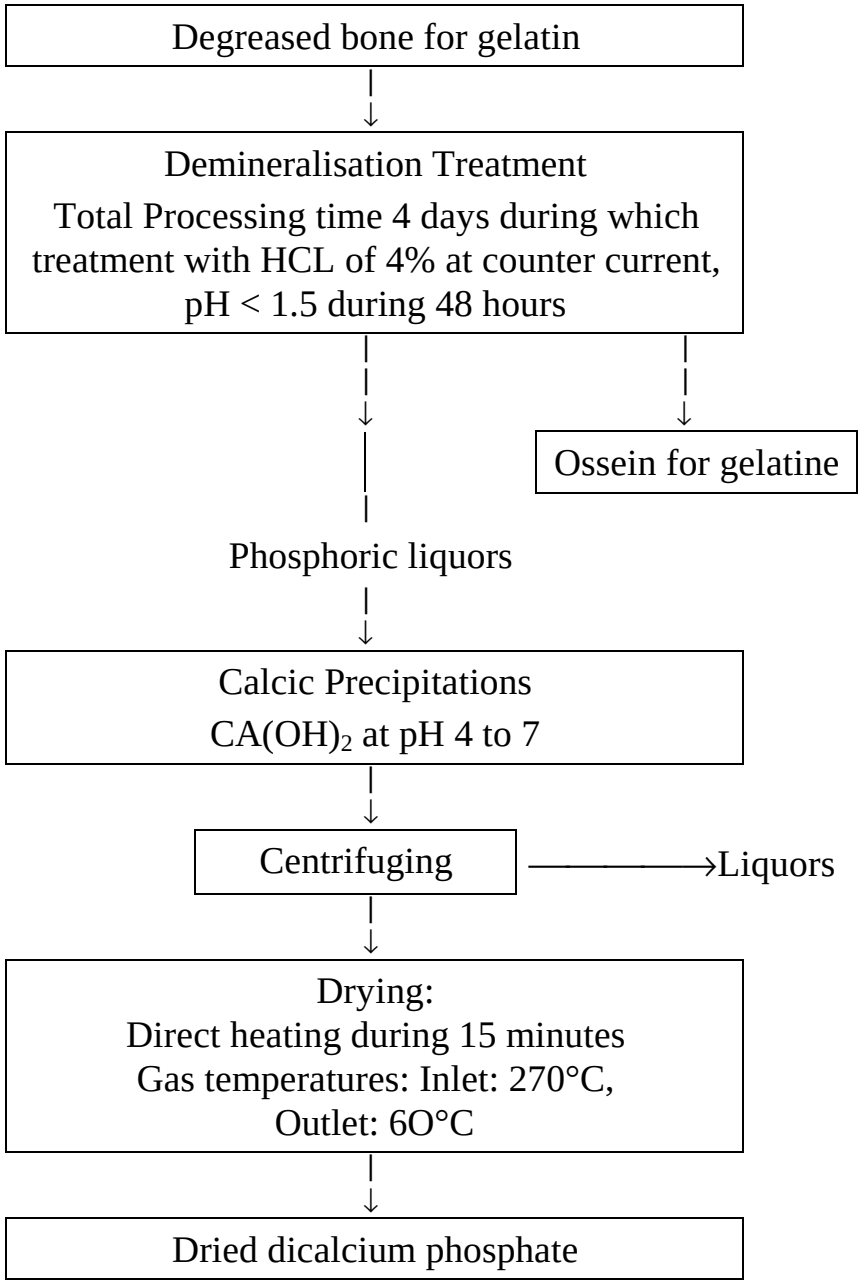
The end use of this dicalcium phosphate seems only intended for animal nutrition.

3. CONSIDERATIONS REGARDING THE SAFETY OF DICALCIUM PHOSPHATE.

Di-calcium phosphate being a co-product during the gelatine production process, is indicated to mention here some of the considerations made by the Scientific Steering Committee in its report and opinion on the safety of gelatine, adopted on 26-27 March 1998. The two first production steps (degreasing and demineralisation) are indeed common to both production processes.

Sample typical production process of Dicalcium phosphate precipitated from bones (after GME, 1997)

Minimum manufacturing conditions:



Humidity: < 5%, Phosphorus content: > 17%, impurities of proteinaceous nature: approx. 0.5%

3.1 General: the opinion of the association Gelatine Manufacturers of Europe (GME) on the quality and the sourcing of raw material

Raw material for one given plant may originate from several sources and may be a mixture of materials from different slaughterhouses and suppliers. The number of critical points[3] in the whole production chain from source to final product which need to be controlled to minimise or neutralise the risk of possible residual infectivity of the final product, is large and their monitoring may not always be easy and evident.

