



EUROPEAN COMMISSION
HEALTH & CONSUMER PROTECTION DIRECTORATE-GENERAL

Scientific Steering Committee

**UPDATED OPINION AND REPORT ON
THE SAFETY OF DICALCIUM PHOSPHATE (DCP) AND
TRICALCIUM PHOSPHATE (TCP) FROM BOVINE BONES,
USED AS AN ANIMAL FEED ADDITIVE OR AS FERTILISER**

SUBMITTED TO THE SCIENTIFIC STEERING COMMITTEE

AT ITS MEETING OF 6-7 MARCH 2003

OPINION

MANDATE AND BACKGROUND

This opinion addresses the following questions regarding the safety of dicalcium and tricalcium phosphate produced from ruminant bones:

"Can dicalcium phosphate and tricalcium phosphate (DCP and TCP) 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 use in animal feed or as a fertiliser?"

On 26 June 1998, the Scientific Steering Committee (SSC) adopted for the first time an opinion on the safety of dicalcium phosphate. The report attached to the opinion was updated by the SSC at its meeting of 26-27 October 2000. Early 2002, the results of a recent TSE inactivation study became available, as well as new information on the production, raw materials and uses of dicalcium phosphate. The TSE/BSE *ad hoc* Group evaluated this new information and prepared the attached report, which served as basis for the updated SSC opinion hereafter.

SCIENTIFIC OPINION

General:

BSE can be found in various animal species, but as far as the production of phosphates from animal bones is concerned, only bovines and pigs are of interest. Bones from pigs are not considered to pose a BSE risk because no BSE has been reported in this species under field conditions. The opinion hereafter applies to phosphates derived from bovine bones. They may be contaminated with infectivity, for example if skull or vertebrae were accidentally included in the raw materials. Also, in one bovine experiment, bone marrow has been found infectious during part of the life cycle¹. A separate risk assessment may be required for phosphates derived from small ruminant bones, should BSE in small ruminants be present or probable and should their bones be used for the production of phosphates. Such risk assessment should take into account the fact that GBR-C (Geographical BSE risk for cattle) related sourcing is not necessarily (yet) applicable in case of small ruminants as the GBR-S (GBR for small ruminants) takes an additional number of risk factors into account compared to GBR-C.

The concentration levels of dicalcium phosphate (DCP) and tricalcium phosphate (TCP) in feed are low (below 1% of the total feed dry matter consumed per day). However, there is existing evidence of the possible presence of remaining impurities of a proteinaceous nature (approx. 0.50-0.60 %²). Only preliminary analysis results are available on the composition of these impurities.

¹ An experimental error or laboratory contamination has, however, not been excluded.

² Calculated from Nitrogen content multiplied by 6.25

Bovine-bone derived Di- and Tricalcium phosphates used as feed additives

For cattle from countries with a geographical BSE risk level I (GBR-C I) the existence of a BSE risk is highly unlikely, and the same is valid for the phosphates obtained from the bones from these cattle.

a. Dicalcium phosphate:

The residual risk in dicalcium phosphate derived from bovine bones from countries where the BSE risk in cattle is not negligible (GBR-C II, III and IV countries) is negligible, under the following conditions:

The raw material for the production of bovine bone dicalcium phosphate should be obtained from appropriate tissues (i.e., from animals fit for human consumption³, exclusion of specified risk materials including skull and vertebrae and avoidance of cross-contamination with these bones) and submitted to a production process that has a proven TSE infectivity reduction capacity.

An example of such a production process is the following:

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 with inlet temperatures of 65°C-325°C and end temperatures between 30°C-65 °C.

A recent validation study shows that together, the acid process after degreasing and demineralisation has a total clearance of infectivity of 2.6 log₁₀. The dicalcium phosphate production process as a whole will reduce the infectivity further up to 3.8 to 3.9 log₁₀.

