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*Pre-authorisation Evaluation of Medicines for Human Use*

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**BIOTECHNOLOGY WORKING PARTY  
Meeting 11-13 February 2002**

**PUBLIC REPORT**

**RISK AND REGULATORY ASSESSMENT OF LACTOSE AND OTHER PRODUCTS  
PREPARED USING CALF RENNET**

**1. Introduction**

The Biotechnology Working Party (BWP) of the Committee for Proprietary Medicinal Products (CPMP) has been requested to conduct a risk and regulatory assessment on lactose prepared using calf rennet. The mandate of this review is as follows:

- a. to examine the available information regarding the manufacture of lactose and preparation of calf rennet;
- b. to conduct a risk assessment of lactose prepared using calf rennet, taking into account the regulation already in place governing the food and pharmaceutical sectors.;
- c. to consider whether there is scientific and regulatory justification to exempt lactose prepared using calf rennet from the scope of the TSE guideline (see below); and
- d. to report its findings to the CPMP, the Mutual Recognition Facilitation Group (MRFG) and Committee for Veterinary Medicinal Products (CVMP) to form the basis of their advice to the European Commission.

**2. Background**

Lactose is a natural sugar present in milk. Bovine lactose is a pharmaceutical excipient commonly used as a bulking agent in pharmaceutical formulation for both human and veterinary medicinal products.

In September 2001, the MRFG was informed by the United Kingdom Medicines Control Agency (MCA) about a problem with the regulatory compliance of a lactose preparation with the current CPMP/CVMP *Note for Guidance on minimising the risk of transmitting animal spongiform encephalopathy agents via human and veterinary medicinal products* (TSE Guideline). The TSE guideline has been given force of law by virtue of the Commission Directive 1999/82/EC. It had been brought to the MCA's attention that a particular pharmaceutical lactose preparation is produced using a bovine reagent (calf rennet). The MRFG referred this matter to the BWP to seek clarification regarding the scope of the TSE guideline.

The BWP gave an opinion on 15 October 2001 stating:

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*“Milk and materials derived only from milk are excluded from the scope of the guideline provided the following two conditions are satisfied:*

- *Milk is sourced from healthy animals in the same conditions as milk collected for human consumption;*
- *The milk derivatives are prepared without the use of other ruminant materials”*

From the stated scope of the TSE guideline, the BWP advised that this lactose preparation was not excluded from the scope of the guideline because calf rennet is used in its production. The BWP however also gave a preliminary opinion to the effect that there was no significant public health risk with lactose prepared using calf rennet.

On 4 December 2001, the BWP heard a presentation given by the lactose manufacturers. Following this, the BWP re-affirmed its position to the CPMP that there was no significant public health risk associated with lactose. However, it was the view of the BWP that:

*“Before lactose produced using calf rennet can be excluded from the scope of the TSE guideline, there is a need to formalise the scientific risk assessment, taking into account the available scientific opinions and the European legislation enacted for the control of TSE.”*

An Ad Hoc TSE Expert Drafting Group was constituted in December 2001 consisting of members of the CPMP BWP and the CVMP Immunologicals Working Party.

### **3. Evaluation**

In this review, the BWP considered:

- a. technical information already provided to the European Medicines Evaluation Agency (EMA) secretariat and the CPMP BWP by the lactose and calf rennet manufacturers with regard to the generic manufacturing process of lactose and the preparation of calf rennet;
- b. the regulation already in place governing food and pharmaceutical sectors for the purpose of this review.

Definition of lactose: The European Pharmacopoeia monograph for Lactose (monohydrate) sets out the physico-chemical characteristics and tests to be performed to render the lactose pharmacopoeia compliant. Tests include appearance, acidity/alkalinity, specific optical rotation, absorbance, heavy metals, water, sulphated ash and microbial contamination. Lactose is a low molecular weight, hydrophilic carbohydrate with low protein content (see below: estimated to be approximately no more than 70 µg per kg of lactose, but in general none detectable).

#### 3.1 Manufacturing process of lactose

Lactose is prepared from a by-product of cheese production. During cheese production, milk is coagulated by a variety of means to produce the curd. Coagulation of milk is brought about most commonly by using calf rennet, an extract of digestive enzymes derived from the abomasum (stomach) of milk-fed calves. The starting material for the production of lactose is whey, a spent liquid fraction. Other methods involve specific micro-organisms or recombinant DNA derived enzymes. According to the lactose manufacturers, approximately 90% of pharmaceutical lactose is produced from rennet-derived whey.

