EUROPEAN COMMISSION
HEALTH & CONSUMER PROTECTION DIRECTORATE-GENERAL

Scientific Steering Committee

PRELIMINARY REPORT ON

QUANTITATIVE RISK ASSESSMENT ON THE USE OF THE VERTEBRAL COLUMN FOR THE PRODUCTION OF GELATINE AND TALLOW.

SUBMITTED TO

THE SCIENTIFIC STEERING COMMITTEE

AT ITS MEETING OF 13-14 APRIL 2000

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COMMENTS UNTIL 10 JUNE 2000
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Please pay special attention to the assumptions made and to the proposed probability distributions (including their key-values).

QUANTITATIVE RISK ASSESSMENT ON THE USE OF THE VERTEBRAL COLUMN FOR THE PRODUCTION OF GELATINE AND TALLOW.

PRELIMINARY REPORT FROM THE WORKING GROUP

I. THE QUESTIONS AND MANDATE

Following the decision of the UK to lift its national ban of bone-in beef, and the justification provided by MAFF-UK, the Commission requests the Scientific Steering Committee to address the following questions:

1. Is there new evidence or are there reasons to reconsider the validity of the various SSC opinions directly or indirectly related to the safety of bones opinions or to amend/update the listed conditions[1]? In particular, if and under what conditions may vertebral column and dorsal root ganglia in view of their relative risk be considered as safe for human and animal consumption? Do factors like the incidence (prevalence) of the disease and effective enforcement of general risk reduction measures such as (other) specified risk material removal rules, feed bans and age reduction at slaughter (Over Thirty Month Schemes) effect the level of risk associated with vertebral column and dorsal root ganglia ?

2. Are the answers to the previous questions also valid for sheep and goats; if not, how should they be amended?

The Scientific Steering Committee established various Working Groups to prepare scientific reports on the above questions. The present report deals only with the *Quantitative Risk Assessment on the Use of the Vertebral Column for the production of Gelatine and Tallow*. Two other reports deal with the *UK decision to lift the ban on the consumption of bone-in meat* and with the *Re-assessment of the safety with respect to TSEs, of certain types of specified risk materials of small ruminants* and.

II. BACKGROUND

1. Regarding the use of the skull, the vertebral column and other bones as raw material for ***derived processed products*** such as tallow and gelatine, the opinions the ***Scientific Steering Committee*** be summarised as follows.

   <u>In the United Kingdom [and other high risk countries]:</u>

   All bovine materials are excluded, except if they comply with the DBES criteria. In the latter case, the conditions for lower BSE risks should apply. [This means:

[1] For example, geographical source, herd source, individual animal source (e.g., age, progeny line, …), processing, intended end-use, risk of cross- contamination.

skull and vertebral column excluded; other bones can be processed provided they are sourced from animals that comply with the DBES criteria.]

In other countries not free of BSE:

- The opinion of 9 December 1997, *Listing of Specified Risk Materials: a scheme for assessing relative risks to man*, suggests dorsal root ganglia to be taken out of the food and feed chains on the basis of a risk analysis carried out for the UK Authorities by DNV (DNV, 1997). The skull and the vertebral column are classified as a SRM, because of the risk of contamination with brain or spinal cord material and because of the likely presence of remaining trigeminal or dorsal root ganglia.

  Other bones are not listed as causing a risk, but implicitly it should be understood that they should be obtained from animals fit for human consumption, that cross-contamination is avoided and that appropriate processing standards are respected.

The reason why additional processing is required for the manufacturing of derived products is clarified in the SSC's opinion on the Safety of meat-and-bone meal of 26-27 March 1998, which states:

"The Scientific Steering Committee recognises that the fact of combining both the requirements of using animals that are fit for human consumption and of submitting the material to a production process respecting conditions of 133°C during 20 minutes at 3 bar, or an equivalent process with demonstrated efficacy in terms of inactivating TSE agents, may be perceived as too precautionary. Accepting that this combination of conditions should not necessarily become a general principle, the SSC nevertheless is of the opinion that reaching the maximum possible level of safety should be the objective, in order to prevent a possible build up of circulating TSE-agents in the animal population as a result of sporadic outbreaks of TSE, even if these have not yet been shown to occur. Whilst an additional submission to 133°C during 20 minutes at 3 bar of material already declared fit for human consumption and to be used as human food cannot realistically be envisaged, the manufacturing of meat and bone meal for animal consumption does accept such conditions. As the TSE transmission barrier between animals of a same species is lower than between animals used as food and humans[2], to prevent also for these countries the possible building up of circulating TSE-agents as a result of sporadic spontaneous cases, even if these have not yet been shown to occur, and more generally, because of the risk of microbiological contamination in rendering and processing plants, this combination of conditions increases the safety of the animal as human food."

For what concerns the use of such products (e.g., gelatine), for human consumption, it should be noted that small amounts of possibly contaminated material, may eventually end up in a very large number of individual doses. (See: The SSC opinion on Human Exposure Risk, adopted on 9-10 December 1999.) Appropriate processing would therefore reduce the possible risk.

[2] This statement may need to be updated in the light of the forthcoming opinion on HELL

III. RISK ASSESSMENT

Preliminary remark:

It is often not possible to distinguish bone-derived fat and fat melted from other carcass parts. Many rendering industries do not collect separately bones and other tissues anymore. The collection of slaughter residues is likely to result in a mixture of: slaughter residues directly obtained from abattoirs; residues of bones and other tissues obtained from butcher's shops and supermarkets; restaurant and canteen residues. In the present report, the safety of bone-derived fat is addressed as a separate issue and the results can therefore not necessarily be extrapolated to fat rendered from a mixture of tissues.

III.2. BONE MARROW, SPINAL CORD, DORSAL ROOT GANGLIA, ETC., AS CONTAMINANTS OF VERTEBRAL COLUMN AND OTHER BONES USED FOR THE PRODUCTION OF GELATINE AND TALLOW FROM BOVINE BONES.

III.2.1. Introduction

The issue addressed in this report is not whether brain and spinal cord should be removed or not from the raw bone material used for the production of gelatine and/or tallow. As stated in the SSC opinion of December 1997, brain and spinal cord of ruminants (cattle) above 12 months *are* specified risk materials and therefore should be removed whenever a TSE risk in ruminants exists.

Infectivity has never been detected in the bone material itself. However, raw bone material may be contaminated with spinal cord, ganglia or (if it is infectious) bone marrow that remain even after their careful removal. The question needs therefore to be addressed whether these contamination levels are such that the possible residual infectivity of the final product, after its processing, constitutes a risk for humans and animals.

Regarding bone marrow no infectivity has been detected so far by mouse bioassay in field cases with clinical BSE. Data for BSE are based, however, on transmissions attempted from a very small number of animals[3]. Nevertheless, these findings are, in general, consistent with those in studies of the pathogenesis of BSE in cattle after oral challenge (Wells *et al*, 1996, 1998), with the exception of the detection of infectivity in distal ileum and, in a level close to the limit of detectability by mouse bioassay, in the sternal bone marrow from animals killed in the clinical phase of the disease at 38 months p.i. (but not before and not after) in this experimental study of BSE in orally exposed cattle (Wells *et al*, 1999). The inconsistent result of the absence of detectable infectivity in bone marrow in this study at the later time point of 40 months p.i. has raised, amongst other alternative explanations, the possibility that the finding of infectivity at 38 months p.i. may have been the result of an accidental procedural contamination. Nevertheless, there is limited evidence from previous studies of other

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

TSEs that infection of bone marrow, although not part of the general pathogenesis pattern, could be a rare event occurring late in the incubation period.

III.2.2. Method

To carry out the risk assessments, the working group used a spreadsheet model in Microsoft Excel, provisionally called *BSE&Risk*, that was developed for the European Commission by Berends (2000) of the University of Utrecht (NL). In this spreadsheet model several input parameters are linearly linked to estimate the remaining level of infectivity in animal products, such as gelatine and tallow produced from bones.

As a first step, a deterministic (fixed) risk assessment was carried out for three basic scenarios (best, most likely and worst case). As a second step, the model was also used for a stochastic (Latin Hypercube) simulation with the aid of the special software '@Risk' (Palisade Corporation, 1996). This add-on programme for Microsoft Excel enables the introduction of probability distributions describing the uncertainty and variation in the input parameters.

Basic rules of calculation in BSE&Risk

The most important risk calculations that are being used in the spreadsheet are:

i) the probability that a particular event, such as the slaughtering of TSE positive animals for food, will happen at least once;

ii) the average expected number of this particular event.

In general, the probability that a particular event will happen at least once during n repetitions is calculated best with the formula:

$$P_{(\text{event one or more times})} = 1-(1-P_{(\text{singular probability of event})})^{n}$$

The average expected number of that particular event can be calculated with the formula:

$$\text{Exp.} = n*P_{(\text{singular})}.$$

For example, if the singular probability of disease following a single oral exposure to a paticular pathogen is 0.5, the probability of that event occuring at least once during 6 different exposures is 0.984 (i.e. $1-(1-0.5)^{6}$), and the average expected number of times that this event will actually occur is 3 (i.e., 6*0.5).