This production should have resulted in a residual proteinaceous fraction not exceeding 0.60 % and with 98% of it having a molecular weight below 10000 Daltons. ⁴

The risk of DCP used in feed should of course also be negligible if the bones are exclusively sourced from non-ruminant species and processed under the above conditions, or if cross-contamination with bovine material possibly posing a BSE risk is excluded.

b. Tricalcium phosphate as feed:

Because of the presence of residual proteins (17% of gelatine), the SSC considers that in the light of current knowledge, tricalcium phosphate produced from bovine bones does not represent a BSE risk in animal feed provided the conditions

³ The definition of animals fit for human consumption varies according to the GBR level. For example, in the UK the OTMS applies for animals above 30 months. Pending the outcome of a quantitative assessment of the residual risk in phosphates derived from OTMS bovines from GBR IV countries, it can not be assessed whether it would be significantly higher than for GBR II and GBR III countries.

⁴ See attached report page 11.

similar as for the production of gelatine under heat/saturated pressure⁵ are respected: sourcing from animals fit for human consumption, removal of SRMs, respect of the appropriate production process conditions, avoidance of risk of contamination, etc.

An appropriate heat/saturated pressure production process, is the following (see also the attached report):

- *Degreasing of the bones in counter-flow with hot water (bone chips less than 14 mm);*
- *Continuous cooking with steam at 145°C during 30 minutes at 4 bars;*
- *Separation of the protein broth from the hydroxyapatite (tricalcium phosphate) by centrifugation;*
- *Granulation of the tricalcium phosphate after drying in a fluid bed with air at 200°C.*

This process can be accepted to clear TSE infectivity by approximately 4 log₁₀.

Di- and tricalcium phosphate, used as fertiliser.

- a. Cattle from countries with a geographical BSE risk level I (GBR-C I) the existence of a BSE risk is highly unlikely, and the same is valid for the phosphates obtained from the bones from these cattle.
- b. Di- and tricalcium phosphates that comply with the above feed-quality criteria and used as a fertiliser, will not pose a risk to animals that would consume residues of DCP or TCP fertilisers. The likelihood of exposure is low, also because the fertilisers would be ploughed in or not persist massively on above ground parts of plants.
- c. Because of the longevity of the TSE agent protein in soils⁶, the risk of accumulation in the environment of possible residual risk is not completely excluded if applied in large quantities or repeatedly on a same area. Environmental pathways for the proliferation of TSE infectivity have been evidenced for scrapie in sheep, but so far not for in bovines. Also the latter possibility can theoretically not be excluded a priori⁷. The SSC, however, considers that, regardless of the GBR-C level, the risk from using DCP and TCP, produced according to feed standards, and used as fertiliser in normal quantities would be remote taking into account the above mentioned low levels of impurities and the clearance by processing.

⁵ See Updated Opinion on The safety with regard to TSE risks of gelatine derived from ruminant bones or hides from cattle, sheep or goats. Adopted by the Scientific Steering Committee at its meeting of 6-7 march 2003.

⁶ See: Scientific Report on The risks of non conventional transmissible agents, conventional infectious agents or other hazards such as toxic substances entering the human food or animal feed chains via raw material from fallen stock and dead animals (including also: ruminants, pigs, poultry, fish, wild/exotic/zoo animals, fur animals, cats, laboratory animals and fish) or via condemned materials. Submitted to the Scientific Steering Committee at its meeting of 24-25 June 1999

⁷ See also: Opinion on the Hypotheses on the origin and transmission of BSE. Adopted by the Scientific Steering Committee at its meeting of 29-30 November 2001 .



EUROPEAN COMMISSION
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Scientific Steering Committee

**REPORT IN SUPPORT OF THE UPDATE OF THE SSC'S OPINION
ON THE SAFETY OF DI-CALCIUM PHOSPHATE (DCP) AND
TRICALCIUM PHOSPHATE (TCP) FROM BOVINE BONES AND
USED AS AN ANIMAL FEED ADDITIVE OR AS FERTILISER**

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I. BOVINE BONES-DERIVED DICALCIUM PHOSPHATE (DCP) AND OF TRICALCIUM PHOSPHATE (TCP)

I.1. DEFINITIONS AND USE

Dicalciumphosphate ($\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$) is a chemical produced through precipitation from bones or inorganic material.