According to the association of Gelatine Manufactures of Europe (GME), which represents most of the EU's gelatine producers, all of their associated gelatine-manufacturing sites in the European Union are certified according to ISO 9000 international standards. The GME's gelatine manufacturers claim to respect the following sanitary guarantees, which are also recommended in OIE documents: no sourcing from countries with high BSE infectivity (UK); sourcing only from countries with low infectivity or BSE free. Bones and skins are collected from the meat industry controlled by the official veterinary services; they come from animals recognised as suitable for human consumption. For each gelatine lot (even from outside E.U. countries) full documentation allows manufacturers to trace the raw materials "origin" from their reception in gelatine plants. Upstream, ruminant bones are subject to a similar traceability in the degreasing plants.

However, given the complexity and multitude of critical points in the overall production process, and given the fact that they are not limited to the conditions within the factory, the SSC is of the opinion that respecting ISO 9000 standards is probably not a sufficient guarantee of the safety of the end product, but that the respect of HACCP[4] procedures should be guaranteed and documented. Some of these points are (non exhaustive list): traceability, the source of the raw materials which may be multi-country and multi-supplier and the treatment at origin (e.g. removal of specified risk materials) of the raw material, and the degreasing step.

3.2. Reduction of infectivity during the degreasing step.

In the production process of gelatine, it is interesting to note that German researchers (Manzke et al., 1996) have shown that during the degreasing step 98-99% of the protein of nervous origin (e.g. S100[5], GFAP[6] and others) are removed. The method used (ELISA test) was very sensitive with a detection threshold from 30 picogr. for S100 and 7 picogr. for GFAP.

The likelihood that animal bones in continental Europe are contaminated with nervous tissue from animals suffering from BSE was previously estimated to be at most 0.0005 (weight) % (Schrieber and Seybold, 1993). It was also noted that total protein from bones before degreasing was 12.9 g/kg and was reduced to 2.4 g/kg after degreasing. (=82% reduction). Further analyses carried out after the succeeding step in gelatine manufacture, the acid treatment of degreased bones (HCl 4%) during 4-5 days, resulted in no longer detectable specific nerve proteins.

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3 In terms of possible hazards in terms of risk for remaining BSE infectivity in the final product

4 HACCP: Hazard Analysis Critical Control Points

5 S100 is a nervous protein, soluble in 100% saturated ammonium sulphate.

6 GFAP stays for glial fibrillary acid protein.

In an other experiment, finely crushed bovine heads were used which implies extremely high contamination with brain tissue. Since 1 September 1997, heads as such are no longer used in routine gelatine manufacture. The results obtained confirm those obtained with crushed bone chips: a reduction of specific nerve tissue proteins by 98-99% after degreasing, additionally, total protein content is reduced from 31.8 g/kg to 3.7 g/kg (88%) and no specific nerve proteins were detectable after the acid treatment step using degreased heads.

The authors conclude that "*there is hardly any reason to assume that prions would not be removed similarly as nervous proteins*."

The Scientific Steering Committee comments that TSE infectivity is not limited to nervous (brain) proteins but is also present in the lympho-reticular system of sheep but not so far in BSE infected bovines, even after spleen and lymph nodes were injected intercerebrally into cattle. The SSC also notes that the above conclusion may be valid for the reduction in protein levels, but not necessarily for infectivity. Prions are not necessarily removed in the same way as nervous proteins.

3.3. Reduction of infectivity during the demineralisation step.

With respect to the possible BSE transmission through gelatine, the *Gelatine Manufacturers of Europe* (GME) took the initiative for a validation study on the removal/inactivation capacity of a typical gelatine manufacturing process, assumed to be the most stringent one in terms of possible reduction of TSE infectivity. For establishing this opinion, the final report presenting the results after 18 months had been made available by GME (Inveresk Research International, 1998b).

Two key chemical treatments in the manufacturing process of gelatine were validated for BSE inactivation: the acid treatment and the liming treatment. In the context of the present report, only the acid treatment is relevant.

The material used consisted of scrapie infected mouse brain ($\log_{10}$ ID_{50}=7.44) for the acid treatment and $\log_{10}$ ID_{50}= 7.90 for the liming treatment. This material was inoculated intracerebraly to susceptible mice to calculate the reduction factors of infectivity in the two respective steps of the gelatine manufacturing process.