General structure of BSE&Risk

To keep the spreadsheet model as versatile as possible, the spreadsheet is subdivided into 3 sections:

I. Risks connected with the slaughter of cattle

II. Risks connected with the processing of (materials from) slaughtered animals.

III. Risks connected with the actual processing of the raw materials and the consumption of the end-product.

BSE&Risk outputs

The outputs of the sections I, II and III are:

1) The ultimate probability that TSE+ animals and materials become one or more times released into home and foreign markets
2) The average expected number of TSE+ animals/carcasses, or batches of slaughter products and/or slaughter by-products, that become released into home and foreign markets in a period of one year.
3) The probability that a given batch of fresh materials, to be processed further into, for example, gelatine or meat and bone meal, is TSE+.
4) The average expected number of TSE+ batches of fresh materials.
5) The average expected number of positive animals per positive batch
6) The average potential risks of infection in humans or animals per contaminated batch (of locally processed materials).
7) The total average potential risks of infection in humans or animals by all contaminated batches produced together.

On the basis of several assumptions, which are outlined below, section III also estimates:

8) The infection risks per typical portion of the end-product investigated, such as gelatine or tallow.
9) The ultimate number of infections in the consumer population, given a series of n (daily) oral exposures to contaminated typical edible portions of the end-product under investigation.

BSE&Risk key input parameters:

1) The **incidence** of BSE positive animals that become slaughtered for food. This parameter is in fact depending on the ratio of undiscovered/discovered cases. This ratio is determined by a) the percentage of underreporting of cases of BSE that might take place in a country, b) the number of animals that do show clinical signs, but nevertheless pass the ante mortem examination at the slaughterhouse and c) the percentage of animals that are infected with BSE, show no neurological signs of an infection, but may harbour enough prions to be considered a hazard
2) The yearly **numbers of adult animals slaughtered** determines the probability that at least once per year a BSE+ animal is slaughtered. This parameter can be seen as the number of repetitive trials (n) used in the formula mentioned earlier ($P(total)= 1-(1-P(once))^n$)
3) The **typical batch size** (i.e. the typical number of animals that make up a batch of fresh materials to be processed into something else, such as a batch of fresh bones used for making gelatine) is an important parameter, because the typical batch size determines the probability that a given batch is TSE+. Again, the typical

batch size is to be considered as the number of trials (n) used in the formula discussed earlier ((P(tot.)= 1-(1-P(once))^n)

4) The total **number of batches of fresh materials processed** in a year determines the probability that at least once per year a TSE+ batch is produced This parameter can be seen again as the number of repetitive trials (n) used in the formula mentioned earlier (P(tot)= 1-(1-P(once))^n)

5) **Typical tissue titres of the BSE agent** . The typical tissue titres determine greatly the assessed consumer risks, since they are directly related to the dose-response relationships in animals and humans. Because there is a considerable lack of hard data, in particular in the low-dose areas, it is assumed that the dose-response relationships are linear. This is a prudent approach, because it leads in the low-dose areas almost automatically to a (considerable) overestimate of any of the assessed risks.

6) **The effects of processing** is another key input, because they determine wether or not any of the risks present are reduced significantly.

7) **The typical batch size of the end-product investigated** determines how many 'typical edible portions' can be made with one (contaminated) batch.

8) If it has been possible to determine the **size of a typical edible portion of the end-product under investigation** it is also possible to determine the infection risks per typical edible portion and/or roughly estimate how many people or animals would actually be (n times) exposed to contaminated meals or medicines (e.g. gelatine capsules) and how many of these (truly) exposed would subsequently become infected.

Sensitivity analysis of BSE&Risk

Increases or decreases in all the major input parameters are more or less directly proportional to the risks calculated. This is a natural consequence of the way the calculations are done in the spreadsheet model, and it means in practice that practically all the parameters involved must be considered equally as important.

Furthermore, a simultaneous change in the settings of more than one of the involved parameters results in a shift in ultimate consumer risks that are a multiplication of these individual changes: When, for example, the incidence of BSE in cattle is reduced to halve of its original value, the calculated consumer risks are also reduced to halve, but when two different parameters are each reduced to their halves, the calculated consumer risks are reduced to one quarter, and to one eight if three different parameters are each reduced to their halves.

Summary:

Within each scenario, nine input parameters were thus identified for which average, best and worst assumptions could be made (see also section III.3.3 below). In the deterministic approach, calculations for the best, average and worst case scenarios assume that all nine input parameters would simultaneously either be best, average or

worst, which is highly unlikely. As a result, especially the worst case scenario provided risk estimates that are unrealistic.

In the stochastic (Monte Carlo) approach, during each iteration of the model, i.e. each calculation of the results, a value is selected from each of the nine probability distributions describing those input parameters. Therefore all possible combinations of parameter values are explored during the 10000 iterations selected for the modelling process, and the distribution of outcome values (results from those 10000 iterations) indicate the possible extreme values but – even more important – the most likely outcome (mode of the distribution) for the chosen combination of input distributions.

III.2.3. Scenarios and assumptions

The following scenarios and assumptions were retained by the Working Group as being reasonable in the context of this risk assessment.

A. The **various risk scenarios** taken into consideration were:

Regarding Specified Risk Materials:

- **Scenario A_1**: total exclusion of brain and spinal cord and of all risky bone materials (i.e., skull, vertebral column). Sources of infectivity are (see annex 1) remainders of spinal cord and of dorsal root ganglia and trigeminal ganglia; bone marrow and bone adnexa.
- **Scenario A_2**: as above, however, bone marrow is considered to be *not* infectious.
- **Scenario B_1**: total exclusion of brain, spinal cord and skull but the vertebral column is included. Sources of infectivity are (see annex 1) the possible remainders of the spinal cord; all dorsal root ganglia and trigeminal ganglia; bone marrow and bone adnexa.
- **Scenario B_2**: as above, however, bone marrow is considered to be *not* infectious.
- **Scenario C_1**: brain and spinal cord are included, as well as risky bone materials (i.e., skull, vertebral column). Sources of infectivity are (see annex 1) the complete brain and spinal cord, all the dorsal root ganglia and trigeminal ganglia, bone marrow and bone adnexa.
- **Scenario C_2:** as above, however, bone marrow is considered to be *not* infectious.

Regarding infectivity titres and species barriers:

Taking into account the report and pre-opinion adopted by the Scientific Steering Committee on 2-3 March 2000[4], the Working Group used the following ranges for its risk assessments:

[4] Preliminary opinion on *Oral exposure of humans to the BSE agent: infective dose and species barrier.* Adopted by the Scientific Steering Committee at its meeting of 2-3 March 2000

- **Scenario 1:**

  The infectivity titre in brain and skull is approximately 10 Cattle oral ID_{50} ($CoID_{50}$) per gram as geographic mean (median) value; 1 and 1000 $CoID_{50}$ as extreme values. For the probabilistic approach, a log-normal distribution is assumed. The 95% percentile is 100 $CoID_{50}$.

  [MAFF has carried out oral challenge tests on cattle at the Central Veterinary Laboratory to try and determine the minimum infective dose for BSE infected cattle brain. In the experiment groups of 10 calves were fed 300g, 100g, 10g and 1g of brain tissue from clinically sick animals. All animals in the two higher dose categories came down with BSE, and 7 out of 10 in both the 10 g and 1g trials. The remaining animals in these trials are still alive 95 months post infection, but show some symptoms of the disease (Dr Danny Matthews, personal communication, January 2000). For the 1g trial the mean incubation period is 4.7 years. An extension of this experiment with lower doses has now started, but the results will not be available for at least 5 years. These results indicate that the oral ID_{50} of clinically affected BSE brain for cattle is likely to be somewhat less than 1 gram, although with the incubation period now being close to that observed in the epidemic it may be close to 1 gram. It was decided to take a precautionary view and assume that the mean value of the oral ID_{50} for cattle is 0.1 gram (i.e. 10 oral ID_{50} units per gram)[5].]

  The species barrier varies within the range of 10^0 and 10^4, with 10^3 as average value. For the probabilistic approach, an adjusted triangular (or BetaPert) distribution is assumed.

- **Scenario 2:**

  The infectivity titre in brain and skull is approx. 100 Cattle oral ID_{50} ($CoID_{50}$) per gram as geometric mean (median) value; 1 and 1000 $CoID_{50}$ as extreme values.

  The species barrier varies within the range of within the range of 10^0 and 10^4, with 10^1 as average value.

Summary table of scenarios:

	Scen. A_1	Scen. A_2	Scen. B_1	Scen. B_2	Scen. C_1	Scen. C_2
Brain	OUT	OUT	OUT	OUT	IN	IN
Spinal cord	OUT	OUT	OUT	OUT	IN	IN
Skull	OUT	OUT	OUT	OUT	IN	IN
Vertebral column	OUT	OUT	IN	IN	IN	IN
Bone marrow	Infectious	Not infectious	Infectious	Not infectious	Infectious	Not infectious
Titre and barrier: Scenario 1	**The infectivity titre** in brain and skull is approx. **10 Cattle** oral ID_{50} ($CoID_{50}$) per gram as geometric mean (median) value; 1 and 1000 $CoID_{50}$ as extreme values. **The species barrier** varies within the range of 10^0 and 10^4, with 10^3 as average value.					
Titre and barrier: Scenario 2	**The infectivity titre** in brain and skull is approx. **100 Cattle** oral ID_{50} ($CoID_{50}$) per gram as geometric mean (median) value; 1 and 1000 $CoID_{50}$ as extreme values. The **species barrier** varies within the range of within the range of 10^0 and 10^4, with 10^1 as average value.					

[5] For the efficacy of other routes of infection: see the pre-opinion of 2-3 March 2000 the Scientific Steering Committee on *Oral exposure of humans to the BSE agent: infective dose and species barrier.*

B. The assumptions made were:

a. **1.000.000 slaughtered cattle** would serve, the "gelatine and tallow-from-bone" needs of *roughly* 10.000.000 people in Europe. This estimate is derived from the following simplified reasoning: total European gelatine market (375.000.000 people) is approx. 95.000 tons. 25 tons of bones results in approx. 1 ton of gelatine. Each slaughtered animal produces approx. 29-42 kg bones (depending upon whether or not the skull and vertebral column were removed - see further; 35 kg if skull and vertebral column are removed); 47% of the European gelatine market is produced from cattle (GME, 1998).

All produced gelatine and tallow is consumed by the local population. There are no exports. And all the raw material is derived from local *cattle*, not from imported bones. This assumption introduces a safety margin, as in Europe, more than 60% of the gelatine for human consumption is probably obtained from pig skins. (GME, 1998)

b. The numbers of animals in the final stages of BSE (i.e. with high infectivity levels) that pass the pre-slaughter controls were considered to be: **0.1, 1 and 100 BSE cases per 1.000.000 slaughtered animals per year**. Because of the linearity of the relations in the risk assessment model, anyone could easily extrapolate most of the results for other boundary conditions. The experts considered it impossible to use the number of observed clinical BSE cases as a starting point. The ratio of this number to the number of undetected TSE cases that would be slaughtered largely depends upon the reliability of the surveillance system and upon the effectiveness of other risk management measures. The general assumption that for each declared BSE case, there is one (1) undiscovered being slaughtered as fit for human consumption, has to be taken with caution and could thus easily be replaced by figures judged to be more appropriate for a given country (e.g., 2, 5, 10 or higher). The residual risk will have to be equally multiplied by 2, 5 or 10 or more.

Similar extrapolations could be made for the assumed species barrier, the minimal infective dose, etc.

c. The average weights of the various bones, the levels of contamination with possibly infective tissues (rests) and the **tissue infective load distribution** are as in annex 1. (Sources: Comer, 1997; Berends, 2000).

d. The estimated average total **weights of fresh bone material** per animal and the corresponding estimated infectivity titres for the above scenarios are also given in annex 1.

e. **One batch** of either tallow or gelatine is produced from 5000 animals. (In reality, there is a range of probably 2500-7500; the exact numbers per batch will also depend upon whether or not vertebral column and/or skull were removed. Even for scenario C1 (highest amount of infectivity retained in the system) and with 100 infected / 1 million slaughtered cattle, using 2500 or 7500 animals per batch resulted in a change of the results - when compared to the results for average, best

and worst cases results for 5000 cattle / batch - of between 0% and less than 16%. It might become important in countries or regions with a very high BSE incidence.

f. Infectivity reduction by processing:

For gelatine:

It is assumed that the "long alkaline process" as described in the SSC opinion on the Safety of gelatine (March 1998) in Western Europe is the most representative one. According to this opinion, the acid and alkaline steps would result in an infectivity reduction of approx. 10^3 and additivity with other possible infectivity reducing treatments (e.g., degreasing, sterilisation) is not guaranteed. However, this value may be too conservative, if the effect of the degreasing and sterilisation steps are additive to the alkaline step and/or if the more severe NaOH step as described in the updated SSC opinion on the Safety of gelatine (January 2000) are used. Under optimal conditions (best case) reduction may then reach 10^6. The Working Group therefore tested the following scenarios: worst case: infectivity reduction by a factor 10^3; best case: infectivity reduction by a factor 10^6; average conditions: infectivity reduction by 10^4.

For tallow:

- The worst-case scenario would be one in which all of the infectivity in the raw materials ends up in the tallow after processing. However, such a scenario has not been considered in this risk assessment because there is sufficient evidence to conclude that this is not what actually occurs in practice:

  Epidemiological studies have failed to incriminate the dietary use of tallow in cattle with any risk of developing BSE because the geographical distribution of BSE did not correspond with the known pattern of distribution of tallow for use in cattle-feed (Wilesmith *et al*, 1998). Also, experimental studies have shown that BSE and scrapie infectivity tend to partition preferentially with meat and bone meal, and not with tallow (Taylor *et al*, 1995;1997). In the experiments with the BSE agent, tallow produced under worst-case conditions had no detectable infectivity but the meat and bone meal contained almost as much infectivity as the untreated raw materials (Taylor *et al*, 1995). The data obtained from these studies with regard to BSE infectivity have been used to calculate the degree of any theoretical risk of BSE infectivity being in tallow as follows (D.Taylor, pers.comm., 1999):

  - 12 mice received a total of 6.24ml of 10% unfiltered tallow. If this had contained 1 mouse intracerebral ID_{50},
  - 6 mice would have been affected theoretically. No mice were affected.
  - 6.24ml therefore contains <1/6 of 1 intracerebral ID_{50} = $<10^{-1.5}$ ID_{50}/ml.
  - The neat tallow must therefore have had $<10^{-0.5}$ ID_{50} /ml. This is equivalent to $<10^{-5.5}$ oral ID_{50} /ml (Kimberlin & Walker, 1988;1989).

  These figures are for mice. The potential level of infectivity in bovine-derived tallow (assayed in mice) is based upon intracerebral ID_{50} measurements, despite the fact that the tallow was injected both intracerebrally and intraperitoneally. During the primary passage of BSE or scrapie to mice, the efficiency of intracerebral challenge is much the same as that for

intraperitoneal challenge. This contrasts with the approximately 100-fold greater efficiency of transmitting rodent-passaged TSE agents to rodents by intracerebral, compared with intraperitoneal, challenge. It is known that, following intracerebral injection, cattle are 1,000-fold more sensitive to infection by the BSE agent than mice. If the same differential between cattle and mice applies to the oral route, it can be calculated that tallow could contain $<10^{-2.5}$ cattle oral ID_{50} /ml ($<10^{0.5}$ ID_{50} /litre).

Although it has been calculated that the level of any infectivity in the tallow must have been below $10^{-2.5}$ Co ID_{50}/ml (Taylor, 1997), the titre of infectivity in the corresponding meat and bone meal fraction was around $10^{5.0}$ Co ID_{50} /g. Therefore, 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. (To err on the cautious side, a risk-assessment could assume that this factor is only 10^3.)

- Although the data discussed above indicate that BSE infectivity does not have a predilection to partion with tallow during its extraction from bovine tissues, it must be recognised that there are insoluble solids, including protein material, that end up adventitiously in tallow. Since BSE infectivity has a tendency to fractionate with the proteinaceous rather than the fatty fraction during the production of tallow, the effect of protein contamination of tallow must be separately evaluated. This is an important assessment since it represents what actually occurs in practice.

  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 (Woodgate, 1999). For the purpose of these assessments, the insoluble solids will therefore be considered to consist entirely of protein. The tallow, at the end of the extraction process is purified to a maximum solid content of 0.15%, as recommended in the SSC opinion on the Safety of tallow (March 1998). This result in an infectivity reduction of $1.01*10^3$. [In scenario C, it is assumed that the infectivity in fresh bone materials is 0.19 ID50/gram (see annex); Fresh bones are composed of 55% solids and 45% water. Of the solids, 35% are protein. Therefore fresh bones have 0.55 x 0.35 = 0.193 protein fraction. If it is then assumed that all the infectivity is in the protein (see above), the protein has an infectivity titre of 0.19/0.193 = 0.98 ID50/gram (i.e. about 1 ID50/gram). Finished tallow has a maximum solids content of 0.15%. If this were all protein (worst case) then the infectivity titre would be 0.0015 ID50/g. This is a factor 100 less than the infectivity titre of the raw bones.]

The Working Group therefore tested the following scenarios: worst case: infectivity reduction by a factor 10^2 (for example as a result of inappropriate purification); best case: infectivity reduction by a factor 10^5 (for example if additionally processed according to the 133°C/20'/3 bars" standard); average conditions: infectivity reduction by 10^3.

g. Human or animal consumption versus technical uses. Not all tallow or gelatine are destined for human or animal consumption (food, feed

pharmaceutical and medicinal uses). Technical and industrial uses exist, for example, in photography, in the tyre industry, etc. It is very difficult to find information on the ratio technical uses / total consumption. GME (1998) estimates this ratio, for Europe, at approximately 31/100. (World-wide the ratio seems to be: 23/100). For tallow, hardly any data are available. The working group used the following values:

For gelatine: on average, an estimated 70% of the production goes for human consumption; the best - worst case range is 50% to 90%.

For tallow: Tallow from bones is unlikely to go to humans, but rather to animals (e.g., milk replacers). Therefore, on average, an estimated 10% of the production goes for human consumption; the best - worst case range is 1% to 25% (the latter value for example if blended with other fats).

h. Daily human consumption. Limited information (if any at all) is available for the consumption patterns of gelatine and tallow.

For gelatine, a rough calculation would yield that the human daily average consumption is of the order of 0.2 grams. (European gelatine market in 1998: 95.000 tons, 375 Europeans, 69% of the market goes to food and pharmaceutical products.) However, this average is hardly representative, knowing that very high daily consumption by children of gelatine processed in sweets of up to 17 grams have been observed (P.Grobben, Gelatine Smits' B.V., personal communication, 2000).

On tallow, no precise data are available. Most tallow for human consumption seems to be derived from muscle fat and adipose tissues. Animal feed seems also (mainly) to be obtained from rendered mixtures of tissues and as by-products from other processes such as the production of gelatine from bones. The working Group therefore assumed the following daily consumption patterns:

Gelatine: average human consumption of 1 gram per day. Best-worst range is 0.2 to 20 grams per day.

Tallow: average human consumption of 1 gram per day. Best-worst range is 0.5 to 10 grams per day.[6]

[6] Note regarding tallow in animal feeds. The following *indicative* values may be given for Belgium (Vanbelle, personal communication, 2000):
- calves for fattening (45-200kg): 100 - 700 g fat/day in the milk replacer, out of which on average 10% may consist of tallow;
- dairy cattle: 750 - 1000 g fat/day, out of which on average 10% or more (up to 100%) may consist of tallow;
- beef cattle: 360 g fat/day, out of which on average 10% or more may consist of tallow;
- poultry: 6 - 8 grams fat/day, but little or no fraction of it consists of tallow;
- piglets (0-10 weeks): 10-15 g fat/day, out of which on average 10% may consist of tallow;
- pigs for fattening (less than 50kg): 45 g fat/day, out of which on average 10% may consist of tallow;
- pigs for fattening (50-105kg): 70-100 g fat/day, out of which on average 10% may consist of tallow.

III.2.4. Risk assessment using probability distributions for the key-variables that enter the risk assessment scheme.

The following probability distributions were proposed and tested by the Working Group for the stochastic (Monte Carlo) simulation model. They may however need to be refined according as evidence becomes available:

Number of animals per batch: normal distribution, with a mean of 5000 cattle and a standard deviation of 750.

Bone quantities per animal: normal distribution, with a mean of 29, 35 or 42 kg [7]and a standard deviation of $1/10^{th}$ of the mean value

Infectivity titre in tissues[8]: in brain and skull: log-normal distribution with approx. 10 Cattle oral ID_{50} ($CoID_{50}$) per gram as geographic mean (median) value; 1 and 1000 $CoID_{50}$ as extreme values. The 95% percentile is 100 $CoID_{50.}$

In bone marrow: either it is infective (at a maximum titre of 0.032 $CoID_{50}$), or it isn't infective at all.

Species barrier: adjusted triangular (or BetaPert) distribution within the range of 10^0 and 10^4, with 10^3 as average value

Titre reduction by processing: adjusted triangular (or BetaPert) distribution

For tallow: range of 10^2 and 10^4, with 10^3 as average value

For gelatine: range of 10^3 and 10^6, with 10^4 as average value

Number of BSE animals in 1 infected batch: Poisson distribution with, for an incidence of 1 BSE case per 1.000.000 slaughtered animals, 1 as most likely value (lambda) [This value needs to be modified according to the epidemiological situation of a country.]

Number of BSE positive batches produced per year: Poisson distribution with, for an incidence of 1 BSE case per 1.000.000 slaughtered animals, 1 as most likely value (lambda) [This value needs to be modified according to the epidemiological situation of a country.]

- Sows (120-200kg): 60 g fat/day, out of which on average 10% may consist of tallow.

[7] Depending upon the breed, the bone quantities per adult animal can reach 50kg.

[8] For the efficacy of other routes of infection: see the pre-opinion of 2-3 March 2000 the Scientific Steering Committee on *Oral exposure of humans to the BSE agent: infective dose and species barrier.*

Ratio human consumption/ total: adjusted triangular (or BetaPert) distribution within the range of 30% and 70%, with 50% as average value

Daily consumption:

(gelatine:) adjusted triangular (or BetaPert) distribution within the range of 0.2 and 20 grams, with 1 gram as average value.

(tallow, humans) adjusted triangular (or BetaPert) distribution within the range of 0.5 and 10 grams, with 1 gram as average value.

(tallow, animals) adjusted of 0.5 and 20 grams, with 10 grams as average value.

III.2.5. Using the potential residual infectivity in "133°C/20'/3bars" processed meat-and-bone meal as a possible reference.

a. In order to be able to fully appreciate the results, the Working Group carried out an additional, but similar risk analysis for bovine-derived meat-and-bone meal (MBM) that gets fed back to bovines. The model assumptions were, mutatis mutandis, kept as close as possible to the ones used for tallow and gelatine:

- Total herd size: 10.000.000, all fed with bovine derived MBM enriched feed.
- BSE incidence: 1 case per 1.000.000 slaughtered animals
- Slaughter ratio: about one third of the population (i.e., 3 million cattle slaughtered/year). Thus, on average three BSE positive cattle become slaughtered for food.
- All offals are being rendered and may be fed back to cattle. No fallen stock is included in the rendered material.
- Total average amount of rendered offals per animal slaughtered: 250 kg
- Batch size for MBM production: 5 tonnes of fresh materials consisting of offals from cattle, pigs, sheep and poultry, with per batch the materials of on average 4 heads of cattle (1 tonne of cattle offals per batch). Sheep are in this exercise not supposed to be infected with BSE or scrapie.
- End product batch size (MBM): 5 tonnes of the fresh materials of mixed origin yield 2 tonnes of MBM
- Reduction by processing: 10^3 (average), 10^6 (best[9]) and 0 (worst, i.e., no effects of processing at all);

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

- Percentage MBM contained in the feed: 2% on average (or 200g in a standardized daily ration of 10kg compound), ranging between: 1% (best) and 3% (worst).
- For MBM, the species barrier was always set equal to 1 (= no species barrier). As for gelatine and tallow, two scenarios of tissue infectivity were tested: an **infectivity titre** in brain and skull of approximately 10 Cattle oral ID_{50} ($CoID_{50}$) per gram as geographic mean (median) value (1 and 1000 $CoID_{50}$ as extreme values) and **an infectivity titre** of approx. 100 Cattle oral ID_{50} ($CoID_{50}$) per gram as geometric mean (median) value (and again, 1 and 1000 $CoID_{50}$ as extreme values).
- Ratio animal consumption/total production of MBM: 75% (average), 50% (best) and 100% (worst). The rest is used, for example, as organic fertiliser.

The tables 1 and 2 in Annex 4 show the calculation of the prion titers in the fresh materials of bovine origin used for MBM production.

III.2.6. Summary table of default values, assumptions and scenarios

A summary table presenting the default values, assumptions and scenarios used in the risk assessment, is given in annex 2.

III.2.7. Results

III.2.7.1. Presentation of the results.

The result of the assessments of the residual risk after removal of specified risk materials at various levels and/or processing are expressed as total number of expected vCJD infections (in humans) per year, in a population of 10.000.000 people (or cattle, for the meat-and-bone meal risk assessment). In addition, the 0.5%ile, 5%ile, 95%ile and 99.5%ile are also presented.

III.2.7.2. Number of iterations.

All simulations were run with 100.000 iterations. Preliminary tests carried out by the working group showed that the results in terms of mode and percentiles are not sensitive to the number of iterations which mainly effect the extreme values, especially the minimum and maximum.

III.2.7.3. Results for tallow and gelatine, scenario 1: mean infectivity titre of 10 $CoID_{50}$ per gram and a mean species barrier of 10^3.

Stochastic approach:

Tables 2 and 3 hereafter summarise the results of the stochastic modelling for tallow and gelatine.

Table 2: Summary results for tallow, scenario 1: residual risk expressed as the total number of expected cases per year, in a population of 10.000.000 people.

Scenario Tallow 1 All zero values replaced with 1e-9 for display

Product	Scenario	BM inf.	Cases/Mill.	0.5% Perc	5% Perc	Mode	95% Perc	99.5% Perc
Tallow	A1	yes	0.1	1.000E-09	1.000E-09	1.000E-09	3.776E-05	3.432E-04
Tallow	A2	no	0.1	1.000E-09	1.000E-09	1.000E-09	1.768E-05	1.692E-04
Tallow	A1	yes	1	1.000E-09	1.000E-09	1.000E-09	3.796E-05	3.590E-04
Tallow	A2	no	1	1.000E-09	1.000E-09	1.000E-09	1.734E-05	1.739E-04
Tallow	A1	yes	100	6.828E-06	1.875E-05	1.473E-05	3.527E-03	2.824E-02
Tallow	A2	no	100	2.637E-06	7.590E-06	5.600E-06	1.637E-03	1.392E-02

Product	Scenario	BM inf.	Cases/Mill.	0.5% Perc	5% Perc	Mode	95% Perc	99.5% Perc
Tallow	B1	yes	0.1	1.000E-09	1.000E-09	1.000E-09	1.317E-04	1.348E-03
Tallow	B2	no	0.1	1.000E-09	1.000E-09	1.000E-09	1.081E-04	1.184E-03
Tallow	B1	yes	1	1.000E-09	1.000E-09	1.000E-09	1.349E-04	1.364E-03
Tallow	B2	no	1	1.000E-09	1.000E-09	1.000E-09	8.124E-05	8.173E-04
Tallow	B1	yes	100	1.597E-05	4.942E-05	2.230E-05	1.247E-02	1.077E-01
Tallow	B2	no	100	9.630E-06	3.196E-05	3.652E-05	1.041E-02	9.511E-02

Product	Scenario	BM inf.	Cases/Mill.	0.5% Perc	5% Perc	Mode	95% Perc	99.5% Perc
Tallow	C1	Yes	0.1	1.000E-09	1.000E-09	1.000E-09	1.256E-03	1.291E-02
Tallow	C2	No	0.1	1.000E-09	1.000E-09	1.000E-09	1.257E-03	1.237E-02
Tallow	C1	Yes	1	1.000E-09	1.000E-09	1.000E-09	1.256E-03	1.388E-02
Tallow	C2	No	1	1.000E-09	1.000E-09	1.000E-09	9.140E-04	9.034E-03
Tallow	C1	Yes	100	8.915E-05	3.338E-04	1.327E-04	1.240E-01	1.130E+00
Tallow	C2	No	100	8.267E-05	3.058E-04	2.330E-04	1.177E-01	1.080E+00

Table 3: Summary results for gelatine, scenario 1: residual risk expressed as the total number of expected cases per year, in a population of 10.000.000 people.

Scenario Gelatine 1 **All zero values replaced with 1e-9**

Product	Scenario	BM inf.	Cases/Mill.	0.5% Perc	5% Perc	Mode	95% Perc	99.5% Perc
Gelatine	A1	yes	0.1	1.000E-09	1.000E-09	1.000E-09	1.728E-05	1.590E-04
Gelatine	A2	no	0.1	1.000E-09	1.000E-09	1.000E-09	8.108E-06	8.089E-05
Gelatine	A1	yes	1	1.000E-09	1.000E-09	1.000E-09	1.755E-05	1.676E-04
Gelatine	A2	no	1	1.000E-09	1.000E-09	1.000E-09	8.222E-06	7.590E-05
Gelatine	A1	yes	100	4.879E-06	1.077E-05	7.359E-06	1.649E-03	1.217E-02
Gelatine	A2	no	100	1.808E-06	4.282E-06	2.607E-06	7.887E-04	6.482E-03
Product	**Scenario**	**BM inf.**	**Cases/Mill.**	**0.5% Perc**	**5% Perc**	**Mode**	**95% Perc**	**99.5% Perc**
Gelatine	B1	yes	0.1	1.000E-09	1.000E-09	1.000E-09	6.232E-05	6.330E-04
Gelatine	B2	no	0.1	1.000E-09	1.000E-09	1.000E-09	4.947E-05	4.998E-04
Gelatine	B1	yes	1	1.000E-09	1.000E-09	1.000E-09	6.090E-05	6.140E-04
Gelatine	B2	no	1	1.000E-09	1.000E-09	1.000E-09	3.788E-05	3.669E-04
Gelatine	B1	yes	100	1.099E-05	2.745E-05	4.042E-05	5.945E-03	4.865E-02
Gelatine	B2	no	100	6.169E-06	1.758E-05	3.618E-05	4.809E-03	4.134E-02
Product	**Scenario**	**BM inf.**	**Cases/Mill.**	**0.5% Perc**	**5% Perc**	**Mode**	**95% Perc**	**99.5% Perc**
Gelatine	C1	yes	0.1	1.000E-09	1.000E-09	1.000E-09	5.966E-04	6.330E-03
Gelatine	C2	no	0.1	1.000E-09	1.000E-09	1.000E-09	5.618E-04	5.654E-03
Gelatine	C1	yes	1	1.000E-09	1.000E-09	1.000E-09	5.851E-04	6.054E-03
Gelatine	C2	no	1	1.000E-09	1.000E-09	1.000E-09	4.360E-04	4.379E-03
Gelatine	C1	yes	100	5.897E-05	1.884E-04	1.222E-04	5.731E-02	4.863E-01
Gelatine	C2	no	100	5.134E-05	1.712E-04	2.092E-04	5.459E-02	4.878E-01

III.2.7.3. Results for tallow and gelatine, scenario 2: mean infectivity titre of 100 $CoID_{50}$ per gram and a mean species barrier of 10^1.

Stochastic approach:

A LogNormal distribution of the infectivity titre was not considered as appropriate for the given range of (expert suggested) values (1,100,1000). A BetaPert distribution (1, 100, 1000) was used instead. For the species barrier, a BetaPert distribution between 0 and 1000 with 10 as the most likely value was adopted.

Sample comparison calculations between scenarios 1 and 2 applied to gelatine, show that the increase for scenario 2, when compared to scenario 1, is less than 6.5-fold in the mode and less than 23-fold in the 99.5%ile. (See table 4). For tallow, similar ratios should apply.

Table 3: Comparison of scenarios 1 & 2 - Results for gelatine: total number of expected cases per year, in a population of 10.000.000 people.

Scenarios Gelatine 1 & Gelatine 2 **(All zero values replaced with 1e-9)**

Product	Scenario	BM inf.	Cases/Mill	0.5% Perc	5% Perc	Mode	95% Perc	99.5% Perc
Gelatine 1	A1	yes	0.1	1.000E-09	1.000E-09	1.000E-09	1.728E-05	1.590E-04
Gelatine 2	A1	yes	0.1	1.000E-09	1.000E-09	1.000E-09	9.891E-05	1.290E-03
Gelatine 1	A1	yes	1	1.000E-09	1.000E-09	1.000E-09	1.755E-05	1.676E-04
Gelatine 2	A1	yes	1	1.000E-09	1.000E-09	1.000E-09	1.007E-04	1.234E-03
Gelatine 1	A1	yes	100	4.879E-06	1.077E-05	7.359E-06	1.649E-03	1.217E-02
Gelatine 2	A1	yes	100	1.073E-05	3.008E-05	2.737E-05	1.025E-02	1.141E-01
Product	**Scenario**	**BM inf.**	**Cases/Mill**	**0.5% Perc**	**5% Perc**	**Mode**	**95% Perc**	**99.5% Perc**
Gelatine 1	C1	yes	0.1	1.000E-09	1.000E-09	1.000E-09	5.966E-04	6.330E-03
Gelatine 2	C1	yes	0.1	1.000E-09	1.000E-09	1.000E-09	8.952E-03	1.216E-01
Gelatine 1	C1	yes	1	1.000E-09	1.000E-09	1.000E-09	5.851E-04	6.054E-03
Gelatine 2	C1	yes	1	1.000E-09	1.000E-09	1.000E-09	8.579E-03	1.213E-01
Gelatine 1	C1	yes	100	5.897E-05	1.884E-04	1.222E-04	5.731E-02	4.863E-01
Gelatine 2	C1	yes	100	3.354E-04	1.641E-03	7.920E-04	9.055E-01	1.090E+01

Deterministic approach:

Tables 4 hereafter summarises, for scenarios 1 and 2, the results of the stochastic modelling for tallow and gelatine*.

Table 4: Deterministic approach. Summary results for tallow and gelatine: residual risk expressed as the total number of expected cases per year, in a population of 10.000.000 people.

			Deterministic results			
			Scenario 1		Scenario 2	
Titre			$10CoID_{50}/g$		$100CoID_{50}/g$	
Barrier			1000		10	
Scenario	Bone Marrow infective	BSE cases / 1 Mill. Slaught.	Tallow: Expected*	Gelatine: Expected*	Tallow: Expected*	Gelatine: Expected*
A_1	yes	0.1	2.90E-06	2.03E-06	0.00	0.00
A_2	no	0.1	2.90E-07	2.03E-07	0.00	0.00
B_1	yes	0.1	6.30E-06	4.41E-06	0.01	0.00
B_2	no	0.1	3.33E-06	2.33E-06	0.00	0.00
C_1	yes	0.1	4.12E-05	2.88E-05	0.04	0.03
C_2	no	0.1	3.65E-05	2.56E-05	0.04	0.03
A_1	yes	1	2.91E-05	2.04E-05	0.03	0.02
A_2	no	1	2.91E-06	2.04E-06	0.00	0.00
B_1	yes	1	6.32E-05	4.42E-05	0.06	0.04
B_2	no	1	3.33E-05	2.33E-05	0.03	0.02
C_1	yes	1	4.13E-04	2.89E-04	0.41	0.29
C_2	no	1	3.66E-04	2.57E-04	0.37	0.26
A_1	yes	100	2.78E-03	1.95E-03	2.78	1.95
A_2	no	100	2.78E-04	1.95E-04	0.28	0.19
B_1	yes	100	6.05E-03	4.23E-03	6.05	4.23
B_2	no	100	3.19E-03	2.23E-03	3.19	2.23
C_1	yes	100	3.95E-02	2.77E-02	39.51	27.66
C_2	no	100	3.51E-02	2.46E-02	35.08	24.55

Scenario A1(brain, spinal cord, skull, vertebral column: OUT); **Scenario A2** (as before, but bone marrow not infective); **Scenario B1** (brain, spinal cord, skull: OUT; vertebral column: IN); **Scenario B2** (as before, but bone marrow is not infective); **Scenario C1** (brain, spinal cord, skull, vertebral column: IN); **Scenario C2** (as before, but bone marrow is not infective)

*** Note**:

In Table 4, the "average" values for the **deterministic modelling** were:

? 10^3 (tallow) and 10^4 (gelatine) reduction of infectivity by processing,

? 10^3 as species barrier,

? 10^1 cattle oral ID_{50} per gram of brain from a bovine in the final clinical stage of disease.

Replacing any of these average values by a worst or best case assumption, e.g. as the ones indicated in paragraphs III.2.3. and III.2.4., would result in a linearly decreased or

increased residual risk. Regarding the application of worst case scenarios, it should be clear that combining at once all worst case assumptions in one deterministic scenario, may result in highly unlikely results in terms of possible risk. In the probabilistic approach, this defect is avoided as, for each of the 100.000 model runs, *combinations* of risk values for each parameter are selected at random. [However, *within* the range of one given parameter, the specific probability distribution given in paragraph III.3.4 is respected]. The chance of selecting a combination of "all worst case scenarios in one" thus much lower, but most likely more realistic than a straight forward "best - average - worst case" deterministic scope of events where each event has an equal probability to occur.

[For example, one of the worst case assumptions to make is a complete lack of effective processing, increasing the risk by a factor 1000. Another worst case assumption is that the maximal number of infected animals that could end up in one batch (about 3, which has a probability of less than 10^{-2}) would be present in the maximal number of contaminated batches (about 6, with again a probability of less than 10^{-2}). The probability of such an event is, however, less than 10^{-4} (i.e. less than 0,0001). Thus, it is easy to imagine that the probability that all worst case assumptions become reality at the same time is a manifold smaller than the above calculated likelyhood that two of the nine worst case assumptions become reality at the same time.]

III.2.7.4. Results for Regarding meat-and-bone meal:

Table 5 summarises the results of the stochastic approach for the situation that on average 3 BSE positive cattle become slaughtered for food, and all the cattle offals become processed into Meat and Bone Meal (MBM) that becomes fed back to 10 million cattle.

The **stochastic simulations** with @Risk (1997), whereby, for example, the titre reduction of processing stochastically varied between a millionfold and no reduction at all, with a 1000 fold reduction as the average value, show that the modal outcomes (i.e. the most frequent outcomes of 100.000 iterations) of all six different scenarios (A1 to C2) are well below 1 BSE case per 10 million exposed cattle. The probability distributions of the outcomes of scenarios A1, A2, B1, B2, C1 and C2, show that the probability that 1 ore more BSE-cases arise is 0.8%, 0.2%, 2%, 1.4%, 9.4% and 9.1%, respectively. For the scenarios C1 and C2 the probability that 10 or more cases arise is about 1%, the probability that 100 or more cases arise about 0,1%, the probability that 200 or more arise 0,05%, and the probability that 2000 or more arise 0.03%.

The outcomes of the **deterministic model calculations** with all the input variables set to their average (or expected) value, demonstrate that the scenario's A and B, especially when bone marrow and adnexa are not to be considered infectious, do not have to lead to any new BSE infections in the exposed population. On the other hand, it is also shown that when all SRM materials become included in the process (scenario C1 and C2), it may be expected that on average about 7 new cases/10 million exposed cattle will arise. That is to say, under the assumption that processing with 133 degrees Celcius and 3 bar for at least 20 minutes will on average lead to a titre reduction of 1^{E}-3 (i.e. a 1000 fold titre reduction). Any better effect of this processing will lead to a proportional decrease in expected BSE cases, of course.

Table 5: Summary of results of the calculations regarding expected cases of BSE in one year in a population of 10 million cattle via the feeding of bovine derived MBM, when on average 3 BSE-positive animals become slaughtered for food and aal the bovine slaughter offals are used for making MBM.

Scenario	Marrow infective?		Stochastic approach: Mode	Deterministic approach: Expected value
Scenario 1:	**Results in the situation that the mean (modal) titre of neural tissue is 10 CoID50/gramme**			
A_1	Yes		**0,0024**	**0,6**
A_2	No		**0,0004**	**0,1**
B_1	Yes		**0,0049**	**1,2**
B_2	No		**0,0041**	**0,6**
C_1	Yes		**0,0316**	**7,5**
C_2	No		**0,0285**	**6,8**
Scenario 2:	**Results in the situation that the mean (modal) titre of neural tissue is 100 CoID50/gramme**			
A_1	Yes		**0,0023**	**1,0**
A_2	No		**0,0022**	**0,6**
B_1	Yes		**0,0256**	**6,5**
B_2	No		**0,0244**	**5,9**
C_1	Yes		**0,5994**	**68,2**
C_2	No		**0,4590**	**67,5**

Scenario A_1 (brain, spinal cord, skull, vertebral column: OUT); **Scenario A_2** (as before, but bone marrow not infective) **Scenario B_1** (brain, spinal cord: OUT; skull & vertebral column: IN); **Scenario B_2** (as before, but bone marrow is not infective) **Scenario C_1**(brain, spinal cord, skull, vertebral column: IN); **Scenario C_2** (as before, but bone marrow is not infective)

IV. ELEMENTS OF DISCUSSION

The judgement of the acceptability of risk levels is beyond the mandate of the Working Group. The WG therefore only limits itself to signal a number of additional elements that may have to be taken into account when interpreting/exploiting the results presented in the previous tables.

a) On the fat quality and EU treatment 133°C/3 bar/20'.

The negative effect of "133°C/20'/3 bars" treatment on fat quality (notable fat browning) depends on the modalities of treatment:

> raw material (muscular tissues, viscera, bones, adipose tissue - fresh slaughtering wastes) treatment by heating of the mass at 133°C in autoclave and with pressure obtained by steam derived from tissues' water: during the

heat treatment, there is a at separation in autoclave and a mass mixing, but there are not valuable negative effects on fat quality;

> fat separation by pressure following heat treatment at ambient pressure and subsequent "133°C/20'/3 bars" treatment by steam injection of degreased meat (treatment with added steam, not derived from tissues' water): the separated fat subsequently treated in autoclave directly at 133°C/3 bar/20' is altered (browning);

> fat separation by pressure following heat treatment at ambient pressure and subsequent "133°C/20'/3 bars" treatment by steam injection of degreased meat (treatment with added steam, not derived from tissues' water): the fat treated in autoclave according EU with abundant insufflation of water and steam, do not brown.

b) Extract from the Report and Opinion Evaluation of the *"133°/20'/3 bars heat/pressure conditions"* for the production of gelatine regarding its equivalency with commonly used industrial gelatine production processes in terms of its capacity of inactivating/eliminating possible TSE infectivity in the raw material, adopted by the Scientific Steering Committee at its meeting of 21-22 January 1999:

"The bone material used for this particular preparation may potentially be cross-contaminated with (dried) brain, spinal cord and bone marrow[10].

It has been reported that it becomes more difficult to inactivate scrapie-infected brain-tissue by heat after it has been dried (Asher et al, 1986; 1987). However, it seems (Gelatine Delft, 1998) that the degreasing step, which precedes the drying of the bones, and carried out at a pilot scale which represent the commercial degreasing process under laboratory conditions[11], reduces the brain protein levels by a factor 300-800. It may be expected that, under operational conditions, this reduction is higher because the same laboratory experiments at pilot scale resulted in degreased bone with a fat content of 6%, compared with 3% in the commercial process."

V. ACKNOWLEDGEMENTS:

The Working Group was composed of the following experts: Dr.M.Doherr (rapporteur, bone-in meat), Dr.D.Taylor (Rapporteur, tallow), Dr.Ph.Comer (rapporteur, gelatine), Prof.Dr.M.Vanbelle, Prof.Dr.G.Piva, Prof.Dr.G.Poli, Dr.B.Urlings, Dr.B.R. Berends (*BSE&Risk* spreadsheet and the MBM calculations)

[10] Preliminary results of the still ongoing BSE pathogenesis experiment in cattle (Wells et al, 1998) are not fully conclusive: the (mice) tests for infectivity of bone marrow were only positive in the group killed at 38 months after infection with BSE, when clinical disease was evident in the cattle, and not at an earlier (2 to 36 months) or later (40 months) time after exposure to BSE. The current SEAC conclusion (SEAC, 1998) is that "*the positive result at 38 months cannot be discounted and may indicate that infectivity in bone marrow occurs occasionally, when clinical signs are apparent and there are already very high levels of infectivity in the central nervous system.*" It is noted that BSE infectivity in bovine bone-marrow has been detected in only one still ongoing experiment, and only after the onset of clinical signs.

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

VI. MATERIAL USED FOR THE RISK ASSESSMENTS AND OTHER LITERATURE REFERENCES

@Risk. Add in computer programme for Monte Carlo and Latin Hypercube simulations for risk analysis in Microsoft Excel and Lotus 1-2-3 spreadsheet models. Newfield NY (USA), Palisade corporation, 1997.

Anderson, R.M., Donnelly, C.A., Ferguson, N.M., Woolhouse, M.E.J., Watt, C.J., Udy, H.J., MaWhinney, S., Dunstan, S.P., Southwood, T.R.E., Wilesmith, J.W., Ryans, J.B.M., Hoinville, L.J., Hillerton, J.E., Austin, A.R., Wells, G.A.H., 1996. Transmission dynamics and epidemiology of BSE in British cattle. Nature, **382**, 779-788.

Asher, D.M. et al (1987) Attempts to disinfect surfaces contaminated with etiological agents of the spongiforrn encephalopathies. Abstracts of the VIIth International Congress of Virology, Edmonton, 9-14 August, p. 147.

Asher,D.M. et al (1986) Practical inactivation of scrapie agent on surfaces. Abstracts of the IXth International Congress of Infectious and Parasitic Diseases, Munich, 20-26 July.

Bader,F., Davis, G., Dinowitz, B., Garfinkle, B., Harvey, J., Kozak, R, Lubiniecki, A.., Rubino, M., Schubert, D., Wiebe, M., Woollet, G. 1997. Assessment of Risk of Bovine Spongiform Encephalopathy in Pharmaceutical Products. Pharmaceutical Research and Manufactures of America (PhRMA) - BSE Committee. Technical document, Washington D.C. (USA). 58 pp

Berends, B.R., 2000. *BSE&Risk.* Spreadsheet model in Microsoft Excel for a deterministic risk assessment and a stochastic risk assessment with @Risk. Department of the Science of Food of Animal Origin, Faculty of Veterinary Medicine, Utrecht University, The Netherlands, 2000.

Cohen, C., et al., 2000. Is Bovine Spongiform Encephalopathy (BSE) disappearing from Europe? Forecasts of BSE epidemic in Switzerland differ from the United Kingdom. [Confidential pre-publication information, to be submitted for publication]

Comer, Ph.J., 1997. Assessment of Risk from Possible BSE Infectivity in Dorsal Root Ganglia, carried out for the UK Ministry of Agriculture, Fisheries and Food and the UK Spongiform Encephalopathy Advisory Committee. Det Norske Veritas Ltd., London, 14 pp + annex.

Donnelly, C.A., Ferguson, N.M., Ghani, A.C., Anderson, R.M., 2000. The impact of control measures on the decline in the incidence of BSE in Great Britain from 1998 to 2001. [Confidential pre-publication information, to be submitted for publication]

European Commission, 1997. Listing of Specified Risk Materials: a scheme for assessing relative risks to man. Opinion of the Scientific Steering Committee of 9 December 1997

European Commission, 1998. Opinion on the revised version of the UK Date Based Export Scheme and the UK proposal on compulsory slaughter of the offspring of BSE-cases, submitted on 27.01.98 by the UK Government to the European Commission. Opinion of the Scientific Steering Committee of 19-20 February 1998.

European Commission, 1998. Opinion on the safety of bones produced as by-product of the Date Based Export Scheme. Opinion of the Scientific Steering Committee of 22-23 October 1998

European Commission, 1998. Report on the UK Date Based Export Scheme and the UK proposal on Compulsory Slaughter of the Offspring of BSE Cases. Opinion of the Scientific Steering Committee of 9 December 1997

European Commission, 1999. Opinion on Monitoring some Important aspects of the evolution of the Epidemic of BSE in Great Britain (Status, April 1999) 18-19 March 1999. Opinion of the Scientific Steering Committee of 27-28 May 1999

European Commission, 1999. Opinion on the Scientific Grounds of the Advice of 30 September 1999 of the French Food Safety Agency (the *Agence Française de Sécurité*

Sanitaire des Aliments, AFSSA), to the French Government on the Draft Decree amending the Decree of 28 October 1998 establishing specific measures applicable to certain products of bovine origin exported from the United Kingdom. Opinion of the Scientific Steering Committee of 28-29 October 1999

European Commission, 1999. Summary Report based on the meetings of 14 and 25 October 1999 of the TSE/BSE *ad-hoc* group of the Scientific Steering Committee on the Scientific Grounds of the Advice of 30 September 1999 of the French Food Safety Agency (the *Agence Française de Sécurité Sanitaire des Aliments*, AFSSA), to the French Government on the Draft Decree amending the Decree of 28 October 1998 establishing specific measures applicable to certain products of bovine origin exported from the United Kingdom.

G.M.E. (Gelatine Manufacturers of Europe), 1998. Letter and attachments of 8 January 1998 from J.Thomsen, GME Secretary General, to P.Vossen, SSC secretary. 11 pp.

Gelatine Delft Company, 1998. Information on the results of laboratory tests carried out at pilot-scale on the efficacy of the degreasing step with respect to the reduction of brain material possibly present in raw bone material. Provided in the letter of 4.12.1998 of Taylor, D., member of the Working Group, to the SSC secretariat.

Kimberlin, R.H. & Walker, C.A., 1988. Pathogenesis of scrapie. In *Novel Infectious Agents and the Central Nervous System.* Ciba Symposium No. 135 (G Bock & J Marsh, Eds): 37-62. Wiley. Chichester.

Kimberlin, R.H. & Walker, C.A., 1989. Pathogenesis of scrapie in mice after intragastric infection.*Virus Research* 12, 213-220.

Ockerman, H.W. and Hansen, C.L., 1988. Animal By-Product Processing. Cambridge, New York, Basel, Weinheim: VCH Verlagsgesellschaft mbH; Chicester (UK): Ellis Horwood Science and Technology Publishers.

Pearson, A.M. and Dutson, T.R., 1992. Inedible Meat By-products. Series: Advances in Meat Research, Volume 8. London and New York: Esevier Science Publishers, 1992.

Taylor, D.M., Woodgate, S.L. and Atkinson, M.J., 1995. Experimental rendering studies with bovine spongiform encephalopathy agent. *Veterinary Record*, 137, 605-610.

Taylor, D.M., Woodgate, S.L., Fleetwood, A.J. and Cawthorne, R.J.G., 1997. Effect of rendering procedures on scrapie agent. *Veterinary Record* 141, 643-649**.**

United Kingdom, 1999. Bone-in beef and cattle bones: further advice of 30 July 1999 to the Government from the Chief Medical Officer Prof. L.Donaldson. 12 pp.

Wells, G.A.H., Dawson, M., Hawkins, S.A.C., Austin, A.R., Green, R.B., Dexter, I., Horigan, M.W., Simmons, M.M., 1996. Preliminary observations on the pathogenesis of experimental bovine spongiform encephalopathy. In: Bovine spongiform encephalopathy: the BSE dilemma (Ed. Gibbs CJ Jr) Springer-Verlag, New York pp. 28-44.

Wells, G.A.H., Hawkins, S.A.C., Green, R.B., Austin, A.R., Dexter, I., Spencer, Y.I., Chaplin, M.J., Stack, M.J., Dawson, M., 1998. Preliminary observations on the pathogenesis of experimental bovine spongiform encephalopathy (BSE): an update.. Vet.Rec. **142**: 103-106.

Wells, G.A.H., Hawkins, S.A.C., Green, R.B., Spencer, Y.I., Dexter, I., Dawson, M., 1999. Limited detection of sternal bone marrow infectivity in the clinical phase of experimental bovine spongiform encephalopathy (BSE). Vet. Rec. **144:** 292-294.

Wilesmith, J.W. *et al*, 1988. Bovine spongiform encephalopathy: epidemiological studies. *Veterinary Record* 123, 638.

Woodgate, S.L., 1999. Personal communication from SL Woodgate (Beacon Research) to DM Taylor**.**

Annex 1: Bone weights, levels of contamination with possibly infectious tissues (resets) and tissue infective load distribution

Weights of bones from adult prime quality beef cattle (Data from EU, 1997; Pearson & Dutson, 1992; Ockerman & Hansen, 1988)**:**

Skull	7		
Vetebral column	6	6	
Os coxeae & scapulae	9	9	9
Legs	12	12	12
Ribs & sternum	8	8	8
Total	**42 kg**	**35 kg**	**29 kg**

Cow oral ID50 titres: [Data from EU, 1999]

Tissue	**Total mass in carcass**	**Titre/gramme**	**Total titre**	**Titre as a %**
Brain	500 g	10,000 /g	5000	56,21 %
Spinal cord	200 g	10,000 /g	2000	22,48 %
Trigeminal nerve Ganglia	20 g	10,000 /g	200	2,25 %
Dorsal Root Ganglia	30 g	10,000 /g	300	3,37 %
Ileum	800 g	0,32 /g	260	2,92 %
Spleen	800 g	0,032 /g	26	0,29 %
Eyes and rest of head	11600 g	0,032 /g	371	4,17 %
Bone marrow (40%ww)	16800 g (max)	0,032 /g	538	6,04 %
Bone adnexa (15% ww)	6300 g (max)	0,032 /g	202	2,27 %
Other tissues	512950 g	0,000 /g	0	0,00 %
Totals	*550000 g*	*0,016 /g*	*8897*	*100,00 %*

Additional assumptions for Scenarios A, B and C:

Sorting out of vertebrae	10% failures
Removal of spinal cords at slaughter	0.2% failure with 20 gram residue left+ 5% of every spinal cord not washed away
Removal of brains	Scenario B: 1% of brain tissue spilled during slaughter/removal of heads/skulls
Trigeminal Ganglia	10% of TRG tissue (2 g) spilled during slaughter/removal of heads

A: Total exclusion of all 'risky' bone materials (i.e., no vertebral columns and skulls used)

Tissue	**Total mass in fresh materials**	**Cow oral ID50 Titre/gramme**	**Total titre**	**as %**
Brain	0 g	10	0	0 %
Sp. cord	1,04 g	10	10,4	2 %
DRGangl.	3 g	10	30	5 %
TRGangl.	2 g	10	20	4 %
Bone marrow (40%ww of bones)	11600 g	0,032	371,2	65 %
Bone adnexa (15% ww of bones)	4350 g	0,032	139,2	24 %
Totals	*15956 g*		*570,8*	*100 %*
Total weight fresh bone material:	29,00 kg			
Titre/gramme fresh bone materials:	0,020 *(with bone marrow and adnexa infectify: A1)*			
Titre/gramme fresh bone materials:	0,002 *(without bone marrow & adnexa infectivity:A2)*			

B: Inclusion of vertebal column, but with skull brains and spinal cords removed

Brain	5 g	10	50	4 %
Sp. cord	10,4 g	10	104	8 %
DRGangl.	30 g	10	300	23 %
TRGangl.	20 g	10	200	16 %
Bone marrow (40%ww of bones)	14000 g	0,032	448	35 %
Bone adnexa (15% ww of bones)	5250 g	0,032	168	14 %
Totals	*19315,4 g*		*1270*	*100 %*
Total weight fresh bone material:	35 kg			
Titre/gramme fresh bone materials:	0,036 *(with bone marrow and adnexa infectify: B1)*			
Titre/gramme fresh bone materials:	0,019 *(without bone marrow & adnexa infectivity: B2)*			

C: Inclusion of all risk materials

Brain	500 g	10	5000	61 %
Sp. cord	200 g	10	2000	24 %
DRGangl.	30 g	10	300	4 %
TRGangl.	20 g	10	200	2 %
Bone marrow (40%ww of bones)	16800 g	0,032	537,6	7 %
Bone adnexa (15% ww of bones)	6300 g	0,032	201,6	2 %
Totals	*23850 g*		*8239,20*	*100 %*

Total weight fresh bone material: 42,00 kg

Titre/gramme fresh bone materials: 0,196 *(with bone marrow and adnexa infectifity: C1)*

Titre/gramme fresh bone materials: 0,174 *(without bone marrow & adnexa infectivity: C2)*

Annex 2:Summary table of default values and assumptions and scenarios

	Gelatine				Tallow			
	Average	**Best**	**Worst**	**Prob.Dist.**	**Average**	**Best**	**Worst**	**Prob.Dist.**
BSE Incidence per 1.000.000 slaughtered cattle (3 scenarios)	1 / 100 / 0.1			None	1 / 100 / 0.1			None
Human population	10.000.000			None	10.000.000		-	None
Number of animals per batch	5000	2500	7500	Normal (5000,750)	5000	2500	7500	Normal (5000,750)
Amount of fresh bone material per animal slaughtered	A: 29kg B: 35kg C: 43 kg			Normal (mean, 0.1*mean)	A: 29kg B: 35kg C: 43 kg			Normal (mean, 0.1*mean)
Species barrier, Scenario 1 Scenario 2	1000 10	10.000 10.000	1 1	BetaPert(b, a,w)	1000 10	10.000 10.000	1 1	BetaPert(b, a,w)
Inf. titres $CoID_{50}$/g, Scenario 1 Scenario 2	10 100	1 1	1000 1000	See text	10 100	1 1	1000 1000	See text
Infectivity reduction by processing and/or handling	10^4	10^6	10^3	BetaPert(b, a,w)	10^3	10^5	10^2	BetaPert(b, a,w)
Number of BSE positive animals per positive batch, for each incidence	1.003 1.2 1	1 1 1	3.95 2 1	Poisson	1.003 1.2 1	1 1 1	3.95 2 1	Poisson
Number of BSE positive batches, for each incidence:	1 80 0.1	0 50 0	3.9 105 1	Poisson or Normal	1 80 0.1	0 50 0	3.9 105 1	Poisson or Normal
Ratio human uses/total	70%	50%	90%	BetaPert(b, a,w)	10%	1%	25%	BetaPert(b, a,w)
Daily consumption	1	0.2	20	BetaPert(b, a,w)	1	0.5	10	BetaPert(b, a,w)

Annex 4: Table 1. Example calculation of the BSE titers used in the scenarios A to C1 for bovine derived MBM fed back to cattle.

A: Total exclusion of all 'risky' bone materials (i.e., no vertebral columns and skulls used)

Tissue	Total mass in fresh materials	Cow oral ID50 Titre/gramme	Total titre	as % of total
Brain	0,00 g	10,00000	0,00	0 %
Sp. cord	1,04 g	10,00000	10,40	1,22 %
DRGangl.	3,00 g	10,00000	30,00	3,53 %
TRGangl.	2,00 g	10,00000	20,00	2,34 %
Spleen	800 g	0,03200	25,60	3,00 %
Ileum	800 g	0,3200	2560	30,03 %
Bone marrow (40%ww)	11600,00 g	0,03200	371,20	43,54 %
Bone adnexa (15% ww)	4350,00 g	0,03200	139,20	16,34 %
Totals	*17556,04 g*		*852,40*	*100 %*
Total weight fresh material	250,00 kg			
Titre/gramme fresh material	0,0034'	*(with bone marrow and adnexa infectifity: Scenario A1)*		
Titre/gramme fresh material	0,0001	*(without bone marrow & adnexa infectivity: Scenario A2)*		

B: Inclusion of vertrebal columns, but with skull, brains and spinal cords removed

Tissue	Total mass in fresh materials	Cow oral ID50 Titre/gramme	Total titre	
Brain	5,00 g	10,00000	50	3,22 %
Sp. cord	10,40 g	10,00000	104	6,70 %
DRGangl.	30,00 g	10,00000	300	19,33 %
TRGangl.	20,00 g	10,00000	200	13,89 %
Spleen	800 g	0,03200	25,60	1,71 %
Ileum	800 g	0,3200	256,00	17,14 %
Bone marrow (40%ww)	14000 g	0,03200	448	28,87 %
Bone adnexa (15% ww)	5250 g	0,03200	168	11,83 %
Totals	*20915,40 g*		*1551,60*	*100 %*
Total weight fresh material	250,00 kg			
Titre/gramme fresh material	0,0078	*(with bone marrow and adnexa infectifity: Scenario B1)*		
Titre/gramme fresh material	0,0037	*(without bone marrow & adnexa infectivity: Scenario B2)*		

C: Inclusion of all risk materials

Tissue	Total mass in carcass	Cow oral ID50 Titre/gramme	Total titre	
Brain	500,00 g	10,00000	5000	58,68 %
Sp. cord	200,00 g	10,00000	2000	23,47 %
DRGangl.	30,00 g	10,00000	300	3,52 %
TRGangl.	20,00 g	10,00000	200	2,35 %
Spleen	800 g	0,03200	25,60	0,30 %
Ileum	800 g	0,3200	256,0	3,00 %
Bone marrow (40%ww)	16800,00 g	0,03200	537,6	6,31 %
Bone adnexa (15% ww)	6300,00 g	0,03200	201,6	2,37 %
Totals	*25450,00 g*		*8520,80*	*100 %*
Total weight fresh material	250,00 kg			
Titre/gramme fresh material	0,0341	*(with bone marrow and adnexa infectifity: Scenario C1)*		
Titre/gramme fresh material	0,0311	*(without bone marrow & adnexa infectivity: Scenario C2)*		

Annex 4: Table 2. Summary of BSE titers/g mixed cattle offals for the scenarios A to C1 and the possible effects of feeding bovine derived MBM back to cattle.

	1: With assumed infectivity for marrow	2: Without infectivity for marrow
1. Titers of neural tissues 1 COid50/g (best case)		
Scenario A	2,228^{E}-03 COid50/g	2,2111^{E} –04 COid50/g
Scenario B	4,249^{E}-03 COid50/g	1,3687^{E}-03 COid50/g
Scenario C	5,993^{E}-03 COid50/g	3,1169^{E}-03 COid50/g
2: Titers of neural tissues 10 COid50/g (expected average)		
Scenario A	2,488^{E}-03 COid50/g	4,464^{E}-04 COid50/g
Scenario B	5,285^{E}-03 COid50/g	2,821^{E}-03 COid50/g
Scenario C	3,316^{E} -02 COid50/g	3,020^{E}-02 COid50/g
3: Titers of neural tissues 1000 COid50/g (worst case)		
Scenario A	2,202^{E}-02 COid50/g	2,002^{E}-02 COid50/g
Scenario B	1,172^{E}+00 COid50/g	1,169^{E}+00 COid50/g
Scenario C	2,921^{E}+00 COid50/g	2,918^{E}+00 COid50/g