For the purpose of the present report *dicalcium phosphate* ($\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$) is defined as the chemical product obtained from degreased bones which are demineralised by hydrochloric acid treatment. The product is precipitated by a saturated lime solution.

The end use of this bovine-derived phosphates is in principle only intended for animal nutrition (as an additive), but may also be used as a fertiliser.

I.2. THE PRODUCTION PROCESSES.

I.2.1. THE PRODUCTION OF DICALCIUM PHOSPHATE (SEE ANNEX).

Di-calcium phosphate is a co-product from the gelatine manufacture. A sample typical production process description is given in the figure **in annex** (after GME, 2000 and EMFEMA, 2002).

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. The osseine obtained is further used for the production of gelatine. The liquor, after treatment with lime, will give a precipitate of dicalcium phosphate. This precipitate is essored and air dried with inlet temperatures of 65°C-325 °C and outlet temperatures of 30°C-65°C.

A typical composition from bones serving for gelatine and dicalcium phosphate 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 finally air dried with inlet temperatures of 65°C-325 °C and end temperatures between 30°C-65°C.

This $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$ contains 20.3% bound water aside less than 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; EMFEMA, 2002).

I.2.2. THE PRODUCTION OF TRICALCIUM PHOSPHATE (TCP)

The production process of tricalcium phosphate from bones involves (Protein s.a., 2002):

- The degreasing of the bones in counter-flow with hot water (bone chips less than 14 mm);
- Continuous cooking with steam at 145°C during 30 minutes at 4 bars;
- Separation of the protein broth from the hydroxyapatite (tricalcium phosphate) by centrifugation;
- The granulation of the tricalcium phosphate after drying in a fluid bed with air at 200°C.

This tricalcium phosphate is not pure and is in average composed of 75% hydroxyapatite, 17% gelatine⁸, 4% fat and 4% moisture.

II. TSE INFECTIVITY IN BONE MATERIAL.

- a. So far, bones, a raw material for the production of gelatine and dicalcium phosphate, have been considered as a material with no detectable infectivity.
- b. However, dorsal root ganglia and spinal cord may constitute possible sources of contamination of (vertebral column) bones. Whether bone marrow may possibly be infectious is less clear:

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).

Early studies with mice intracerebrally injected with bone marrow from cattle with *spontaneous* clinical BSE has not demonstrated infectivity (SEAC, 1994). These data for BSE are based on transmissions attempted from a very small number of animals⁹ but they are, in general, consistent with those in studies of the pathogenesis of BSE in a single group of experimentally challenged cattle *with 100g of BSE infected brain tissue* (Wells *et al*, 1996, 1998), with the exception of the detection of infectivity, in a level close to the limit of detectability by mouse bioassay¹⁰, in the sternal bone marrow from animals killed in the clinical

⁸ This is most likely collagen, as it concerns the substance before extraction, filtration and final processing

⁹ The experiments were limited and not all the different bone marrow bones, at different stages of incubation have been tested.

¹⁰ *2 of 18 mice developing late clinical disease after having been injected with marrow from cattle of 38 months post oral infection with 100 g untreated BSE infective brain material. Another 3 mice*

phase of the disease at 38 months p.i. (but not before and not after) in this experimental study of BSE in orally exposed cattle (Wells *et al*, 1999). The inconsistent result of the absence of detectable infectivity in bone marrow in this study at the later time point of 40 months p.i. has raised, amongst other alternative explanations, the possibility that the finding of infectivity at 38 months p.i. may have been the result of an accidental procedural contamination. Nevertheless, there is limited evidence from previous studies of other TSEs that infection of bone marrow, although not part of the general pathogenesis pattern, could be a rare event occurring late in the incubation period.

