SCIENTIFIC STEERING COMMITTEE

REVISED OPINION AND REPORT ON:

THE SAFETY OF TALLOW OBTAINED FROM RUMINANT SLAUGHTER BY-PRODUCTS

ADOPTED BY THE SCIENTIFIC STEERING COMMITTEE AT ITS MEETING OF 28-29 JUNE 2001

REVISED OPINION ON THE SAFETY OF TALLOW OBTAINED FROM RUMINANT SLAUGHTER BY-PRODUCTS

The Scientific Steering Committee (SSC) was asked to address the following question: "*Under which conditions can industrially produced tallow obtained from ruminant slaughter by-products be safely used for food, feed or other applications?"*. Based on the attached report from the TSE/BSE *ad hoc* Group, the SSC adopted the following updated scientific opinion on the safety of tallow which replaces the SSC opinions on tallow and/or rendered fats adopted on 26-27 March 1998 and of 12 January 2001.

1. General principles

a. There is no evidence that tallow derived from ruminant animals would constitute a TSE risk. The SSC considers that possible TSE risks associated with tallow will result from protein impurities that may be present in the end product, because it is expected that TSE agents, if present in the product, would be associated with these impurities.

b. As the safety of most products[1] can currently not (yet) be assessed exclusively on the basis of the infectivity reduction capacity of a production process alone, consideration needs also to be given to safety criteria such as the geographical source of the raw materials, the individual animal source of the by-products, the presence of specified risk materials, the risk of (cross-) contamination, the level of residual impurities and the intended use.

In this opinion the tallow production has been assessed in three parts: (1) sourcing of the animals and the tissues, (2) the fat extraction processes and (3) the possible sterilisation processes.

1. Sourcing of the animals and the tissues.

The SSC considers that the main protection against TSE infection of tallow is safe sourcing of animals and materials based on the recommendations made in the various SSC opinions on product safety, geographical risk, specified risk materials and avoidance of cross-contamination. The risk associated with raw materials from animals fit for human consumption, following *ante- and post mortem* inspection[2] and after careful removal of the specified risk materials is considered to be sufficiently low for them to be used for the production of tallow.

For certain raw materials, inclusion or contamination with infectious materials cannot be excluded, as parts of specified risk materials might accidentally be mixed with the raw materials. This risk is considered to be negligible for discrete fat tissues that are intended for or associated with products destined for human consumption and that are handled as such, for example: fat and certain bones sold on the meat, discrete fat tissues

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1 The SSC is aware of the limitations of the currently available methods and techniques for the assessment of (residual) TSE infectivity in products and tissues These are detailed in the attached report or discussed in other opinions of the SSC, for example the opinion of 13-14 April 2000 on *Oral exposure of humans to the BSE agent: infective dose and species barrier*.

2 If and where appropriate: including a rapid BSE test.

removed during meat cutting or from carcass parts approved for human consumption and entering a dedicated storage, transport and production line.

The risk of inclusion or contamination with infectious materials is considered to be higher for slaughter by-products that initially are not intended for direct human consumption. These are, for example: slaughter by-products such as bones and certain slaughter offals. The risk is expected to be the higher for bones and (mixtures of) slaughter offals (by-products) as compared to discrete adipose tissues (provided the intestine-associated risks are excluded[3].)

2. Fat extraction processes.

   A priori the fat extraction procedure excludes most proteinaceous material and hence probably most infectivity, unless there is selective partitioning of infectivity into the fat phases during extraction. There is little evidence for or against this possibility. Filtration steps in the process may trap some infectivity, if infectivity follows the bulk of proteinaceous material. However it is probable that significant reduction of infectivity occurs during processing by reducing the non fat matter during the filtration and purification stages.

3. Sterilisation processes

   Some TSE strains are not completely inactivated at 133°C during 20 minutes even when fully hydrated. When dehydrated greater survival of infectivity at higher temperatures is found. In general, TSEs are more readily inactivated by wet than dry heat. This may be because hydrated infective agent is more susceptible to inactivation than dehydrated (or otherwise fixed) agent. The hydration state of protein, or specifically TSE infectivity, after the fat extraction procedure is not known, nor is it known how it might change when exposed to wet heat sterilisation at 133°C. If the extraction procedure dehydrates (or otherwise fixes) the infectivity then it is more likely to survive.

c. The SSC points out that the list of specified risk materials is different for small ruminants and cattle. This may imply that in practice most fatty tissues of small ruminants may pose a risk if transformed into feed for certain animal species, if the presence of BSE infectivity cannot be excluded.[3]

d. For all countries[4], sourcing of raw materials *from animals declared fit for human consumption following appropriate ante- and post-mortem inspection* will reduce TSE risk for humans and animals.

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3 See the following opinions of the SSC: (1) *Pre-emptive risk assessment should BSE in small ruminants be found under domestic conditions* adopted on 8-9 February 2001 and (2) *Adipose tissue associated with the digestive tract of cattle, sheep and goats: an appreciation of possible TSE risks* adopted on of 29-29 June 2001

4 Please refer to the SC opinion of 24-25 June 1999 on "Fallen stock", which recommends the exclusion of fallen stock also in GBR I countries, to avoid recycling of unrecognised first cases.

For countries where the presence of BSE is highly unlikely (GBR level I), additional conditions regarding minimal production processes, purification levels or removal of specified risk materials such raw materials will not result in an additional (TSE) risk reduction. For other countries, the additional exclusion of specified risk materials will further significantly reduce the possible risk of residual TSE infectivity being present in tallow.

e. Regardless the source of the material and of the type of material, tallow can be safely used as raw material for the production of tallow derivatives if it is sourced from countries where BSE is highly unlikely or from countries where BSE is unlikely but not excluded (GBR levels I and II) but provided the production process uses the appropriate, validated and scientifically most up-to-date methods in terms of inactivating the BSE agent. Several amongst them have been listed in the scientific opinion of the Scientific Committee on Cosmetology[5] (for cosmetic products) and in the opinions of Committee for Proprietary Medicinal Products (CPMP) of the European Agency for the Evaluation of Medicinal Products (EMEA) [6] (for medicinal products). The SSC recognizes that other methods may exist, but they should be evaluated and acknowledged as regards to their safety on a case by case basis.

For countries where BSE is likely but not confirmed or confirmed at a lower level and for countries where BSE has been confirmed, at a higher level (GBR levels III and IV) the material should in addition be sourced at least according to the recommendations made in the SSC opinion of 25 June 1999 on "Fallen stock"

f. The SSC considers that separate storage, transport and use of industrial tallow is needed to avoid possible mixed uses or contamination with food or feed-grade tallow. Also, if the intended end use cannot be verified and controlled to exclude any human or animal consumption or use, then the conditions outlined for food-standard tallow should apply also for tallow for industrial or technical use.

2. Specific recommendations

Remark: For countries with a geographical BSE risk level IV additional recommendations that have been made on a case-by-case basis in various SSC opinions, remain valid.

a. Tallow obtained from melting discrete adipose tissues sourced from animals fit for human consumption.

- The SSC considers that tallow derived from discrete fat tissues that were intended for or associated with carcass parts intended for human consumption and that were handled as such, is as safe as meat and can therefore be used for all applications. Such raw materials are, for example:

5 Of 24 June 1997

6 Of 16 April 1996 and of 17 December 1997.

fat and certain bones sold on the meat, discrete fat tissues removed during meat cutting or from carcass parts approved for human consumption. Dedicated storage, transport and production lines for these raw materials will further minimise the risk of contamination with TSE agent.

- Other discrete adipose tissues not intended for direct human consumption as such can also safely be used for all applications, with the exception of certain digestive tract-associated discrete adipose tissues as described in the SSC opinion of 28-29 June 2001 [7], and provided the fat collection procedure is able to prevent contamination with potentially BSE infected materials and provided dedicated manufacturing lines are used.
- The SSC considers that the current practice adhered to by most European tallow industries to purify the tallow derived from these source materials to a maximum level of insoluble impurities of 0.02%, will reduce the residual risk, if any, to a negligible level.

Notes:

- For uses which imply a contact with skin or open wounds (e.g., as a constituent of ointments) the use of a food-standard tallow is recommendable.
- The Scientific Steering Committee has examined the existing licensed uses in the E.U. of tallow and is not aware of any licensed use of tallow as an injectable product or for possible parenteral pharmaceutical applications. If it were to be used as such, the SSC would need to issue an additional opinion.

b. Tallow obtained from rendering other tissues sourced from animals fit for human consumption.

These tissues can be safely used for the production of tallow in feed (but excluding calf feed[8]) and petfood, industrial/technical tallow or tallow for tallow derivatives, under the following conditions.

- The produced tallow should be purified to levels below 0.15% insoluble impurities. Because of the higher risk of inclusion or contamination with infectious materials pointed out in section 1, a sterilisation process should in addition be applied.

  So far, only the "133°C/20'/3 bars" process, applied as a pre-sterilisation to a mixture of fresh tissues has been validated in terms of TSE infectivity clearance. Tallow derived (pressed and filtered) from such pre-sterilised material can be considered to pose no risk, although it is recognised that the end-product is likely to be of a minor quality.

  No validated and operationally exploitable results are available on the clearance achieved by other processes, for example post-sterilisation of fats (e.g., 133°/20'/3 bars; the current deodorization processes, …). Although it is recognised that post-sterilisation processes have some clearance capacity,

7 SSC Opinion on *Adipose tissue associated with the digestive tract of cattle, sheep and goats: an appreciation of possible TSE risks*. Adopted on 28-29 June 2001.

8 Tallow in calf feed (including in milk replacers) is excluded here because of the high amounts of such tallow that may be consumed by the young animals and because the susceptibility to infection of young animals may be higher.

they should first be validated before they can be accepted as equivalent to pre-sterilisation.

Tallow derived from (a mixture) of tissues other then discrete adipose tissues and not submitted to a pre-sterilisation should therefore, pending the validation of the TSE infectivity clearance capacity of post-sterilisation processes, only be used for certain industrial applications and for the production of tallow-derivatives.

The SSC recommends that research is done on the TSE clearance capacity of processes, for example of post-sterilisation.

C. Tallow obtained from rendering other tissues sourced from animals possibly not fit for human consumption.

Tissues can be safely used for the production of for industrial / technical tallow if the animals from which the raw material is derived are not fit for human consumption, the recommendations in the SSC opinion of 25 June 1999 on "Fallen stock"[9] should be complied with. In addition, the specified risk materials should be removed (if applicable), the raw material should be submitted to minimal conditions of 133°C at 3 bar during 20 minutes or equivalent process as specified in that opinion and it should be purified to max. 0.15% insoluble impurities.

9 Complete title: *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.*

SUMMARY OVERVIEW OF SAFETY CRITERIA FOR TALLOW OBTAINED FROM RUMINANT SLAUGHTER BY-PRODUCTS

TALLOW FROM MELTING DISCRETE ADIPOSE TISSUES FROM ANIMALS FIT FOR HUMAN CONSUMPTION		TALLOW FROM RENDERING OTHER TISSUES FROM ANIMALS FIT FOR HUMAN CONSUMPTION		TALLOW FROM CERTAIN TISSUES FROM ANIMALS NOT FIT FOR HUMAN CONSUMPTION
GBR LEVEL I	***GBR LEVELS II, III, AND IV***	***GBR LEVEL I***	***GBR LEVELS II, III AND IV***	
	If specified risk materials removed* and if either: - discrete fat tissues that were intended for or associated with animal products intended for human consumption and that were handled as such (see Section 2.a. of the text) or: - Other discrete adipose tissues not intended for direct human consumption as such, with the exception of certain digestive tract-associated discrete adipose tissues as described in the SSC opinion of 28-29 June 2001 [10], and *provided the fat collection procedure is able to prevent contamination with potentially BSE infected materials and provided dedicated manufacturing lines are used.* Note: Filtration to 0.02% insoluble impurities will reduce any possible residual risk to a negligible level. **Tallow for all uses (e.g., food, pet food, feed, milk replacers, industrial uses, tallow derivatives, cosmetics, …)**		If specified risk materials removed *: - Filtration to maximum 0.15% insoluble impurities and pre-sterilisation at 133°C/20'/3bars": **Tallow for restricted uses (Including feed but excluding, for example: food and calve feed.**	If specified risk materials removed and provided that the "Fallen stock" opinion of 25 June 1999 is respected: - Filtration to max. 0.15% insoluble impurities. **Tallow for restricted uses such as industrial ones and tallow derivatives**.

(*) It is noted that, with respect to the production of tallow and with regard to the risk of intra-species recycling of TSEs, the list of specified risk materials is different for small ruminants and cattle. This may imply that in practice most fatty tissues of small ruminants may pose a risk if transformed into feed, if the presence of TSE infectivity cannot be excluded. (See SSC opinion of 28-29 June 2001 on *Adipose tissue associated with the digestive tract of cattle, sheep and goats: an appreciation of possible TSE risks*)

10 SSC Opinion on Adipose tissue associated with the digestive tract of cattle, sheep and goats: an appreciation of possible TSE risks. Adopted on 28-29 June 2001.

REVISED REPORT ON THE SAFETY OF TALLOW OBTAINED FROM RUMINANT SLAUGHTER BY-PRODUCTS

I. ON THE PRODUCTION OF TALLOW

In order to express an opinion on the safety of tallow it is important to keep into account a number of aspects of the fat production methodologies and conditions.

I.1 DEFINITIONS

Definitions used in the context of the present report:

"Tallow" covers a very broad spectrum of products. The term 'tallow' is a name applied to a whole range of fats, produced by many different techniques dependant on the tissues used as raw materials and the end use of the product. The spectrum ranges from human edible products derived from materials destined for human consumption (meat products) to fuel products manufactured from SRM for example. Edible type tallows would never start at high levels of impurity prior to settling and, according to EFPRA (2001d) are usually produced with final total impurity levels of <0.02%.

Within the context of this report it is defined as fats obtained by extraction and separating by melting or by processing such as rendering and extracted by pressing, drawing off or centrifugation down from certain[11] ruminant (by-)products such as certain discrete adipose tissue masses[12], trimmings, bones, certain slaughter offals, etc. It can be further purified by centrifugation, filtration or treatment with phosphoric acid or also by thermal refining.

Discrete adipose tissue is internal and external body fat removed during the slaughter and cutting process, in particular fresh fat from the heart, caul, kidneys and mesentery of bovine animals, and fat from cutting rooms. Discrete adipose tissue is not bone fat, nor is it rendered fat from multiple source tissues from multiple species whether derived from animals fit for human consumption or from other sources.

Fit for human consumption refers to material from animals that passed both *ante-* and *post mortem* inspection by an competent veterinary authority and that are certified and identifiable as fit for human consumption on the basis of the existing national and EU legislation. (Under certain conditions, this may imply that materials are considered fit for human after a *post mortem* inspection following an unsatisfactory *ante mortem*.)

A purification process consists of adequate filtering and/or centrifugation and/or coagulation and results in levels of remaining total insoluble impurities

11 The term "animal by-products" covers a larger scope of materials then the ones possibly used for tallow and may also include ova, embryos, semen, digestive tract content, manure, etc.

12 This term is used to describe those reserves of fat which can be removed readily during slaughter in the abattoir or at meat-cutting plants (e.g., fat trimmings). It does not refer to lipid extracted from mechanically recovered meat or from many other tissues, or at a later stage in the production process. It is signalled that the safety of intestine-associated discrete adipose tissues is addressed in the SSC opinion of 28-29 June 2001 on Adipose tissue associated with the digestive tract of cattle, sheep and goats: an appreciation of possible TSE risks.

below a given percentage in weight or residual nitrogen below a given % (determined by the Kjeldahl method[13]) or residual peptides or polypeptides with a molecular weight below a given value.

Specified risk materials refer to all tissues listed in the opinions of the Scientific Steering Committee (SSC) adopted on 9 December 1997 of 28 November 2000, of 11-12 January 2001 and of 28-29 June 2001 (on Adipose tissue associated with the digestive tract of cattle, sheep and goats).

Industrial use means that the end product is neither for direct nor for indirect human or animal consumption or use, including as a cosmetic nor as a pharmaceutical product.

I.2. ON THE PRODUCTION OF TALLOW

A wide range of systems exist for the production of tallow. They can broadly be grouped as follows:

a. Melting of fatty tissues. The raw materials used are fresh slaughter-fats (discrete adipose tissues). For melting slaughter-fats fit for human consumption dedicated processing is common and widely used. The ruminant fatty tissues are gently heat-treated to maintain the high quality of tallow and it is purified, mainly by separation and filtration in order to reduce any residual insoluble impurities.

Note: According to EFPRA (2001a), when fatty tissues are melted for food and feed applications, the raw materials used are fresh slaughter-fats (discrete adipose tissues) fit for human consumption. Slaughter-fats are considered to be meat. For melting slaughter-fats fit for human consumption dedicated processing is common and widely used. *Premier Jus*, the highest quality of tallow is produced with the fat melting system for food application, for example in soups, sauces, margarine and frying and for in Calf Milk Replacers industry. The ruminant fatty tissues are gently heat-treated to maintain the high quality of tallow and it is purified, mainly by separation and filtration in order to reduce any residual insoluble impurities to no more than the commercial values of 0.02% insoluble impurities for edible fat for food and calf milk replacers.

b. Rendering (a mixture of) other tissues such as bones, trimmings, meat rests and slaughter offals into tallow. The rendering is done at a temperatures well below 100°C. The produced tallow is purified and a sterilisation process may be applied.

Note: According to EFPRA (2001a), when rendering a mixture of tissues into tallow is performed by renderers for feed, petfood and technical applications. The produced tallow is purified to levels below 0.15% insoluble impurities. The rendering activity is divided into processing (a mixture of) animal by-products fit but not destined for human consumption ***or*** high risk materials. In the rendering industry a sterilisation process is used to produce meat and bone meal and animal fat. Dedicated production is also occurring in the rendering industry e.g. materials of porcine and avian origin.

[13] Regarding the nitrogen levels, not published laboratory analyses (Piva, 1997) show nitrogen levels of 0.01 - 0.02 % corresponding to impurity levels of 0.15%.

c. Fats pressed after rendering at "133°C/20'/3 bars" of (a mixture of) tissues such as bones, trimmings, meat rests and slaughter offals into tallow.

The various tallow qualities that are produced are schematically summarised in Table 1 (EFPRA, 2001b). In annex, a more detailed description of the processing systems of animal by-products as applied by the members of the European Fat Processors and Renderers Association is provided as an example. (See: EFPRA, 2001a; EFPRA, 2001b). Note that this annex does not cover fats from other species (e.g., poultry, pigs, …)

SCHEMATIC SURVEY OF TALLOW QUALITIES

TALLOW TYPE	PREMIER JUS	BEEF TALLOW	CALF FAT	TALLOW
Usual production process	Wet melting	Dry rendering	Wet/dry melting	Wet/dry rendering
Quality characteristics				
FFA (%)	Max 0,5	Max 2	Max 0,75	Max 15
POV (meq/kg)	Max 2	Max 3	Max 2	Max 10
IV (g I_2/kg)	37-42	40-50	47-54	
Slip melting point (°C)	Min 43	Min 40	Min 35	
Color	Yellow	Yellow/brown	White/yellow-brown	Brown
Gardner color	max 3	max 8	max 2 / 7	max 9
Insoluble impurities	Max 0.02	0.15	0.02	0.15
Odor / flavor	Fresh, slightly butter-like	Fresh, fried greaves-like	Fresh typical	Typical
Fatty acid composition in %				
Lauric acid C-12:0			2	
Myristic acid C-14:0	4	3	8	
Palmitic acid C-16:0	26	26	25	
Stearic acid C-18:0	22	24	16	
Oleic acid C-18:1	36	36	34	
Linoleic acid C-18:2	3	2	6	

I.3. IMPURITIES LEVELS IN FATS.

When it is sold, tallow will typically be decanted from large holding tanks in which it has settled. The level of insoluble solids in such tallow can be as high as 0.5%. Although the proportion of protein in the insoluble solids is not known with any degree of accuracy, it is considered to be potentially high According to experts of the industry, who met on 6 June 2000 in a Working Group meeting, up to 85% of the impurities may be proteinaceous impurities[14]. For a total level

[14] Analyses reported on by EFPRA (2001a) and carried out in 1998 show much lower values. Crude protein levels in the impurities, determined for 6 samples, ranged from 5 to 16 %. The crude protein is calculated with the assumption that all nitrogen is present as protein molecules (which is not certain, because of the possible presence of NPN -Non Protein Nitrogen-) with 16%N (factor 6.25).

of impurities of 0.15%, this would correspond with 0.127% of proteinaceous impurities in the finished tallow. The value of 85% is a estimate, not based on any analytical results[15]. In order to set an acceptable upper limit for impurities a maximum level of total insoluble impurities in weight and/or a maximum level of nitrogen content (determined by the Kjeldahl method[16]) and, if possible, an upper limit of the size of residual peptides or polypeptides may be proposed[17].

The separation of fat from proteins and from all the other impurities can be realised through the following steps:

- ***centrifugation:*** highly efficient process, but it doesn't always guarantee the complete elimination of the residues; efficacy will depend upon the achieved clearances, e.g., particle sizes.
- ***filtration***: some methodologies of microfiltration on ceramics, on filtering beds (earth filtering, clays, bentonite, montmorillonite, philipsite) (20-25µ) with appropriate filtration co-adjuvants (celite) are available. Aid free bag filters are normally used in the fat melting industry to produce edible tallow (premier jus) with commercial values of residual insoluble impurities of max 0.02%.

  These methodologies are effective and lead to very low residues levels. Afterwards it is necessary to eliminate the filtration supports (filtering beds and co-adjuvants) which cause costs and environmental problems.

- ***treatment with phosphoric acid*** homogenate with a phosphoric acid water solution and afterwards centrifugation. Residual nitrogen levels are less than 0.01%.

All these processes are usually realised at a temperature around or over 80°C. The quality of the filtration (in terms of remaining level of impurities) depends upon the quality of the raw tallow before filtration (for example, from which type of tissues it was derived) and depends also upon the type of production process used (for example, mechanical pressure combined with heat treatment or tallow obtained after a heat treatment).

Note on filtration and centrifugation:

In 1998 higher levels of impurities were common, prior to the current legislation requiring maximum impurity levels of 0.15%. The calculated level of protein is theoretical and assumes all nitrogen is present as protein which will probably not be the case.

15 According to EFPRA (2001d) referring to crude inedible tallow held in the large holding tanks after pressure cooking of the lowest grades of 'high risk' raw material. This is before any settling of the impurities which, at that stage, could be expected to have suspended particles of MBM making up perhaps as much as 85% of the impurities - itself with a crude protein content of about 50%. Settling and then decanting the tallow from these holding tanks will reduce this massively as will the other purification techniques (such as centrifugation and filtering) employed after settling.

16 Regarding the nitrogen levels, not published laboratory analyses (Piva, 1997) show nitrogen levels of 0.01 - 0.02 % corresponding to impurity levels of 0.15%. The above analyses reported on by EFPRA (2001b, 2001d) show values between 0.001 and 0.004% corresponding to impurity levels of 0.13-0.27.

17 Research is ongoing on the composition of insoluble impurities. As soon as the results are available they will be included into this report.

The clearance, e.g., particle sizes, achieved by the filtration and centrifugation steps will depend upon the conditions used and should be validated for TSE removal. On the latter, no specific data are available.

I.4. DEODORIZATION PROCESS (CONTINUOUS) .

According to EFPRA (2001b), fat is pre-treated to de-aerate and de-gum the fat and then heated in a deodoriser to 240 degrees C under vacuum. In the deodoriser vessel, steam is "sparged" (released under pressure) through the fat as it moves (falls) through 3 separate levels. The process is dynamic, i.e. there is intimate mixing activity during this period. The total residence time in the deodoriser is 1 hour. The volatile matter removed by the steam is condensed separately to produce a Fatty Acid Distillate (not used for human food). The deodorised fat is stored at about 65 degrees C under a nitrogen atmosphere prior to use. According to D.Taylor (pers.comm, 18 may 2001), this process must be regarded as enhancing the safety with regard to the BSE agent. Firm evidence with regard to this hypothesis is, however, not available.

II. SOME CONSIDERATIONS REGARDING THE SAFETY OF TALLOW.

II.1. CONSIDERATIONS EXPRESSED BY THE SSC IN ITS OPINION OF MARCH 1998 AND COMMENTS IN THE LIGHT OF PRESENT KNOWLEDGE (JUNE 2001)

a. Wilesmith *et al* (1988) indicated that the geographical variation in the incidence of BSE in the UK is not consistent with the distribution and use of tallow in cattle feed.

Comments:

BSE control measures in countries such as the UK, Switzerland and Ireland[18] with a significant BSE epidemic were mainly targeted towards the exclusion from the ruminant feed of ruminant meat-and-bone meal. The subsequent reduction of the incidence and the varying rate of decrease which followed the perceived level of enforcement of the feed bans (including the risk of cross-contamination), show that ruminant meat-and-bone meal was/is indeed the main common source of the epidemic.

Also, the known patterns of commercial distribution of tallow failed to show an association with the distribution of BSE in the UK but did show an association with the distribution of Meat-and-bone meal, and it was concluded that (Wilesmith,1988 *et al*). Tallow was not responsible for the majority of cases in the epidemic because, unlike MBM, it went to a rather small number of blenders all of which distributed nationwide. The geographical variation could therefore not be explained from a tallow source.

However, the epidemiological evidence for a common main source of infection (meat-and-bone meal in the case of BSE in bovines) does not necessarily imply that other products are infectivity-free:

Although there is little doubt about the Wilesmith *et al* (1988) epidemiological conclusion that MBM was driving the BSE epidemic in the UK, the question must be raised whether the limited number of cases (a few hundreds of animals) which was enough to find the main infectious source, also allows to explicitly exclude tallow from the list of infectious materials. This number may be sufficient to identify the major cause of transmission, but more information than currently available in

[18] S.Ward (pers.comm., 2001, on controlling of the epidemic in Ireland.

the publication would need to exclude definitely tallow as a (less efficient) source of infection.

It should also be verified (1) whether Wilesmith's data could be consistent with the idea that MBM was responsible for the more efficient, but more geographically restricted transmission of BSE while tallow would be responsible for the countrywide distribution, but with a lower efficiency; (2) whether the use of milk replacers (with limited infectivity) could be an explanation of the "maternal risk enhancement" factor needed in mathematical modelling of the BSE epizootic; and (3) whether the second BARB case could be explained by the exposure to low infectious tallow, e.g. milk replacers.

No results from other epidemiological studies on the source of the epidemic exploitable in the context of the present report seem to be available and no further studies seem to be planned.

b. Normal industrial tallow production processes - even the ones using the lowest time/temperature combinations - and corresponding research have shown to result in a product which is free from detectable TSE infectivity (injection into the brain of mice), even if the source material was highly infective. (See also Taylor et al., 1995; Taylor et al., 1997; MAFF et al., 1997). The explanation of this result was considered to lie in the fact that "*because of the proteinaceous nature of the TSE agents they would tend to remain with the cellular residues of meat and bone meal during extraction process, rather than be extracted with the lipids of tallow*." (Millson et al, 1976 in Wilesmith *et al*, 1988; WHO, 1995).

   It was signalled that the experiments, because of their scope, size and duration, have not been repeated in other laboratories. The experiments were simulations carried out at a pilot scale and the extrapolation of the results (scaling up) into the real operational industrial conditions may therefore not be convincing. No test results, confirming the hypothesis that tallow is 100% safe, are available from operational rendering plants. On the other hand, the above pilot-scale experiments were not simply laboratory approximations of rendering processes, but were carried out in actual (although pilot-scale) rendering equipment. In collaboration with the industry it was determined how the pilot-scale equipment could be operated to provide a realistic representation of what occurs in full-scale rendering. Also, most validation studies done on the safety of a wide variety of biopharmaceutical products with respect to TSE agents, are almost always carried out on scaled down versions of the manufacturing processes that are spiked with TSE agents.

c. The mice infection tests which are in most cases carried out to detect TSE infection, may not be as sensitive as a system of homologous detection between animals of the same species (e.g., from bovine to bovine). The sensitivity of the mouse bioassay for assaying TSE agents from cattle or sheep will be compromised by the species barrier. Cattle-to-cattle transmission of BSE by intracerebral route is estimated to be about 1.000-fold more effective than cattle-to-mouse transmission by the same route. Superficially, this might appear to compromise any conclusions drawn from the rendering studies with regard to the safety of tallow. However, in assessing risks related to the consumption of tallow, the much greater

efficiency of establishing infection in mice by the intracerebral (compared with the oral) route of infection must be considered. For example, the difference in efficiency between these two routes for scrapie in mice is 100.000-fold (Kimberlin, 1996). Also, it has been calculated that the transmission of BSE to mice by the oral route is 200.000-fold less efficient than by intracerebral challenge (Kimberlin, 1994). These data seem to indicate that the negative results from the mouse bioassays of tallow in BSE and scrapie-spiked rendering studies can be viewed with some confidence with regard to any risk from infection by its consumption. On the other hand, certain strains of natural scrapie are transmitted as easily by the peripheral as by the central route. For example, the infection of mink by the BSE agent is almost equally effective by the oral route as by the mixed parenteral/intracerebral route (Robinson *et al*, 1994).

Comments:

The Working Group is aware the limitations of the currently available methods and techniques for the assessment of (residual) TSE infectivity in products and tissues which previously have been signalled by the SSC. The main limitations are:

- The number of infectivity inactivation studies and experiments is limited; as a result, a TSE on which more research has been done (e.g., scrapie) often serves as model for another (e.g., BSE) which may however be different for certain characteristics (e.g. sensitivity to heat treatment);
- It is not valid to extrapolate ic/oral comparison applied to a specific TSE model within a single species to interspecies transmissions. BSE can be orally transmitted to mice with 300mg infected cattle brain (Taylor *et al*, 2001) See also: E.C., 2000a.

  Also, the quoted efficiency of the i/c over the oral route is correct in the rodent models then available but should be worked out again from data now available from cattle studies.
- The fact that the disease was not transmitted does not necessarily mean that there is no infectious agent; it indicates that the level of infectivity is lower than the detection threshold of the method used, with two important limiting factors[19]:

  i) the quantity of the inoculable sample, in particular since it is an intracerebral inoculation,

  ii) the species barrier, since the inoculation is performed on a different species from the host species, such as the mouse. It is generally admitted that the strength of the "cattle-mouse" species barrier is approximately 1000, i.e. under most current experimental systems it is not possible to measure a level of infection below 10^3 lethal doses 50%/g of inoculated tissue (this estimate does not take into account the limitations of sampling).

  Furthermore, the experiments on the basis of which infectivity is evaluated do not take into account the possible effect of repeated doses.

  The safety of most products can thus currently not (yet) be assessed exclusively on the basis of the likely infectivity reduction or -elimination capacity of a production process alone, but consideration needs also to be given to safe geographical sourcing of the raw materials, safe individual animal sourcing,

[19] See also: Opinion forwarded on 18 September to the Director-General of the French Food Safety Agency by the French Interministerial Committee on Transmissible Subacute Spongiform Encephalopathies.

tissue infectivity distribution, avoidance of (cross-) contamination, the possible presence of impurities, etc. (see also the overview table in annex to this opinion).

Until further studies are done, tallow itself should therefore not be considered by definition as 'risk-free':

d. Depending upon the strain and the host, it is possible to have differences in incubation times, pathogenesis, distribution of the lesions in the central nervous system, amount of infective PrP^{Res} and its location inside the central nervous system, etc. (e.g., Lasmézas *et al*., 1996; Kimberlin *et al*., 1983; Dickinson *et al*, 1989; Bruce *et al*, 1994). There are also known differences between some strains of scrapie agent in terms of their thermo-stability (Dickinson and Taylor, 1978; Kimberlin *et al*, 1983).

Comments: Available evidence indicates that BSE and derived strains are amongst the most thermo-stable. (Schreuder, 1997, Taylor, 1999).

II.2. CONSIDERATIONS IN THE LIGHT OF RECENT INFORMATION.

a. The question has been raised whether the fats extracted in rendering plants from the remainder of the material by pressing using the heat/pressure procedure (133°C/20'/3bar) and purification so that impurities insoluble in petroleum ether make up no more than 0.15% of the original material, constitute a greater, comparable or lower risk of contamination with TSE agents than animal meal, if agents have entered the process.

   A hypothesis is that TSE agents (studied using the example of the scrapie agent) do not partition from the fat phase, but in certain cases may even have a certain affinity with the fat phase. According to Groschup (2000), this may be illustrated by an experiment among others in which brain homogenates from hamsters suffering from scrapie, from which the large cell debris have been separated by conventional centrifuging, were then separated by gradient ultracentrifugation, in which the fractions partition by specific gravity. When these fractions were analysed by means of bioassay, considerable concentrations of agents were found in both the pellet and the meniscus (floating fat phase) of the gradient. The experiment shows that the agents can be associated with the fat phase and cannot be separated with sufficient certainty using methods based on differences in specific gravity or the size of their aggregates.

b. A recent study (Appel *et al*, 2001) reported on the effectiveness of inactivating prion rods by autoclaving when they are suspended in different concentrations of lipid. The study reports that the autoclaving process became less effective as the concentration of lipid was increased.

The TSE/BSE *ad hoc* Group discussed the above two issues as follows:

a. <u>On the partitioning of the abnormal prion protein with tallow</u>

There is evidence that TSE agent does not preferentially migrate into the tallow fraction during rendering but tends to remain in the meat and bone meal fraction (Taylor *et al*, 1995; 1997). An experiment is currently ongoing

(J.Wilesmith, personal communication, 2001) of looking at the partitioning of the agent in a small scale rendering system. The experiment is still in its early days and there will not be any results for some time.

Taylor *et al* (1995; 1997) tested tallow production processes with the highest and lowest capacity to inactivate the BSE agent. Normal abattoir waste spiked with infected bovine brain at a level of 10%.

The process that was perceived to have the best capacity for inactivating the BSE agent was one in which the raw materials were autoclaved at 145^{o}C for 18 minutes. This produced *meat-and-bone meal* with no detectable infectivity. 10% suspensions of the unfiltered and filtered *tallow* were each injected into 24 mice. None of these 48 mice developed any signs of neurological disease, and 17 of these survived through to 904 days after injection, when the experiment was terminated.

The worst-case scenario involved the Carver-Greenfield process.. This process produced *meat-and-bone meal* containing almost as much infectivity as there was in the unprocessed raw materials. Again, 10% suspensions of unfiltered and filtered *tallow* from this process were each injected into 24 mice. None of these 48 mice developed any signs of neurological disease, and 24 of these survived through to 904 days after injection, which is when the experiment was terminated. Tallow produced under worst-case conditions had thus no detectable infectivity but the meat-and-bone meal from the same process and also administered to mice contained almost as much infectivity as the untreated raw materials.

On the basis of Taylor *et al* (1995, 1997), EFPRA (2001a) estimates that infectivity appears to be less likely to be present in tallow, compared with protein (meat and bone meal), by a factor of around 10^{7}. However, the TSE/BSE *ad hoc* Group considers that the data obtained from these studies are too little to absolutely exclude any risk of BSE infectivity being in tallow. Given the scarcity of data and given the limitations inherent to biological experiments when extrapolating their results and the estimate should be used with a broad enough safety margin**.**

b. <u>On the inactivation of TSE agents</u>

<u>In a fatty environment (post-sterilisation):</u>

Some TSE strains are not completely inactivated at 133°C even when fully hydrated. When dehydrated greater survival of infectivity at higher temperatures is found. In general, TSEs are more readily inactivated by wet than dry heat. This may be because hydrated infective agent is more susceptible to inactivation than dehydrated (or otherwise fixed) agent. The hydration state of protein, or specifically TSE infectivity, after the fat extraction procedure is not known, nor is it known how it might change when exposed to wet heat sterilisation at 133°C. If the extraction procedure dehydrates (or otherwise fixes) the infectivity then it is more likely to survive.

Appel *et al* (2001) report on the effectiveness of inactivating prion rods by autoclaving when they are suspended in different concentrations of lipid[20]. The study reports that the autoclaving process becomes less effective as the concentration of lipid was increased[21] and under lipid-rich conditions, scrapie agent might survive autoclaving at temperatures of up to 170^{o}C for 20 minutes. The presence of fat reduced thus the velocity of the destruction of prion rods (prion rods heat treated in water as compared to prion rods heat treated in fat/water mixtures)[22].

The practical conclusions regarding the BSE-related safety of tallow and tallow-derived products drawn from this study should be assessed in the following context:

- The Appel *et al* study used aggregated protein in the form of prion rods to spike the material and does not deal with the natural distribution of infectivity between the different phases, but found a stabilisation effect if infectivity was introduced into the fat phase in a model study. The conclusions are arrived at by using purified prion rods (analogous to SAF), and are based upon biochemical observations rather than bioasssays. The paper only measured changes in biochemical properties of PrP. There were no measures of infectivity. PrP was measured using a monoclonal antibody. It is not possible to distinguish whether the epitope is specifically altered or that PrP as a whole has been affected.
- It should be noted that some separation of infectivity from PrP^{Sc} is possible under certain conditions, so using PrP^{Sc} as a surrogate for infectivity in assessing separation or inactivation steps is invalid.
- Appel *et al* (2001) further acknowledges that PrP^{res} in the brain has a more diffuse structure (than SAF). In the hamster scrapie model used by Appel *et al* (2001), amyloid plaques are not found in the brain[23]. The

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20 Inactivation was measured by Western Blot; for detection of infectivity, animal assays would be needed.

21 From the Appel *et al* (2001) research it appears that with increasing temperatures above 130°C in pure lipid environment, PrP monomers disappear and dimers (60KD), tetramers (120KD) and octomers (240KD) are formed. The question needs to be addressed as to whether these polymers are still infectious and whether they can be absorbed via the enterocytes and/or taken up by Peyer patches.

22 This is also what occurs with conventional micro-organisms (e.g., bacterial spores) (Sendhaj, 1997; Sendhaj and Loncin, 1977).

23 The precise nature of the unconventional agents that cause transmissible degenerative encephalopathies (TDEs) such as bovine spongiform encephalopathy (BSE) and Creutzfeldt-Jakob disease (CJD) in humans has still to be established. Nevertheless, it is widely agreed that a physically (but not chemically) altered form of the normal PrP protein in the host is at least a component of the infectious moiety. The normal form of the PrP protein is often referred to as PrP^{sen} in recognition of its relative sensitivity to degradation by proteolytic enzymes. In contrast, the disease-specific form is described as PrP^{res} because of its relative resistance to degradation by proteolytic enzymes. Because it is relatively resistant to proteolytic digestion, PrP^{res} forms pathological accumulations in the brains of affected individuals to varying degrees. The presence of PrP^{res} in brain-tissue can be confirmed by subjecting the brain-tissue to a detergent process and then examining it under the electron microscope. This reveals fibrillar structures composed of PrP^{res} that are referred to as scrapie-associated fibrils (SAF) and are diagnostic for TSEs. However, it is important to note that SAF are not always actually present as such in infected brain-tissue but can result from the detergent process used to prepare samples for electron microscopy.

aggregated form of PrP^{res} used in these studies represents an artificially high degree of challenge compared with the infected brain-tissue that would be used in studies of this nature.

- The study covers an experimental situation in which prions are deliberately added to liquid fat without any separation steps. In the processing industry of animal by-products a rendering process is effective with separation and purification technologies.

Accordingly it is possibly inappropriate to use Appel *et al* (2001) for the assessment of the safety of tallow produced as described in the present report. But in any case, it must be expected that, in terms of TSE infectivity reduction, the application of an "133°C/20'/3bars" autoclaving process on materials that are mainly composed of fat masses will be less effective than if applied to a mixture of fresh animal by-products.

If fat is submitted to a sterilisation process, then a possible further TSE infectivity reduction can only be expected if the sterilisation is wet and and as effective as possible in terms of its potential of TSE infectivity reduction.

EFPRA (2001b), anticipating that infectious prions are inactivated under similar conditions as bacterial spores, deduced a number of the conditions under which a sterilisation at 133 °C, 20 min at 3 bars steam pressure should optimally be performed. They are attached as Annex 3.

Overall there is insufficient data to evaluate the effectiveness of steam sterilisation as described in annex 3. Little or no data are available permitting to exactly define the temperature / time / pressure conditions of a possible optimal sterilisation process applicable should TSE infectivity be present in [the residual impurities in] fats.

It is appreciated that there may be other methods (such as a cascade system) that might effectively steam sterilise tallow but these would need to be considered on a case-by-case basis.

Conclusion:

Overall there is insufficient data to evaluate the effectiveness of steam sterilisation but one can [should] expect that it will enhance safety if the process guarantees (see Annex 3):

> Sufficient water to assure a water activity close to 1[24] in the fat phase during sterilisation. Processed fats contain little moisture and during sterilisation water must be added to ensure proper sterilisation. The amount of water needed increases with the temperature.

However it is noted that Somerville *et al* (1988), following research in rodents, concluded that SAF are not detergent-induced artefacts but most probably assemble *in vivo***.** In contrast, discrete amyloid plaques can be found in some individuals, and these contain SAF.

24 A water activity close to 1 could mean a water activity between 0,9 and 1, where the rate of inactivation of the scrapie agent is decreasing by a factor of 3 with respect to the inactivation at water activity 1. This is about the error (for 95% confidence) of bioassay based titration data used for the evaluation of inactivation experiments (cf. Havekost, U.: Scrapie-Inaktivierung in der Fleischmehlindustrie, Diplomarbeit, Universität Gesamthochschule Paderborn 1999)

> Proper agitation and dispersion of water to ensure good contact between the fat and the water/steam.
> Heating from app. 100°C to 133°C by live steam injection into the fat to obtain a fine dispersion of water in the fatty phase.
> homogeneous achievement of the temperature throughout the tallow.

The exact amounts of water [moisture contents] needed to reach saturation depend upon the temperature and upon the type and quality of tallow. For example, animal fat containing 6% free fatty acids is saturated at 0.4 % in weight at 100°C and at 0.6% in weight at 133°C.

Inactivation in the raw material prior to fat extraction (pre-sterilisation):

It has been customary to render first by a conventional rendering system at atmospheric pressure, and then take off the tallow before then subjecting the remaining solids to the 133°C/20 minute/3 bar process for the production of sterilised proteins (e.g., meat-and-bone meal). However, there is no, or little, experience of processing raw materials through the 133°C/20 minute/3bar process for the production of tallow. One might probably expect raw materials processed in this fashion to become "muddy" during processing if they were initially of poor quality (i.e. not fresh, or with a high gut content) but this is much less likely if the raw materials are fresh and have a low gut content.

According to EFPRA (2001b), the effect of pressure cooking on the quality of edible fats remains uncertain. For indirect heating with saturated steam and low moisture content the quality of rendered tallow is expected not to decrease substantially. Pressure cooking with a higher degree of moisture, proteins and direct heating is suspected to seriously deteriorate the quality of tallow on colour, free fatty acids, peroxide value, odour, etc. On this subject additional research is necessary.

Conclusion:

The possibility of tallow being obtained by pressing the (133°C/20'/3bars") processed materials rather than trying to skim it off seems to be feasible but presumably produces tallow with much more protein contamination compared with tallow that is floated off. However, at the moment there is no information available about the quality of such a product, which could be altered due to the impact of the high temperature treatment. It can therefore not be excluded that this type of processing leads to a product of inferior quality, which is not suitable for a wider application.

c. Note: risks of transmission through a processing plant, despite the existence of a sterilisation stage (e.g. through product spillage, unreliable temperature control in the sterilisation stage, poor batch mixing, etc).

The safety of tallow can be influenced by the dynamics of the rendering and extraction operations employed. The existence of an appropriate prion inactivation stage in a given processing plant does not necessarily guarantee that the end product will be free of infectivity. This can arise where end-

product safety is compromised by inappropriate procedures in the plant. Therefore, it is important to model plant operations and assign associated risk values. An example of a risk assessment model of the rendering process is tteh one developed by Brereton *et al* (2000). This program, *RenderSim*, enables the various product flows and processes in operation in a given rendering plant to be assessed in the context of the risk of infectivity passing through to the end-products (e.g. tallow and meat and bone meal).

Also, recent literature provides indications that the presence of water in lipids could expedite reduction of TSE infectivity. A shortage of water could lead to a less efficient sterilisation. If this process goes along with a less efficient denaturation of the proteins in the lipids, immunoassay analysis of the solid phase in the processed tallow should give a positive response. The feasibility of such an approach could be evaluated by analysing raw tallow (i.e. its proteinaceous fraction) after the sterilisation process but prior to the subsequent purification step.

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ANNEX: PROCESSING ANIMAL BY-PRODUCTS AS APPLIED BY THE MEMBERS OF THE EUROPEAN FAT PROCESSORS AND RENDERERS ASSOCIATION (EXTRACTS FROM EFPRA, 2001a AND EFPRA, 2001b)

(Status, May 2001)

The production activities of the European Fat Processors and Renderers Association (EFPRA) members are split up into fat melting and rendering.

The fat melting activity consists of processing animal by-products for food and feed applications. The raw materials used are fresh slaughterfats (discrete adipose tissues) fit for human consumption originated from animals approved fit for human consumption. Slaughterfats are considered to be meat. For melting slaughterfats fit for human consumption dedicated processing is common and widely used. Premier Jus, the highest quality of tallow is produced with the fat melting system for food application, for example into soups, sauces, margarine and frying and for the Calf Milk Replacers industry. Ruminant tissues are gently heat-treated to maintain the high quality of tallow and it is purified, mainly by separation and filtration in order to reduce any residual insoluble impurities to no more than the commercial values of 0.02% insoluble impurities for edible fat for food and calf milk replacers (the legal fixed value is max 0.15% insoluble impurities). Material fit for human consumption is processed by fat melters in conformance with 92/5/EC (meat products; revised former 77/99/EC).

Processing solely Low Risk Material exclusively from healthy animals is performed by fat processors for feed, petfood and technical applications. The produced tallow is purified to levels below 0.15% insoluble impurities and processed in conformance with 90/667/EC.

The Rendering activity is divided into processing Low Risk Material, High Risk Material and the processing of Specified Risk Materials. As from the first of March 2001 dead animals are not allowed any more for feed application. With the change of the health rules the raw material in the rendering industry for feed purposes is limited to Low Risk Materials from healthy animals. In the rendering industry a sterilisation process is used to produce meat and bone meal and animal fat. Tallow is purified to a maximum level of 0.15% insoluble impurities. Renderers are processing animal by-products in conformance with 90/667/EC. Dedicated production is also occurring in the rendering industry e.g. materials of porcine and avian origin.

Notes:

Low risk materials are animal by-products derived from healthy animals. The by-products are obtained from animals slaughtered in an approved slaughterhouse, that passed the health inspection[25] in accordance with the Community legislation. The specified risk materials have been removed from the carcass. These animal by-products are fit but not destined for human consumption e.g. for commercial reasons. The by-products themselves consist of (a mixture) of bones, trimmings, meat rests, etc.

[25] This currently (May 2001) implies that cattle for human consumption above 30 moths are tested on BSE and positive animals removed and completely destroyed; other animals must be certified to be under thirty months old.

High risk materials[26] present animals by-products with a risk of spreading communicable diseases to animals and humans (i.e. cadavers, animals which has died with clinical signs of diseases, animals killed in the framework of disease eradication plan, slaughterhouses' condemned material). The highest risk materials include animal by-products presenting a risk related to TSE or an unknown risk or risk related to the presence of residues of prohibited substances (i.e. hormones. B-agonists, etc.) or residues of environmental contaminants (i.e. dioxines, PCBs, etc.). The animal by-products belonging to this category, must be completely disposed of as waste by incineration, co-incineration or landfill (See SSC opinion on "Fallen Stock" of 25 June 1999).

The table hereafter summarises the sector of animal by-products.

26 It is noted that the definition of high risk materials may vary according to the country.

SPECIFICATION RUMINANT TALLOW PRODUCTION

Type of tallow produced	**Edible tallow, Premier jus, FFA max 0,50% (legally max 0,75%)** **Edible tallow, others, FFA max 1,25%** **Tallow for refining, FFA max 3,0%**	**Ruminant tallow, FFA max 1% to max 15%**
Type of industry	Fat processors	Renderers
Legislation	92/5/EEG (amended and updated 77/99/EEG)	90/667/EEG
Intended use (tallow)	Human or animal use	Animal or industrial use
Animal class	Approved fit for human consumption	Approved fit for human consumption (cat.3 : low risk material)
Animal by-product class	Fit for human consumption	Fit for animal consumption : low risk material (dead animals banned from feed as from 1 March 2001)
Type animal by-products	Fresh slaughterfats from bovines : fatty tissues from the kidney area, « mesogastrium » mesentery and cutting fats (minimal) readily removed during slaughter in the slaughterhouse or cutting plants (fat content fatty tissue from kidney area : 80% fat)	Animal byproducts from bovine, ovine and caprine All other ruminant tissues (SRM excluded) : cutting fats, bones, etc.
Bones as raw material	Bones are not used as raw material	Bones are used as raw material. In specific cases, bones are processed separately to produce bonemeal (40% protein) and bonefat (e.g. bone processors)
Specie dedicated	Always applied	Is occurring in specific cases
Other products produced	Wet greaves, proteinwater, greavesmeal (80% protein)	Meat meal, meat and bone meal, bonemeal (protein 40-60%), greavesmeal (80% protein)
Tallow production process	1. Wet melting process (premier jus) Mincing, direct steam injection (95°C), purification by decantation, centrifugation and filtration (bag filters, aid free) 2. Dry melting process Mincing, indirect heating (e.g. 135°C ; disc dryer), purification by decantation, pressing, centrifugation and filtration	1. Dry rendering process : the tallow is separated from the proteins after indirect drying ;: mincing, indirect heating (e.g.135°C disc dryer), purification by decantation, pressing, centrifugation and filtration. Pre- or post-sterilisation can be applied. 2. Wet rendering process : the tallow is separated from the proteins before removal of the water ; mincing, heating, purification by decantation, centrifugation and filtration. Pre- or post-sterilisation can be applied.
Sterilisation 133°C/20'/3 bars	Only applied on animal protein destined for feed (temporary banned)	For both methods pre sterilisation on raw material or post sterilisation on purified tallow and/or meal is used. The sterilisation of animal proteins destined for petfood and tallow from LRM destined for feed/petfood is not required (derogations on sterilisation 1999/534/EC are applied in practice).
Residual insoluble impurities	Max 0.02%	Max 0.15%
Applications tallow	Food : soups, souces, margarine, frying medium Feed : mainly calf milk replacers Petfood : petfood ingredient	Feed : feed ingredient Petfood : petfood ingredient Industrial :oleochemistry, cosmetics, soaps, detergents, fuel
Remarks	For commercial reasons tallow is refined or deodorised (e.g. removal of FFA, odour, colour and impurities) Refined tallow FFA max. 0,30%	

Notes (EFPRA, 2001c):

Dry rendering is a process where proteins and fats are dried together and the fat is removed after drying. Fat removal is normally done by pressing. To obtain a clean fat further centrifugation is performed.

Wet rendering is a process where proteins and fats are separated before the drying process. The separation is done by pressing or centrifugation. The wet defatted protein fraction is dried separately. The fat fraction is separated from the process water by centrifugation and the process water (glue water) is normally concentrated and dried together with the protein fraction. Further fat cleaning is done by centrifugation (or filtration).

In as well the dry and the wet rendering process sterilisation can take place at the beginning of the process (pre-sterilisation) or the final products, meal and fat can be sterilised in steam atmosphere.

Depending on the nature of the raw material pre-sterilisation might give technical problems due to cooking out of glue from the proteins or from creating fines. Glue will give difficulties during drying and fines will give difficulties cleaning the fat by centrifugation (or filtration).

A post-sterilisation process can therefore sometimes but not always be changed to a pre-sterilisation process as this might give technical problems. Furthermore a pre-sterilisation process will give a darker coloured fat due to the heating process in contact with the proteins.

ANNEX 3 CONDITIONS UNDER WHICH A STERILISATION AT 133 °C, 20 MIN AT 3 BARS STEAM PRESSURE SHOULD OPTIMALLY BE PERFORMED (EFPRA, 2001B)

EFPRA (2001b), anticipating that infectious prions are inactivated under similar conditions as bacterial spores, deduced a number of the conditions under which a sterilisation at 133 °C, 20 min at 3 bars steam pressure should optimally be performed. They can be summarised as follows:

- To have maximum effect of the sterilisation this should be performed at water activity close to 1. To have a water activity of 1 in fat the fat must be saturated by water. Hilder (1968 and 1971) reported on the amount of water for the saturation of fats, expressed as molar fraction, as a function only of temperature in all types of oils and fats. The higher the temperature the more water is needed for saturation. This means that by heating of fat saturated with water the water activity will go down. Therefore additional water must be added since a water activity close to 1 is required during heating[27].

  The amount of water to ensure saturation of animal fats is rather low. As an example animal fat containing 6% free fatty acids is saturated at 100 °C at 0.4% w/w moisture content. If heated to 133 °C 0.6% w/w water is needed for saturation. If saturation is ensured there is no reason to anticipate a better sterilisation effect by adding more water.

  Processed fats often contain less than 0.6% w/w moisture and during sterilisation water must be added to ensure proper sterilisation.

- To have the water dissolved in the fat phase a proper agitation is essential to maintain the water/vapour in fat dispersion/emulsion which assures the contact between the water/steam and the fat for diffusion of water into the fat phase. Dissolving water into fat is speeded up at elevated temperatures.

  From measurements of fat viscosity at different temperatures an average viscosity of 4 mPa*s at 133 °C can be extrapolated. The corresponding diffusion coefficient of water in fat at the same temperature can be estimated to be in the order of $4*10^5$ cm^2/s from which an average distance of displacement of a water molecule in fat within 1 minute of 0,7 mm can be calculated (cf. Perry 1973). On the other hand the mean distance between droplets at a given water concentration of 3% and a droplet diameter of 0,25 mm is 0,8 mm from geometrical considerations. Hence, it can be assumed that water saturation can be reached even during the heating time of the fat by direct steam injection into the fat, which takes several minutes.

  From a typical energy intake into the fat through stirring in the range of 0,2 kW/kg in a typical cooker the maximum diameter of water droplets is estimated to be in the range of 0,25 mm in good agreement with the above assumptions taking into

[27] Senhaji and Loncin (1977) described detailed experiments on heat inactivation of bacterial spores in fat/water model systems at different water activities are. Considerably increased heat resistance was found at low water activities (<0.5). This is an additional phenomenon which can be expected at low water content, where the ratio of water vapour pressure of the system with respect to the corresponding vapour pressure of pure water at the same temperature (= water activity) decreases. In the experiments of Appel *et al* (2001) however, the water activity can be assumed to be close to 1, because the water activity only starts to decrease at water contents <1% in fat/water mixtures and at water contents <20% in the mixed abattoir offal.

account the surface tension of water and the density of fat as further parameters (cf. Grassmann 1970). This indicates that a fine dispersion of droplets (cf. Re 3) is easily maintained in an ordinary 10000-kg-batch-cooker under normal working conditions (about 13 rpm).

- In fat/water systems with low water content it is essential that heating takes place by live steam injection into the stirred liquid fat, as this gives condensation of steam in small droplets adding water to the system in a fine dispersion being maintained by adequate stirring (cf. Re 2). This condensation will yield enough water to saturate the fat. As an example it can be calculated that heating of fat from 100 °C to 133 °C will add 3% water to the fat. This is 5 times more than necessary to saturate the fat and will ensure a water activity close to 1.

  It must be avoided to heat fat with low water content to elevated temperatures by indirect heating and then add steam under pressure to the fat. This might lead to overheated steam with no water droplets in the fat phase, and a poor sterilisation effect can be expected.