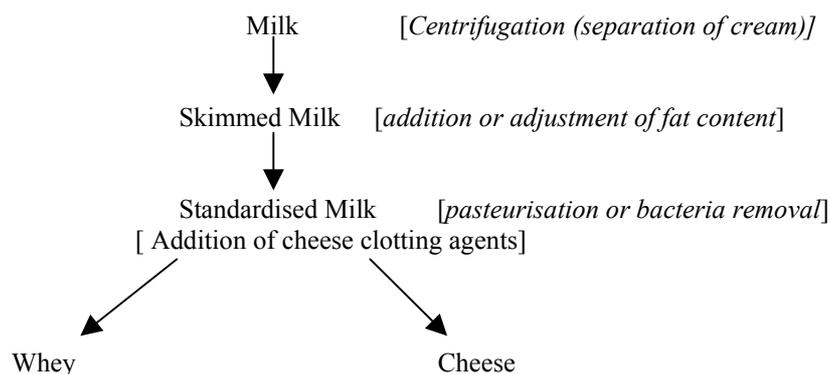


Figure 1

Whey as a by-product of cheese production is then processed through a number of steps as follows:

- Concentration: the whey is concentrated in a vacuum evaporator until the dry matter content reaches approximately 60%. During the process the temperature exceeds 60°C for some minutes
- Crystallisation: the concentrate is cooled down and lactose crystals form
- Separation: the lactose crystals are separated with the help of decanters/screen centrifugation
- Washing: the lactose crystals are washed several times with drinking water
- Refining: A 60% solution is obtained from washed crystals by adding hot water (drinking water). Activated coal/inorganic filter auxiliaries are added. Refining takes place at more than 97°C and during a period of more than 30 minutes.
- Filtration: refining is followed by filtration at approximately 95°C.
- Crystallisation: the pure lactose solution is cooled down and crystals form.
- Separation: the lactose crystals are separated by decanters/screen centrifugation.
- Drying: drying of lactose crystals at more than 70°C
- Finishing: desired granulation or modification
- Lactose for pharmaceutical use

### 3.2 Preparation of Calf Rennet

According to the information provided by the Milchindustrie Verband e.V., the following outlines the typical procedure for the isolation of abomasum in Europe.

The slaughter of animals, according to the documentation provided, has to follow the hygiene Council Directive 93/43/EEC which sets out the rules governing the hygiene for foodstuffs and the procedures for verification of compliance with these rules. The principles of hazard analysis and critical control points (HACCP) have to be applied. Council Directive 64/433/EEC (Meat Hygiene Directive) is also applied.

- Stunning of animal (normally calves not older than 6 months)
- Bleeding in hanging position
- Incisions for the frequently applied rodding and bagging<sup>1</sup> and longitudinal cut for evisceration. Rodding is particularly recommend for heavier/older animals.
- Removal of complete intestines
- Separation of the abomasum from paunch.
- Excision of the abomasum by cutting through the bible (third stomach) and by clipping off the abomasum approximately 1 cm distal of the pylorus muscle.

<sup>1</sup> Rodding: closure of the oesophagus to prevent the contamination of the carcass with stomach content; Bagging: enclosure of the rectum into a bag to prevent faecal contamination

- Expel the abomasum content through the gut-end (distal part) by passing the abomasum between the forefinger and thumb. A few passes may be needed to remove all contents. No washing or rinsing of any part of the abomasum at any time.
- The abomasum is hung and all adhering fat and other tissues are removed with a knife avoiding any damage to the abomasum.
- No water is to be used in the removal and transport of the abomasum to the packaging area.
- Usually the material is sold with a certificate of origin and approval of the veterinary authority.

Also, according to the lactose manufacturers, the stomach is removed before removal of the spinal cord.

### 3.3 Production of calf rennet

- Frozen abomasum is milled and extracted with sodium chloride and water
- The crude extract is acidified to activate chymosin
- Inorganic filtration auxiliaries are added for subsequent filtration
- Sodium chloride and preservatives are then added prior to sterile filtration to produce refined activated enzyme extract.
- For powder preparation, the liquid preparation is lyophilised.

### 3.4 Source of Animals

According to the information provided by the lactose manufacturing industry, abomasum is obtained from milk suckling calves or milk-replacer fed calves, normally not more than 6 months old, in order to maintain the chymosin:pepsin ratio within the abomasum of at least 75:25. Animals are sourced from Australia, New Zealand, USA and Europe but not from countries within Europe with a high BSE incidence, i.e. UK, Portugal and Ireland. Ruminant proteins with the exception of milk protein are prohibited in the feed. Composition of milk replacers has been provided (see later).

## **4. Risk Assessment**

The following factors are considered critical for performing this risk assessment:

- a. source animals (including feeds used)
- b. procurement of the abomasum and the subsequent preparation of calf rennet
- c. tissue infectivity of abomasum
- d. manufacturing process and control of lactose

### 4.1 Source Animals

In order to ensure a chymosin:pepsin ratio of at least 75:25, calves not normally more than 6 months of age are used for the preparation of calf rennet. The Association of Manufacturers of natural Animal derived Food Enzymes (AMAFE) indicated that “*Usually calf vells (calf stomachs) are used from animals that are less than 6 months of age. Vells from animals older than 12 months are **not** used.*” In the assessment previously performed by the European Commission’s Scientific Steering Committee (SSC), the SSC was of the opinion that in classifying tissue infectivity in ruminants<sup>2</sup>, an age cut-off of 12 months was recommended. However, in countries specified as at high risk of BSE, the SSC recommended it appropriate to reduce further the age limit for these tissues from 12 months to 6 months. The SSC also considered that in high-risk countries, all tissues from cattle over 30 months are of greater risk and should be classified as specified risk material (SRM). This requirement has already been written into the relevant European legislation.

The latest review, conducted for the United Kingdom Government by an Advisory Committee chaired by Professor (Sir) Gabriel Horn MD, DSc FRS [the Horn Committee] and published on 5 July 2001,

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<sup>2</sup> ruminants: cattle, sheep and goats

addressed the possible route of transmission of BSE including that from dams to calves. The Committee advised amongst other things:

*“The evidence is very strong that the spread of BSE to the point at which it became an epidemic arose through the use of meat and bone meal (MBM) in cattle feed. If transmission from mother to calf, contamination of pastures and/or the use of veterinary preparations played a part in the transmission of BSE, their effects are likely to have been small.”*

In September 2001, the CPMP/CVMP Ad Hoc TSE Expert Group noted this finding at its preparatory meeting for the revision of the TSE guideline. In November 2001, the SSC concurred with Horn’s report. In addition, the SSC was of the following opinion with regard to maternal transmission of BSE in cattle:

*“Maternal transmission is theoretically a possible route of transmission, and has been investigated (Wilesmith et al 1997; Donnelly et al 1997; Curnow and Hau 1996) if only for the reason that it would appear to occur in natural scrapie. Furthermore, in sheep a plausible mechanism has been identified. That is to say from the placenta of infected sheep. However, comparable investigations in cattle have led to different results (no experimental transmission) and thus different conclusions i.e. that if maternal transmission occurs, either the placenta (and other reproductive tissues and milk) are not involved or that the event is infrequent.”*

The calves are fed on milk or milk replacers. Milk is classified as category IV material (no detectable infectivity) according to the TSE guideline. This classification is in concordance with that given by the World Health Organisation and the SSC. The UK Department of Environment, Farming and Rural Affairs (DEFRA, formerly MAFF) has published that milk obtained from clinically affected cattle contains no detectable infectivity as measured by a standard bioassay.

A milk replacer is legally defined in two European legal instruments:

- a. Article 2(j) of Council Directive 79/373/EEC defines milk replacer feeds as compound feedstuffs administered in dry form or after dilution in a given quantity of liquid for feeding young animals as a supplement to, or substitute for, post-colostral milk or for feeding calves intended for slaughter.
- b. Article 5(1) of Commission Regulation 2799/1999 defines “compound feedingstuffs” to contain per 100 kilograms of finished product the following constituents
  - i. not less than 50 kilograms and not more 80 kilograms of skimmed milk powder; and
  - ii. not less than 5 kilograms of non-butter fats and at least 2 kilograms of starch or puffed starch, or
  - iii. not less than 2.5 kilograms of non-butter fats and at least 2 kilograms of starch or puffed starch in cases where 5 kilograms of Lucerne meal or grass meal containing at least 50% of particles not exceeding 300 microns are incorporated per 100 kilograms of skimmed milk powder.

EU legislation defines milk replacer as a compound feedingstuff. Thus the EU Council Directive 95/53/EU on the official control of feedingstuffs also applies to milk replacers. The composition of a milk replacer must be declared on the label according to the EU Council Directive 79/373/EEC on the Marketing of compound feedingstuffs. Official control of the product information on the label is part of national official feed control programme in all EU Member States according to EU Council Directive 95/53/EU. The national official feed control programme is subject to the supervision of the EU Food and Veterinary Office in Dublin.

According to the information provided to the EMEA by the European Feed Manufacturer’s Federation (FEFAC) (original text in German), a variety of feed constituents as indicated below are generally used:

Table 1: Composition of milk replacers

<b>Skimmed milk powder</b>	<b>Magermilchpulver</b>
<b>Powder made whey</b>	<b>Molkenpulver</b>
<b>Wheat gluten (soluble)</b>	<b>Weizenkleber (löslich)</b>
<b>Soya protein isolate</b>	<b>Sojaproteinisolat</b>
<b>Synthetic amino acids</b>	<b>Synthetische Aminosäuren</b>
<b>Fats (refined plant oils or pork fats)</b>	<b>Fette (raffinierte Pflanzenöle, Schweineschmalz)</b>
<b>Starch Products</b>	<b>Stärkeerzeugnisse</b>
<b>Lactose</b>	<b>Lactose</b>
<b>Vitamins and trace elements</b>	<b>Vitamine und Spurenelemente</b>

None of these constituents listed above are considered to carry any measurable TSE risk, however, mindful of the fact that lactose is the subject of this risk assessment.

It should be noted that the legal definition of milk replacers does not exclude the use of bovine non-dairy fat. There is insufficient information at this juncture on the procurement of such fats and potential cross-contamination with high risk tissues is a concern. As such, there is uncertainty in relation to the origin and nature of the fats used in milk replacers<sup>3, 4</sup>.

#### 4.2 Procurement of the abomasum and the preparation of calf rennet

In 1987, DEFRA commenced experiments to determine whether or not infectivity was present in the tissues of clinically BSE affected cattle by inoculation of mice with brain from such cattle. By 1988, there was clear evidence that BSE was transmissible to mice via inoculation of brain tissue. Therefore, infectivity assay has been performed using the standard mouse bioassay. The following gastrointestinal tract tissues from clinically affected cattle contain no detectable infectivity by parenteral inoculation of mice:

Table 2: Gastrointestinal tract tissues with no detectable infectivity

<b>Gastro-intestinal Tract</b>	<b>Abomasum</b>	
	<b>Colon</b>	<b>Distal</b> <b>Proximal</b>
	<b>Oesophagus</b>	
	<b>Omasum</b>	
	<b>Small intestine</b>	<b>Distal</b> <b>Proximal</b>
	<b>Rectum</b>	
	<b>Reticulum</b>	
	<b>Rumen</b>	<b>Oesophageal groove</b> <b>Pillar</b>
	<i>Information obtained from DEFRA website; Wells et al 1998</i>	

Thus, based on the previously published information, abomasum is considered to have no detectable infectivity. In orally challenged cattle, infectivity is detectable by the mouse assay in the distal ileum from 6 months after challenge. This is the only non-nervous tissue that appears to be consistently infected. Eighteen months post-challenge, infectivity was detected in the intestine, at which time the animals were clinically affected, as well as in the brain, spinal cord, dorsal root and the trigeminal ganglia beginning 3 months before clinical onset. Bone marrow was slightly infectious when clinically affected.

<sup>3</sup> Some national legislation has been issued recently prohibiting the use of bovine fat in milk replacers (French decree came into force on 1 January 2000).

<sup>4</sup> The issue of calf rennet and milk replacers is being reviewed by the Scientific Steering Committee ([http://europa.eu.int/comm/food/fs/sc/ssc/out265\\_en.pdf](http://europa.eu.int/comm/food/fs/sc/ssc/out265_en.pdf))

In a recently published SSC opinion<sup>5</sup>, abomasum is included in the list of tissues from a confirmed case of BSE in which no infectivity was detected by bioassay in mice injected both intracerebrally and intraperitoneally.

The procedure for the removal and preparation of the abomasum follows the rules as laid down in Council Directive 93/43/EEC which sets out the requirements for the development of HACCP. Reference is also made to the recommended International Code of Practice, General Principles of Food Hygiene of the Codex Alimentarius. Although recognising that this Directive does not address the risk of TSE, the procedure as set out above for the isolation of the abomasum and the adherence of the principles for good food hygiene practice appear to provide an assurance of the quality and safety (from the cross-contamination viewpoint, e.g. with distal ileum) of the abomasum procured.

The Association of Manufacturers of Natural Animal-Derived Food Enzymes (AMAFE) indicated that most of their members operate under a certified quality management system of ISO 9000, and that the tracing and tracking of calf stomachs is included in the procedures.

European Council Regulation 999/2001/EC and Commission Decision 2000/418/EC govern the use of material presenting risks as regards transmissible spongiform encephalopathies, for example in the food sector. These regulations provide amongst other things that skull, brains and eyes, tonsils, the spinal cord of bovine animals aged over 12 months and the intestines from the duodenum to the rectum of bovine animals of all ages are considered as an SRM. For the UK and Portugal, the following tissues are also considered as SRM:

*“the entire head excluding the tongue but including the brains, eyes, trigeminal ganglia and tonsils, and the thymus, the spleen and spinal cord of bovine animals aged over six months; the vertebral column, including dorsal root, ganglia of bovine animals aged over 30 months”.*

In the information provided by the lactose industry, there is no evidence to suggest abomasum is exposed to or contaminated with the aforementioned SRMs as defined in the European legislation.

The preparation of freeze-dried calf rennet involves a series of extraction, acidification, filtration and lyophilisation steps. None of these steps has been validated for the removal of prions. Nor is there any information currently available to assess the process capacity for removing prions.

However, given the age of source animals, the tissue used to produce calf rennet as well as the removal procedure, as outlined by the lactose manufacturers, the TSE risk associated with the use of this tissue is small.

#### 4.3 Manufacturing Process of Lactose

The starting material of the manufacturing process is whey which is the spent liquid fraction following milk coagulation using calf rennet. The process for lactose production seems, at least on a theoretical basis, to be able to separate the protein (calf rennet) from the carbohydrate fraction (hydrophilic). None of the processing steps have been validated to demonstrate their effectiveness to inactivate or remove a TSE agent. The following information was provided to the EMEA and the BWP in relation to the calculation of the protein content of each intermediate step of the process and in the finished lactose.

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<sup>5</sup> Opinion on TSE infectivity distribution in ruminant tissues (state of knowledge, December 2001) adopted by the Scientific Steering Committee at its meeting of 10-11 January 2002.

Table 3: Lactose production process

Manufacturing step	Carbohydrate component	Protein content	
Cheese production	100 kg milk	20 g liquid rennet	
	100 kg milk	Approximately 200mg rennet protein	Liquid rennet contains 1% rennet protein
	10kg cheese and 90 kg whey		
Whey	90 kg whey	200 mg rennet protein	Worst case: all rennet protein into whey
	5.9 kg dried whey	200 mg rennet protein	Dry matter of whey 6.5%
	1 kg dried whey	34 mg rennet protein	
	Concentrated whey		
SPLIT	50% food grade lactose + 50% delactosed whey powder		
	0.5% protein in dry matter	26% protein in dry matter	Protein Content
	2%	98%	Protein ratio after split
Food grade lactose	1 kg food grade lactose	0.7 mg rennet protein	Rennet protein: 2% of 34 mg
Refining	1 kg pharmaceutical lactose (protein content: 0.05%)  (lactulose protein content: <0.05%)		Protein ratio: 1:10 from food grade lactose to pharmaceutical lactose
Pharmaceutical grade lactose	1 kg pharmaceutical lactose	0.07mg rennet	Rennet protein: 10% of 0.7mg
	1 kg lactulose	<0.07mg rennet protein	

On the basis of the information provided by the lactose manufacturers to date, residual protein present in pharmaceutical grade lactose is very low. Assuming that there is residual infectivity present in the calf rennet, given i) a high dilution, ii) the possible physico-chemical separation capacity of the processing steps, and/or iii) possible preferential partitioning of infectivity into the proteinaceous fraction (assuming prion protein is the surrogate marker for infectivity), the risk of contamination with the BSE agent would appear to be negligible.

## 5. Regulatory Assessment

As indicated previously (see sections 3 and 4), the starting material for the production of pharmaceutical lactose is whey, a by-product of cheese manufacture, the regulation of which falls within the competence of the food sector. It is the view of the BWP that regulatory control over the production of cheese is an important component in its consideration for exempting lactose prepared using calf rennet from the scope of the TSE guideline. The BWP therefore reviewed the existing European legislation and domestic laws currently in operation governing cheese production.

In relation to the regulatory control over cheese production at the European level, Council Directive 92/46/EEC establishes rules for the production and placing on the market of raw milk, heat-treated milk and milk-based products, including cheese. This Directive sets out legal requirements for the quality of the milk. The health status of source animals is defined as indicated below<sup>6</sup>:

<sup>6</sup> Annex A to Council Directive 92/46/EEC of 16 June 1992 laying down the health rules for the production and placing on the market of raw milk, heat-treated milk and milk-based products  
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Raw milk must originate from cows or buffalos belonging to a herd:

- Pursuant to paragraph 1 of Annex A to Directive 64/432/EEC,
  - officially tuberculosis-free;
  - brucellosis-free or officially brucellosis-free
- which do not show any symptoms of infectious diseases communicable to human beings through milk;
- incapable of giving the milk abnormal organoleptic characteristics;
- whose general state of health is not impaired by any visible disorder and which are not suffering from any infection of the genital tract with discharge, enteritis with diarrhoea and fever or a recognisable inflammation of the udder;
- which do not show any udder wound likely to affect the milk;
- which in the case of cows yield at least two litres of milk per day;
- which have not been treated with substances dangerous or likely to be dangerous to human health that are transmissible to milk, unless the milk has complied with an official waiting period laid down in Community provisions or if absent in national provisions.

This Council Directive however does not include specific requirements in relation to the risk of TSE transmission for

- cheese production or
- clotting agents used in the coagulation process.

The BWP has also considered domestic laws that govern cheese production namely those in operation in France, Germany, United Kingdom, Belgium, the Netherlands, Greece and Spain.

In general, domestic laws define that cheese is produced from milk, cream, skimmed milk by means of coagulation with rennet or by other methods such as acidification. Domestic legislation describes requirements for labelling, chemical and biological purity of enzyme preparations used in the coagulation process and allowable additives to the cheese products etc. However, the domestic laws in the member states do not address minimising of the risk of transmitting TSE agents in relation to the calf rennet employed in the clotting process and the control of the procurement of abomasum, similar to the principles in the TSE guideline.

## 6. Conclusions and Recommendations

a. In relation to the risk assessment, the Biotechnology Working Party has considered the following relevant processing parameters or factors:

- age of animals (including their feed);
- tissue used for producing calf rennet;
- the procedure used to procure the abomasum; and
- the lactose processing steps involved (in relation to dilution and partitioning).

Taking all the factors together, the Biotechnology Working Party concludes that the BSE risk in finished pharmaceutical grade lactose is negligible and that pharmaceutical grade lactose can be excluded from the scope of the TSE Note for Guidance<sup>7</sup>, which is currently being revised.

The Biotechnology Working Party however recognises that this conclusion is based on a number of assumptions, particularly in relation to the quality of the milk replacers and the procurement of abomasum.

b. The Biotechnology Working Party also recognises that as this matter has a wider implication beyond the regulation of medicinal products, it merits further scientific discussion by the European Commission's Scientific Steering Committee<sup>8</sup> (SSC). It is therefore recommended that this be referred to the SSC via the European Commission. Furthermore, the BWP considers that the opinion of the SSC<sup>9</sup> would contribute to consolidating the risk evaluation of lactose.

c. Although the risk assessment has been conducted for lactose, the same conclusions could be drawn for other products derived from whey, such as lactulose, galactose, ethanol (which also involves a distillation step).

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<sup>7</sup> Provided the milk is sourced from healthy animals in the same conditions as milk collected for human consumption (this criterion is already included in the present version of the TSE note for guidance).

<sup>8</sup> Commission Decision 97/404/EC establishes the Scientific Steering Committee for providing scientific advice on matters concerning consumer health.

<sup>9</sup> Scientific Steering Committee issued an opinion on the lactose produced using calf rennet and concurred with the risk assessment conducted by CPMP Biotechnology Working Party. Documents can be found on [http://europa.eu.int/comm/food/fs/sc/ssc/out255\\_en.pdf](http://europa.eu.int/comm/food/fs/sc/ssc/out255_en.pdf), [http://europa.eu.int/comm/food/fs/sc/ssc/out265\\_en.pdf](http://europa.eu.int/comm/food/fs/sc/ssc/out265_en.pdf)