On the basis of the above, the TSE/BSE *ad hoc* Group suggests to place bovine bone marrow in the same infectivity category as sheep bone marrow, namely Category 3, i.e., showing low infectivity. The TSE/BSE *ad hoc* Group further proposes, for quantitative risk assessments, to consider both scenarios of bovine bone marrow being either infectious or not.

- c. It has been reported that it becomes more difficult to inactivate (scrapie-) infected brain-tissue by heat after it has been dried (Asher et al, 1986; 1987; see also EC, 1999).

III. BONE-DERIVED PHOSPHATES IN ANIMAL FEED

Dicalcium phosphate:

The daily amount of phosphorus required by animals varies according to the species, its use (e.g., milk or meat production), its production (e.g., expected daily milk production) its age, etc. In terms of BSE risk, cattle can serve as a worst case scenario because there would be no interspecies barrier.

According to data compiled by Tessenderlo Chemie (2002), the amount of phosphorus (P) required by cows ranges approximately as follows:

- Adult milk cow: Average daily P requirement for an adult milk cow of 600 kg, producing 30 kg of milk per day: 73 grams (range: 62 – 85 grams of P per day). Most of this phosphorus is of vegetal origin, and it is estimated that a maximal fraction of 20% is of mineral origin. DCP having a P content of 17 – 18%, this corresponds with approx. 81 – 86 grams of bone DCP per day (included normally in approx. 10 kg of concentrate dry matter).
- Meat cattle: Average daily P requirement for meat cows: 22 grams (range: 7-21 grams for growing dairy heifers; 8-23 grams for growing dairy bulls; 14-42 grams for meat cows). This corresponds with approx. 24 – 26 grams of bone DCP per day (included normally in approx. 5 - 6 kg of concentrate dry matter).

According to Tessenderlo Chemie [pers.comm.] the DCP concentrations (as a percentage of total concentrate dry matter are as follows:

also show immunocytological evidence of the presence of PrP^{Sc}, having been injected with the same bone marrow extract.

	<i>% DCP in feed without phytase</i>	<i>% DCP in feed with phytase</i>
Piglets	1.0% DCP	0.3% DCP
Growing pigs	0.6% DCP	-
Broilers	1.5% DCP	0.8% DCP
Layers	1.3% DCP	0.6% DCP

Remarks:

- o No meat and bone meal in European feeds
- o Not DCP but Monocalcium phosphate (MCP) is mainly used in pig feeds

Note: In reality, bone DCP is almost not used for ruminants due to two reasons:

- 1) Bone DCP is a fine powder and for the preparation of mineral mixtures for ruminants, granulated inorganic phosphorus sources are used.
- 2) Bone DCP has a high phosphorus digestibility of 87% (Jongbloed and Kemme, 1990) and is therefore mainly used in feed for monogastric animals.

The above consumption amounts should thus be considered to be worst case scenarios.

Tricalcium phosphate:

Precise data on the concentration of TCP in animal feed are not available, but it can be expected that it will be approximately as for DCP.

IV. TSE INFECTIVITY CLEARANCE DURING PRODUCTION

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 the version of 26-27 March 1998 of its report and opinion on the safety of gelatine. The two first production steps (degreasing and demineralisation) are indeed common to both production processes.

IV.1. REDUCTION OF INFECTIVITY DURING THE DCP DEGREASING STEP.

- a. 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. S100¹¹, GFAP¹² 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

¹¹ S100 is a nervous protein, soluble in 100% saturated ammonium sulphate.

¹² GFAP stands for glial fibrillary acid protein.

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.

In an other experiment, finely crushed bovine heads were used which implies extremely high contamination with brain tissue. 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."