The acid treatment (demineralisation) shows only limited efficiency in the inactivation of potential prion contamination: after 18 months inoculation, the reduction factor was 1.17 $\log_{10}$ (approx. 10 fold).

The SSC notes that, from the present two sections, appears that there seems to exist only evidence for an approximately log 2 reduction of the specific nervous tissue proteins during degreasing and a log 1 reduction of infectivity during the acid demineralisation process.

3.4. The nature of the residues of the proteinaceous fraction

If the prion theory is correct, the quality controls in relation to possible BSE transmission of all products obtained by processing of bones has also to be focused on the nature of the residues of the proteinaceous fraction.

During the degreasing step of the fresh bones, 98-99% of specified nervous proteins are removed during this step. However, it is unclear whether prions, if present, are also removed by this step. After degreasing the bones are demineralised by a treatment

with hydrochloric acid during 4-5 days. The mineral fraction of the bones is transformed and separated from the organic fraction (collagen) by precipitation with lime. From the impurities remaining after centrifugation and air drying, less than 0.5% is of proteineaceous nature.

Information on the nature and chemical composition of these organic residues especially from the protein fraction are available from various sources. However, these sources all provide only preliminary analysis results:

- SKW Biosystems (1998), determined by chromatograp^hy on polypropelene sulfonate the protein fraction in the mother-liquor (before precipitation) and in the washing water of dried dicalcium phosphate. The results obtained after washing with warm water of the precipitated dicalcium phosphate, showed that 99.96 % of the residual protein fraction has a molecular weight below 10.000 Dalton and that 100% of the proteins have a MW below 12.000 Dalton. No fraction with a molecular weight above 12.000 Dalton was detectable in the washing water. Only some traces (0.13% or 0.5 ppm) with a MW between 30.000 and 40.000 Dalton seemed to present in the mother liquor. (According to the report, the latter traces may well be the tail at the end of the signal representing the analysis results). Of the residual protein in the mother phosphoric liquor, 84.91% has a molecular weight below 10.000 Dalton, 97.89 % has a weight below 20.000 Dalton and 99.87% has a weight below 30.000 Dalton.
- Preliminary results of protein content determinations carried out by PB Gelatins (1998) finally show a protein content in the final dicalcium phosphate product, of 0.4%. All proteins (which are now in part acid hydrolysis products) had a MW below 1800 Dalton (molecular size exclusion chromatography on superose 12 FPLC colon). The amino acid pattern, determined on the basis of the hydroxyproline content) showed that only 20% of the residual protein had a collagen origin, meat, keratine, elastine, …., being the origin of the remaining 80%.

[**Questions to the Industry, industrial associations or research institutions**: was the determination of the above characteristics based on the total of the residual protein? If not, what fraction of the total residual protein was really recuperated and used for the analyses? Are there other data and results available? Which were the methods used for the determination of the proteinaceous fraction? According to the GME (1998d), recent studies have shown that after washing with hot water, nitrogen in the dicalcium phosphate was no longer detectable using the Kjeldahl method. The SSC would welcome the results of the analyses carried out by GME and of similar work by other laboratories.]

10.000 Dalton may be considered as a large safety margin for molecular weight of remaining proteins to be considered as acceptable, as the molecular weight of the assumed infectious prion protein is 27.000 - 30.000 Dalton.

According to the above preliminary results and considerations, dicalcium phosphate can most likely be considered as a safe animal feed. The probability of residual BSE contamination is extremely low and this is the reason why different E.U. Committees have always excluded bone precipitated dicalciumphosphate from the forbidden feed materials for ruminants. The residual protein fraction is small and the molecular weight of the proteins is (largely) below the assumed average weight of prions.

However, the method of determination of the residual proteins using washing with hot water – which is presently the only available method for the determination of the proteinaceous fraction in the presence of high concentrations of minerals - may not necessarily result in a complete extraction of all proteins: part of the fraction may indeed not be soluble in water. The SSC therefore would welcome that research laboratories and the industry make more results available on the concentration and composition and molecular weights of the residual protein fraction in dicalcium phosphate with more precise analytical methods such as the use of the Mass Spectrometry Method (MALDI-MS). [However, most of this latter fraction should then already have been removed during the degreasing step, which removes 98-99 % of the proteins of nervous origin, and/or present in the osseine fraction resulting from the acid treatment and further used for the gelatine production].

III. THE OPINION

Preliminary remark:

Readers should keep in mind that the report and opinion only address the scientific aspects of the risk *assessment* of the issue (e.g., identification of hazards, levels of infectivity in the starting materials and final products, etc.). The risk *management and policing* aspects related to the implementation of an opinion, are not dealt with.

4. THE QUESTION

On the basis of what precedes, the working group addressed the following question*:*

"Can dicalcium phosphate derived from ruminant bones, be considered to be free of BSE infectivity?

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 the animal and/or production process can it be considered as safe?"

6. Scientific opinion

Introductory note:

On 29 May 1998, a new version of the OIE International Animal Health Code Chapter 3.2.13. on Bovine Spongiform Encephalopathy (BSE) was unanimously adopted during the 66th Annual General Session of the International Committee of the OIE. The Code identifies four categories of countries or zones with regard to BSE. These are defined on the basis of criteria of risk analysis, on-going education programmes, compulsory notification and investigation of cattle showing clinical signs compatible with BSE, BSE surveilla,ce and monitoring system and examination of brain and other tissues collected within the framework of the surveillance system. The four categories are:

1. BSE free country or zone
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)

Some important characteristics of the OIE classes need to be further defined.

In its opinion of 22-23 January 1998 defining the BSE risk for specific geographical areas, the Scientific Steering Committee has listed the factors contributing to the incident and propagation risks in a geographical area. On 20 February 1998 the SSC adopted that list, slightly amended, as final opinion. More work needs to be done on the definition of risk regions or countries. The Scientific Steering Committee is preparing a further opinion on the geographical aspects of BSE risks

The four classes of the geographical aspect of BSE risks used in the opinion hereafter, are therefore indicative and, for the time being, are: "high risk countries", "lower risk countries", "countries considered free of BSE or classified as at negligible risk" and "Countries with an unknown TSE status". However, given the fact that a number of elements are still under study, the opinion hereafter may thus possibly be updated in accordance with the forthcoming Scientific Steering Committee opinion on the geographical aspects of TSE/BSE risks and with future updates of the OIE Code.

On the basis of the report of the working group, approved by the TSE/BSE ad hoc group, the Scientific Steering Committee adopted on 14-15 May 1998 the following final opinion on the safety of dicalcium phosphate:

"6.1. <u>Definitions</u>:

- *For the purpose of the present opinion dicalcium phosphate is defined as a chemical ($CaHPO_4.2H_2O$) obtained from degreased bones which are demineralised by hydrochloric acid treatment and precipitated by a saturated lime solution.*
- *The wording "Fit for human consumption" hereafter refers to material from animals that passed both pre- and post mortem inspection and that are certified by a competent veterinary authority and identified as fit for human consumption on the basis of the existing national and EU legislation. The Scientific Steering Committee stresses that positive identification of material not fit for human consumption should be possible, to avoid possible entering of such material in the food or feed chains.*
- *Unless otherwise specified, the wording "Specified risk materials" refers to all tissues listed in the opinion of the Scientific Steering Committee (SSC) adopted on 9 December 1997 and ammended on 10-20 February 1998. However, the SSC intends to consider the possibility of making a selection of specified risk materials on the basis of the results of a risk assessment, which takes into account the geographical origin of the animals, their species and their age.*
- *Appropriate production processes in the opinion hereafter refer to processing bone materials and are those processes which have an appropriate efficacy in terms of eliminating TSE agents. For the*

transformation of bones sourced from countries or regions where the BSE risk is not negligible or zero or where the BSE status is unknown, only those processes are "appropriate" with the highest possible efficacy to eliminating TSE agents. An example of an appropriate production process is: dried bones finely crushed and degreased with hot water, are submitted, over a total period of 4-5 days, to a sequence of solutions with an increasing hydrochloric acid (at a maximum concentration of 4% and pH <1.5) over a period of at least two days. The produced phosphoric liquor is treated with lime, resulting in a precipitate of dicalcium phosphate at pH 4 to 7. The wet precipitated dicalcium phosphate is essored and finally air dried during 15 minutes with inlet temperatures of 270-325°C and end temperatures between 60-65 °C.

Note: The Scientific Steering Committee calls for the results of the research on the TSE agent inactivation during the production process as a whole of dicalcium phosphate - starting with the degreasing step of infected material, and not as individual research studies covering each of the production steps separately - and for specifications on the residual protein fraction to be made urgently available, in order to possibly revise or broaden the above definition of appropriate production processes.

6.2. *Because of existing evidence of the possible presence of remaining impurities of a proteinaceous nature (approx. 0.5%, obtained as Nitrogen content multiplied by 6.25),*

as long as not more details on the composition and molecular weights of the residual protein fraction are available,

and given the fact that the number of critical points[7] in the whole production chain is quite large and that their monitoring may not always be easy and evident,

the Scientific Steering Committee is of the opinion that the optimum level of safety can be obtained from a combination of safe source of raw material used and a well documented process with defined minimum levels of treatment.

6.3. *The Scientific Steering Committee strongly recommends that manufacturers implement and respect HACCP[8] 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 (e.g. removal of specified risk materials) of the raw material, and the degreasing step.*

6.4. *The sections of the opinion hereafter cover the approach to be followed if the risk of infectivity in the remaining impurities is to be reduced to the lowest possible level. As an alternative, a more detailed quantitative risk analysis should be carried out to assess the remaining risk for a herd or individual animals. Such assessment would take account of:*

- *the type of final product and infectivity reduction capacity of the production procedure;*
- *the geographical origin of the raw material;*

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7 In terms of possible risk for remaining BSE infectivity in the final product

8 Hazard Analysis and Critical Control Points

- *the type of raw material, including the age of the animals;*
- *the removal or not of specified risk materials;*
- *the incidence and propagation components of the BSE borne risk, as specified in the opinion of 22-23 January 1998 of the Scientific Steering Committee defining the BSE risk for specified geographical areas.*

This assessment requires results of experiments on and justified estimates of, reduction factors during the various steps of the production process, from sourcing to marketing. Such data are not always available, as some experiments are still ongoing or only in a planning phase. In order to provide the Commission with two alternative choices, the Scientific Steering Committee will eventually complete the in this opinion followed approach to reduce the risk of infectivity in the final product to the lowest possible level with a quantitative risk analysis. The results of the latter analysis may eventually change or ask for an update of the recommendations hereafter.

6.5. *The raw material should be obtained from appropriate sources (geographical, herd, animal and its age), animal species and tissues.*

6.6. *In any case, the raw materials should be submitted to an appropriate production process, as indicated in the above definition.*

6.7. *For countries considered to be 'BSE free or classified as at negligible risk'. Raw material (bovine bones) can be used free without removal of specified risk materials when coming from animals certified as fit for human consumption.*

6.8. *For lower risk countries. Specified risk materials should first be removed to minimise the risks of possible contamination of the bovine bones. The origin of the bovine raw materials should be certified to be exclusively from animals that are fit for human consumption.*

6.9. *For high risk countries. Given the existing production procedures which do not always permit the tracing back of specified risk materials and their geographical origin, the SSC recommends that no sourcing of ruminant raw materials from high risk countries is allowed. However, in certain circumstances, the risk profile can be changed, e.g. on the basis of age of the animals, the origin (source herd) of the animal, etc., provided those circumstances carry no risk and provided the conditions applicable for lower risk countries are respected.*

6.10. *Countries with an unknown BSE status should be evaluated individually on the basis of a detailed evaluation using appropriate criteria. If no judgement on the basis of available evidence or because of a lack of information is possible, they should be considered as high risk countries.*

Remark: The previous statement does not prejudge the opinion of the SSC on the TSE/BSE status of any country. Work on geographical risk assessment is ongoing.

7. Summary table: the safety of dicalcium phosphate derived from bones from ruminants and intended as animal feed

Source:	End use :animal feed (additive)
BSE FREE or NEGLIGIBLE RISK	- Bones derived from animals that are fit for human consumption - Appropriate production process[9]
LOWER RISK	- Bones derived from animals that are fit for human consumption - SRMs[10] excluded - Appropriate production process[9]
HIGH RISK	- Exclude: all ruminant materials[11]; - Appropriate production process[9]
Status unknown	To be evaluated; if no judgement on the basis of available evidence or because of a lack of information is possible: consider as high risk.[12]

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9 See definition in Section 6.1.

10 Specified risk materials refer to the tissues listed in the opinion adopted on 8-9.12.97 and amended on 19-20.02.98. However, the SSC considers the possibility of making a selection of SRMs on the basis of the results of a risk assessment, which takes into account the geographical origin of the animals, their species and their age.

11 In certain circumstances, the risk profile can be changed, e.g., on the basis of age of the animal, the origin (source) of the animal, etc., provided those circumstances carry no risk and provided the conditions applicable for lower risk countries are respected

12 This statement does not prejudge the opinion of the SSC on the TSE/BSE status of any country.

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