- b. According to Gelatine Delft (1998) that the degreasing step, which precedes the drying of the bones, and carried out at a pilot scale which represent the commercial degreasing process under laboratory conditions¹³, reduces the brain *protein* levels by a factor 300-800. It may be expected that, under operational conditions, this reduction is higher because the same laboratory experiments at pilot scale resulted in degreased bone with a fat content of 6%, compared with 3% in the commercial process.
- c. However, in its report attached to the opinion of 27-28 March 1998, the SSC noted 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.

IV.2. REDUCTION OF INFECTIVITY DURING THE DCP DEMINERALISATION STEP.

- a. 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

¹³ Ten grams of pig-brain thoroughly mixed with 1 kg of bone-chips typically used by gelatine manufactures (average particle size: 12 mm, maximum: 20 mm).

treatment. This material was inoculated intracerebrally to susceptible mice to calculate the reduction factors of infectivity in the two respective steps of the gelatine manufacturing process.

The acid treatment (demineralisation) showed only limited efficiency in the inactivation of potential prion contamination: after 18 months inoculation, the reduction factor was 1.2 log₁₀ (approx. 10 fold).

- b. A recent validation study presented in Grobber *et al* (2002) showed that together, the degreasing step followed by acid process for the production of gelatine, have a BSE clearance capacity of 2,6 % log₁₀.

IV.3. INFECTIVITY REDUCTION DURING THE DCP PRODUCTION PROCESS AS A WHOLE.

From the previous two sections, it appeared reasonable to accept an approximately log 2 log₁₀ reduction of the specific nervous tissue proteins during degreasing and a 1 log₁₀ reduction of infectivity during the acid demineralisation process and that a total reduction for both steps of approx. 2,5 log₁₀.

Recently, Grobber *et al* (2003) presented the final research results on TSE infectivity clearance during the DCP production process. They use rodent-passaged TSE agents (the 301V mouse passaged BSE agent and the 263K hamster passaged scrapie agent) in a downscaled model of the DCP production process. The start material had a high initial infectivity titre of 7,7 to 8,0 log₁₀ ID₅₀/g. The results, at 600 days post infection, show an infectivity level between 2,5 and 2,7 log₁₀ ID₅₀/g. The corresponding clearance factor of the process is estimated to be approximately 3,9 log₁₀ for the DCP with the 301V BSE agent and 3,8 log₁₀ for the 263K scrapie strain. I

It seems thus reasonable to accept an overall TSE clearance factor of at least approx. 3,5 log₁₀ during the DCP production process as a whole.

IV.4. INFECTIVITY REDUCTION DURING THE TCP PRODUCTION PROCESS.

It is to be expected that the degreasing treatments applied in the process have a TSE infectivity reduction activity as for DCP and gelatine, i.e., approx. 2 logs. Regarding the heat/pressure/time treatment, a recent validation study for gelatine is available (Grobber *et al*, 2002b) showing a reduction of TSE infectivity of at least 6.8 logs (degreasing and purification steps included).

It should be noted, however, that, contrary to the gelatine production under saturated pressure/heat/time, the bone material for the production of TCP undergoes only once a continuous cooking with steam at 145°C during 30 minutes at 4 bars. Also the degreasing step is less thorough as the final product still contains approx. 4% of lipids¹⁴ and the final production steps are less severe as compared to gelatine. Under the given conditions, and taking into account the SSC opinion on the “133°C/20’/3bars” heat-pressure treatment

¹⁴ As opposed to 0,15% in DCP

meat-and-bone meal, an overall TSE clearance factor of approx. $4 \log_{10}$ during the production process of TCP as a whole can be accepted.

V. THE NATURE OF THE RESIDUES OF THE PROTEINACEOUS FRACTION

In Dicalcium phosphate:

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 proteinaceous 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 chromatography on polypropylene 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 column). 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%.
- Recent analyses (EMFEMA, 2002) of the amount, nature and molecular weight of the proteinaceous fraction associated with bone DCP indicated a maximum amount of 0,56% [0,50 to 0,56%; Khejda method] with 21% to 74% of it having a collagen origin. The major proteinaceous fraction had a molecular weight around 1000 Daltons (with a fraction of 0,53 –

1,21 % having a MW above 10.000 Dalton). This means that the fraction is composed in majority of small peptides.

- In its Updated Report and Scientific Opinion¹⁵ on the safety of hydrolysed proteins produced from bovine hides, the SSC stated: *“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 [...] 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.”*
- Note: The methods of determination of the molecular weight of residual proteins using washing with hot water 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 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).

In Tricalcium phosphate:

According to Proteïn s.a. (2002) tricalcium phosphate is not pure and is in average composed of 75% hydroxyapatite, 17% gelatine, 4% fat and 4% moisture.

VI. CONCLUSIONS

Regarding dicalcium phosphate (DCP):

Cattle from countries with a geographical BSE risk (GBR) level of I do not represent a BSE risk, neither does the dicalcium phosphate sourced from animals from such countries.

Given the low concentration levels of DCP in feed (below 1% of the concentrate dry matter), the BSE risk from Dicalcium phosphate can be considered to be negligible, if sourced from animals from countries with a GBR level of II, III and IV and from animals fit for human consumption and after

¹⁵ 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

SRM removal, and provided the production was carried out as described in the above report. This production should have resulted in a residual proteinaceous fraction not exceeding 0,60 % and with 98% of it having a molecular weight below 10.000Daltons.

Note: The definition of animals fit for human consumption varies according to the Geographical BSE risk in cattle (GBR-C) level. For example, in the UK the OTMS applies for animals above 30 months. Pending the outcome of a quantitative assessment of the residual risk in phosphates derived from OTMS bovines from GBR-C IV countries, it can not be assessed whether it would be significantly higher than for GBR-C II and GBR-C III countries.

Regarding tricalcium phosphate (TCP):

Because of the presence of residual proteins (gelatine), the TSE/BSE *ad hoc* group concludes that in the light of current knowledge, tricalcium phosphate produced from ruminant bones does not represent a BSE risk in animal feed provided the similar conditions as for the production of gelatine are respected: sourcing from animals fit for human consumption (see also above Note), removal of SRMs, respect of the appropriate production process conditions, avoidance of risk of contamination.

Regarding the use of DCP and TCP as fertilisers:

Di- or tricalcium phosphates obtained from animals from GBR I countries, do not pose a BSE risk.

Di- and tricalcium phosphate that comply with the above feed-quality criteria and used as a fertiliser, will not pose a risk to animals that would consume residues of DCP or TCP fertilisers. The likelihood of exposure is low, also because the fertilisers would be ploughed in or not persist massively on above ground parts of plants.

However, because of the longevity of the TSE agent protein in soils¹⁶, the risk of accumulation in the environment of possible residual risk is not completely excluded if applied in large quantities or repeatedly on a same area. Environmental pathways for the proliferation of TSE infectivity have been evidenced for scrapie in sheep, but so far not for in bovines. Also the latter possibility can theoretically not be excluded a priori¹⁷. However regardless of the GBR-C level, the risk from using DCP and TCP, produced according to feed standards, and used as fertiliser in normal quantities would be remote taking into account the above mentioned low levels of impurities and the clearance by processing.

¹⁶ See: Scientific Report on The risks of non conventional transmissible agents, conventional infectious agents or other hazards such as toxic substances entering the human food or animal feed chains via raw material from fallen stock and dead animals (including also: ruminants, pigs, poultry, fish, wild/exotic/zoo animals, fur animals, cats, laboratory animals and fish) or via condemned materials. Submitted to the Scientific Steering Committee at its meeting of 24-25 June 1999

¹⁷ See also: Opinion on the Hypotheses on the origin and transmission of BSE. Adopted by the Scientific Steering Committee at its meeting of 29-30 November 2001 .

VII. ACKNOWLEDGEMENT

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Sample typical production process of Dicalcium Phosphate precipitations from bones (after EMFEMA (2000) and GME (2000))

Minimum manufacturing conditions:

