EUROPEAN COMMISSION
HEALTH & CONSUMER PROTECTION DIRECTORATE-GENERAL

Directorate B - Scientific Health Opinions
Unit B1 - Monitoring and dissemination of scientific opinions

Scientific Steering Committee

OPINION AND REPORT ON :

THE TREATMENT OF ANIMAL WASTE BY MEANS OF HIGH TEMPERATURE (150°C, 3 HOURS) AND CORRESPONDING HIGH PRESSURE ALKALINE HYDROLYSIS.

ADOPTED BY THE SCIENTIFIC STEERING COMMITTEE

AT ITS MEETING OF 16 MAY 2002

OPINION

BACKGROUND AND MANDATE

Commission Services received a submission and accompanying dossier from a commercial company requesting endorsement of a process for the safe disposal of animal waste which may be contaminated by TSEs. This process consists of a treatment of animal waste by means of high temperature (150°C, 3 Hours) and corresponding high pressure alkaline hydrolisis.

Scientific Steering Committee (SSC) was requested to address the following questions:

1. Can the treatment of animal waste, as described by the dossier, be considered safe in relation to TSE risk? Can the liquid residues be considered safe in relation to TSE risk?
2. Can the by-products resulting from this treatment (i.e. ash of the bones and teeth of vertebrates) be considered safe in relation to TSE risk?

It is not in the remit of the SSC to endorse specific commercial products and processes. This opinion therefore relates only to the nature of the process as it relates to possible human health and environmental risks. The opinion does not address practical issues such as economics and potential throughput of carcasses/tissues.

The SSC appointed a rapporteur to address the mandate in a scientific report to be discussed by the TSE/BSE *ad hoc* Group. This report, amended in the light of the discussions by the TSE/BSE ad hoc Group on 2 May 2002, is attached.

OPINION

1. Regarding the first question of the mandate the SSC concludes that the liquid residue after a 3-hour digestion cycle could retain infective potential. Under controlled laboratory conditions in a single experiment the treatment of animal waste by means of high temperature (150°C, 3 Hours) and corresponding high pressure alkaline hydrolysis has been shown to reduce the infectivity of TSE/BSE by a factor of $10^{3.5}$ – $10^{4.5}$.

   No infectivity was found after 6 hours. This may indicate that the clearance after 6 hours processing time is higher than after 3 hours. However, these experiments can only give a measure of the minimum clearance possible and do no permit to exactly quantify the clearance factor after 6 hours.

   Regarding the second question of the mandate, the SSC concludes that, on the basis of the data available, by-products of the 3-hour process could carry a risk of BSE/TSE infectivity and that this risk may decrease with the duration of processing.

   The SSC refers to the attached report for some comments on the experimental conditions, which were considered when drawing the above conclusions.

2. The possible human BSE exposure risk under field conditions not only depends on the maintenance of the efficiency of the equipment during processing, but also on factors such as: the probability that a TSE-infected animal is processed, the type of material

processed (e.g., carcasses as compared to by-products and waste from animals that tested negative for BSE), the relationship between effectiveness and throughput and workplace control and environmental protection measures. The consideration whether or not the inactivation capacity of a process is effective must take fully into account such factors. The SSC opinion of 24-25 June 1999 on "*Fallen Stock"* provides some further guidance on which materials should be disposed of and which ones could possibly be recycled for certain uses[1].

3. The dossier supporting the request for the endorsement of the process states that the levels of 68 priority pollutant semi-volatiles was low and that odour emission was moderate. However, it provides no details of what was looked for, nor of sensitivity limits. Data also needs to be provided on the nature and levels of possible non-volatile chemicals which could be generated by the process.

   The solidification of the digestate is uncommon. However, from the test experiments that exclusively used materials of sheep carcass (with a high fat content) appears that, if hydrolysate is released on a large scale to a sewer in a warm condition without extensive dilution, it might solidify under certain circumstances. In the absence of data to the contrary it must thus be assumed that any residual BSE/TSE material could co-precipitate and thence be accessible to sewer vermin.

   On the evidence of these findings, the SSC considers that the direct discharge of the liquid residues to the sewer without further treatment is not appropriate.

4. No information has been provided on the storage or handling of carcasses which might be contaminated with TSE/BSE. Consequently, it is, like for most other processes, not possible to evaluate the total risk associated with the process as a whole (from animal collection to final disposal of the residues).

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[1] Scientific Opinion 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. Adopted By the Scientific Steering Committee at its meeting of 24-25 June 1999 and re-edited at its meeting of 22-23 July 1999

REPORT ON :

THE TREATMENT OF ANIMAL WASTE BY MEANS OF HIGH TEMPERATURE (150°C, 3 HOURS) AND CORRESPONDING HIGH PRESSURE ALKALINE HYDROLYSIS

FINALISED BY THE TSE/BSE AD HOC GROUP

AT ITS MEETING OF 2 MAY 2002

SUBMITTED TO THE SCIENTIFIC STEERING COMMITTEE

AT ITS MEETING OF 16 MAY 2002

I. MANDATE AND BACKGROUND

According to the EU regulation in force on 1 January 2001, animals, animal waste or products derived thereof (e.g., animal meat and bone meal), potentially contaminated with TSE agent by-products, shall be disposed of by incineration or co-incineration. Alternative ways may be allowed following a positive scientific opinion.

The Scientific Steering Committee (SSC) has previously provided opinions on the use of rendering and incineration to reduce the risk from TSEs in animal tissues and products from these. It has also provided a framework for evaluating the risks involved in the storage, transport and handling of animal materials which may contain TSEs.

The Commission has now received a submission from a commercial company requesting endorsement of a process for the safe disposal of animal waste which may be contaminated by TSEs. This process consists of a treatment of animal waste by means of high temperature (150°C, 3 Hours) and corresponding high pressure alkaline hydrolysis.

The Commission Services therefore submitted the following questions for opinion to the Scientific Steering Committee (SSC):

1. Can the treatment of animal waste, as described by the dossier, be considered safe in relation to TSE risk? Can the liquid residues be considered safe in relation to TSE risk?
2. Can the by-products resulting from this treatment (i.e. ash of the bones and teeth of vertebrates) be considered safe in relation to TSE risk?

The SSC appointed a rapporteur to address the mandate in a scientific report to be discussed by the TSE/BSE *ad hoc* Group. This report, prepared under the rapporteurship of Prof.Dr.J.Bridges, was discussed and amended by the TSE/BSE *ad hoc* Group and finalised at its meeting of 2 May 2002.

III BASIS FOR THE OPINION

III.1. NATURE OF THE PROCESS

A whole carcass or parts of a carcass is placed in a steel alloy container. A measured amount of alkali is added either in solid form or as a solution of NaOH or KOH, (starting concentration: 1 molar), the vessel is sealed and the contents heated at 150° for 3 to 6 hours and at a corresponding high pressure (approximately 5 Bars). (In practice, the volume of alkali solution is and the duration of the process may be adjusted according to the load and composition of the material). In respect to basicity at ~ 1 N base concentration, almost no difference exists between NaOH and KOH. At the beginning of the process, entire animal carcasses can be used. Six parts of aqueous alkaline solution are used to 4 parts of tissue material. The physical energy generated by a constant pumping action continually circulates the liquid material present in the vessel thereby aiding the digestion process.

Under these conditions the tissues are dissolved and bones and teeth softened. The solid residue is a small fraction of the original weight. The use of alkaline conditions minimises gaseous emissions of gases such as CO_2, NO_2, SO_2.

III.2. HUMAN HEALTH AND ENVIRONMENTAL CONSIDERATIONS

In evaluating the process in respect of the questions posed by the Commission Services, three issues need to be addressed:

i) Does the process destroy/inactivate TSEs (including BSE) and if so how effective is the destruction/inactivation?

ii) Are there pathogens and/or chemicals whose potency is not reduced significantly inactivated by the process?

iii) Are any of the end products of the process of concern from a human health risk or an environmental risk viewpoint?

In considering the evidence available to deal with these issues it is important to consider whether the data has been generated under very controlled laboratory conditions or under practical large scale working conditions. In this context it should be noted that it is often the case that data derived under laboratory conditions demonstrates higher efficiency in destruction/inactivation and lower levels of contamination than will occur in regular use at the industrial scale.

III.2.1 Destruction/inactivation of TSEs.

a. General

Alkaline hydrolysis at high temperatures leads to the breakage of ester or amide bonds in particular. As a consequence it will inactivate and degrade proteins, nucleic acids, fats and polysaccharides.

Kaye *et al* (1998) have assessed the efficacy of high temperature and pressure alkaline hydrolysis in destroying a number of pathogens using a commercially available large laboratory animal tissue digester. It should be noted in this study that the digester was operated at 110° /120° for 18 hours at 1516 square inch, different from the here proposed conditions of WR2 of 150°C for 3 hrs at 85 psi . The pathogens examined were *S. aureus, M. fortuitum, C. albicans, B. subtilis, P. aeruginosa, A. fumigatus, M. bovis* BCG, MS-2 bacterophage and *G. muris*. The pathogens were contained in dialysis bags and the animal tissue being digested was kept separate. Within the limits of the sensitivity of the analysis no viable organisms could be detected. Destruction of TSE/BSE was not assessed directly.

The following paragraphs summarise the already published evidence on the TSE inactivation capability of alkaline treatment in combination with temperature. This evidence is also summarised briefly in the table in **annex 1.**

Although autoclaving or exposure to sodium hydroxide *at room temperature*[2] are not completely effective *per se* for inactivating TSE agents, adequate inactivation

[2] Work with sodium hydroxide, involving the BSE agent and rodent-passaged strains of scrapie agent (Taylor *et al,* 1994) has demonstrated that infectivity can be shown to survive exposure to 2M sodium hydroxide for up to two hours at room

has been achieved under various experimental conditions by combining these procedures. Taguchi *et al* (1991) and Ernst and Race (1993) described the successful inactivation of rodent-passaged strains of CJD and scrapie agents respectively by a sequential process involving exposure to 1M sodium hydroxide, followed by autoclaving at 121°C for 30 or 60 minutes respectively. Complete inactivation of a hamster-passaged scrapie agent has also been reported after autoclaving at 121°C for 90 minutes in the presence of 1M sodium hydroxide (Prusiner *et al*, 1984). It has also been observed that when a mouse-passaged strain of scrapie agent is autoclaved at 121°C for 30 min in the presence of 2M sodium hydroxide (without a prior holding period in sodium hydroxide), inactivation can be achieved (Taylor *et al,* 1997). More recently, it has been shown that 301V, which is a high titre and extremely thermostable strain of mouse-passaged BSE agent, can be inactivated by boiling in 1M sodium hydroxide for one minute (Taylor *et al,* 1999). A report by Taylor (2000) addresses specifically the issue of the effects of the process on TSEs. This is based on some direct experiments together with a literature search.

Although each of these studies demonstrates the likelihood of inactivation of a TSE/BSE the extent of inactivation is not entirely clear because the sensitivity of the TSE/BSE detection method is not well defined. It is noted, however, that the WHO recommends a hot alkali process as a means of inactivating a CJD agent.

b. <u>The Somerville (2002) experiments</u>.

The UK Department of the Environment, Food and Rural Affairs (DEFRA) is currently funding a validation study to test (at pilot scale) the effectiveness of the ”pressurised steam with alkaline hydrolysis as a means of inactivating TSE agents[3]“. The study started in August 1999 and is scheduled to end in September 2002. An interim report was released on 26th March 2002 (Somerville R, 2002):

> This investigation examined three alkali digestion conditions each at 150°C: sodium hydroxide for 3 hours, sodium hydroxide for 6 hours and potassium hydroxide for 3 hours. The process duration starts after the heating-up phase and ends before the cooling down phase. The vessel is hermetically closed at the end of the heating up phase, after most of the air has been removed. The pressure achieved is influenced by the temperature/moisture conditions but is of the order of approximately 5 bars. The biological material used was sheeps' heads into which 301v infected mouse brain tissue was inserted by means of a hole drilled in the skull. The hole was then sealed. A 35 litre capacity WR2 equipment was used, its use being supervised by specialists from the company. The resultant hydrolysate from the digestion process was in each case brown and syrupy with a pH of between 10.5 – 11 when warm. It tended to solidify on cooling. This necessitated its disposal by incineration. In order to test for the survival of TSE, small aliquots

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temperature. With hamster-passaged scrapie agent, although more than five logs of infectivity were lost following such treatments, around four logs survived. Other studies on rodent-passaged strains of scrapie agent have reported on the detection of residual infectivity following treatment with 1M sodium hydroxide (Diringer & Braig, 1989; Ernst & Race, 1993) even for periods of up to 24 hours (Prusiner *et al,* 1984). Mouse-passaged CJD agents have also been reported to survive exposure to 1M (Tamai *et al,* 1988) or 2 M sodium hydroxide (Tateishi *et al,* 1988). As with autoclaving, there have been no hydrolytic processes described that can reliably inactivate TDE agents.

[3] The 301V agent is being used for the experiments.

were diluted prior to their injection into naïve mice. Under each hydrolysis condition at 1 in 4 dilution or 1 in 9 dilution no TSE effects could be identified, either from observation of clinical signs or from histo-pathological investigation 500 days after injection. However, in the case of the 3-hour potassium hydroxide treatment (4 out of 12 animals) and the 3-hour sodium hydroxide treatment (1 out of 12 animals) characteristic TSE effects were observed at 1 in 49 dilution. The lack of any dose response relationship and uncertainties regarding method sensitivity do not allow a reliable estimate to be made of the extent of infectivity reduction. Since the data do not fit into a titration series with 100% positive cases at a low dilution and 0% cases at high dilution it is not possible to calculate the titres with precision. The data do indicate that the residual titre is just a little higher than the minimum detectable by the assay. The experiments demonstrated clearance of about 10^4 ID_{50}. There may have been greater reduction of titre in the six hour run but this has not been demonstrated because of the limits on the sensitivity of detection of the assay).

c. Comments on the above experiments:

- The experiments of the types as described above cannot demonstrate a "qualitative" destruction of all TSE infectivity in a test sample, but only a quantitative or semi-quantitative reduction in the amount of infectivity, the "clearance" factor, which is limited by the sensitivity of the assay system and the starting titre of the TSE spiked material. Hence in the Somerville (2002) experiments the maximum clearance that could be demonstrated was about 10^4 ID_{50}. The three hour digestions are very close to this value, since there were positive cases in the test animals. The six hour sample could also be close to this value or be much lower. The limitations on the experiment do not allow us to decide.
- The experiments listed in **Annex 1** do not address the evidence showing that BSE and the BSE derived mouse passaged strain 301V used in the Somerville (2002) are now thought to be more thermostable than the TSE strains used in the experiments cited in the Annex. There are few papers discussing this point e.g. Schreuder *et al* (1998) and Taylor *et al* (1999). Taylor et al (1999) did use 301V in their experiment cited above. However the conditions of exposure differ.

d. Parallel laboratory experiments (WR^2, 2002c; see also **Annex 2**) using less stringent alkaline treatment, showed that the hydrolysate comprised a mixture of amino acids and peptides. It is stated that this destructive process occurs whether or not the amino acid is bound into a peptide or protein. A range of new cross linked peptides and formation of D amino acids was found along with the destruction of serine, glutamine, threonine, cysteine, cystine and arginine particularly. It is claimed that none of the peptides is of more than 25 residues. However, the sensitivity of the method used to detect residual proteins and peptides is relatively poor. A study at De Mulder in March 2002 on the digestate from pig bones and head (WR2, 2002b) indicates a protein recovery of between

7.1 and 8.2 per cent. No experimental details of this study however are available.

e. According to Edinburgh City Council (2002), the composition of of 4 kg of semi-solid mush of deintegrated bones, discrete fragements of bones and a brown coloured liquid resulting from the high temperature (150°C, 3 Hours) and corresponding high pressure alkaline hydrolisis under pressure of a 46 kg sheep carcass is as follows:

Water:	51.0%
Mineral matter:	47.6%
Organic and volatile matter:	1.4%
Calcium (as calcium phosphate):	47.6%
pH value:	12.9
Ammonia (as ammonium hydroxide):	0.06%

No analysis was conducted of the organic matter to identify possible protein and/or peptide content. The Somerville (2002) assays on the residual bone (150°C, 3 Hours) had no positive cases and therefore show similar clearances to the assay on the six hour sample.

f. Note:

In a reaction to Somerville (2002), Kaye (2002), writing on behalf of the Company, considers that the incomplete inactivation after 3 hours may be explained by the specific set up of the experiment which differs as follows from field conditions:

- the hole in the skulls through which the sheep brains were spiked with TSE agent has been sealed with Teflon∑ bungs. This material is highly resistant to alkali treatment and may therefore have constituted an initial barrier to the penetration of the alkali and the hydrolysis of brain material;
- The heads used for the experiment ere frozen. This may have resulted in a reduction of the real active process duration and in a reduction of the concentration of alkali to which the brain was exposed because the alkali would have first reacted with the unfrozen mixture of other tissues that were added in the vessel to simulate a total weight comparable with a sheep carcass.
- For a protection of workers the heads were wrapped in polyethylene bags. These could act as an additional barrier to the exposure to the alkaline solution. Other materials such as polyvinyl alcohol plastics would have been destroyed (much) more rapidly.

In the light of the above, Kaye (2002) concludes that the experimental conditions were really a worst case scenario and that alkali hydrolysis process operating for 3 hours at 150°C would effectively inactivate TSEs to risk levels well below those demonstrated for any other current process.

The TSE/BSE *ad hoc* Group considers that the exposure of the sheep brain material to the appropriate alkali solution is indeed a significant factor. The frozen state of the head and/or the plastic bag might have further delayed the access of the alkaline solution to the brain material prior to reaching the operating temperature. Perhaps the prions were then only accessible once the skin, part of the skull and tissue had been digested and this may have resulted in too great a neutralisation of the NaOH/KOH. It is possible that the prions only experienced a pH of 11-12 (ie: between 1/100 and 1/1000th of the original strength)[4].

On the other hand, there may exist other reasons why the process was/is not fully effective: the relatively long heating phase might for example have induced the conversion of the prion into a more stable and inactivation-resistant form (Somerville *et al*, 2002)..

Little is also known as to how the experimental results would apply under industrial field conditions with several whole carcasses, possibly representing several tons, and also with possible delays in the access of the alkali solution to brain or CNS material.

The Kaye (2000) considerations are therefore insufficient to remove the doubt about the effectiveness of the process as currently applied to dealing with potentially TSE/BSE infected carcasses:

III.2.2. Survival of other pathogens and chemicals

A summary of the known characteristics of the residue after treatment is given in annex 2. The data indicates that pathogens are generally destroyed after the alkaline high temperature and pressure treatment process (see for example Kaye *et al,* 1998). Whether the process will reduce the infectivity of all human pathogens needs further study. A number of chemical agents are highly likely to remain intact however, for example metals.

III.2.3. Chemicals created by the process

The process results in the formation of an alkaline (pH 10.3-11.5) aqueous residue with a high BOD (biological oxygen demand, 50.000-75.000 mg/l) and a higher COD (chemical oxygen demand, up to 100.000 mg/l).

NB: In the digestate analysis study at De Mulder in March 2002 (WR2, 2002b), BOD levels were up to 2 fold higher than these values. Higher ammonia concentrations were also found. Since it is a batch process, if the process is used on a large scale without controlled release, the disposal of this fluid to the foul sewer might cause problems for sewage treatment works.

[4]. It becomes than difficult on the basis of the experiments conducted to identify whether or not significant further degradation of proteins / peptides would occur if the process is extended for 4 or 6 hours. The pH in the incubator at this time was around pH11, which might be insufficient for significant further digestion to take place.

The potential formation of two chemical groups needs to be examined particularly:

a) chlorophenols, dioxins and other polychlorinated hydrocarbons

One of the principal public concerns about the incineration of animal waste is dioxin generation. It is appropriate therefore to consider whether the WR2 process might also generate toxic chemicals. Research has shown (for example: Wikstom & Marklund, 2001) that chlorophenols, dioxins, etc are formed under a number of conditions of relevance to the proposed process i.e.:

- in the presence of weak to strong alkali;
- at process temperatures in excess of 150° (particularly (250-400°);
- in the presence of chloride and/or organochlorine compounds.

Chloride is of course a common element in animal tissues. Since the proposed process conditions involve a strong alkali and a temperature of 150°C, the extent of the possible formation of chlorophenols, dioxins and other chlorinated hydrocarbons needs to be investigated. According to WR^2 (2001) the process does not generate detectable emissions of dioxins[5], furans, SO_2 or NO_x, however no details of what else was looked for, nor of sensitivity limits are available in WR^2 (2001).

b) generation of fumes and gases at the end of the process

Further information is requested on the following:

- greenhouse gas emissions on neutralisation of the alkaline pH, e.g. CO_2
- VOC release, e.g. methanol, phenols, when the vessel is opened at the end of the digestion.

IV. CONCLUSIONS

1. The process appears to cause a very substantial reduction of BSE/TSE infectivity but after 3 hours does not result in complete destruction of TSE/BSE. From the experiments conducted by Somerville (2002) a reduction of infectivity after 3 hours of between $10^{3.5}$ and $10^{4.5}$ seems likely**.** No infectivity was found after 6 hours. The experimental set-up does however not permit to exactly quantify the clearance factor after 6 hours. One problem in interpreting the data in this study is that it is difficult to explain why in the study the highest dilution samples showed the greatest infectivity. It is important to resolve this question.

   The experiments did not investigate the inactivation for durations between 4 and 6 hours. The data indicate that the process applied for 3 hours it is more

[5] Dioxins (the most studied isomer is 2,3,7,8-tetrachlorodibenzo-p-dioxin or TCDD) are formed during burning (incineration) of carbon compounds in the presence of organically bound chorine compounds, typically polyvinylchloride. According to George Russell (WRE, personal communication, 30.04.02Because of the aqueous nature and low temperature (150°C) in the WR^2 process, nor rearrangement of the carbon skeletons of dioxin precursor molecules takes place. He also states that, compounds such as pentachlorophenol can be recovered unchanged after the alkaline digestion process and even extracted from digest if paraffin wax or oil is added during the process. [Patent pending]. The same can be stated for polyvinlychloride (PVC) which can be recovered unchanged but melted if in the form of tygon tubing or PVC gloves from the alkaline digests.

effective than the heat/pressure rendering process at 133°C during 20 minutes and at 3 bars which is currently accepted to have a TSE infectivity reduction capacity of approx. $10^{3.0}$ (EC, 1999). However, such comparisons are problematic because the impact of the rendering refers to the industrial scale process, while the alkaline hydrolysis infectivity reduction study was carried out under laboratory conditions and the equipment was used optimally (supervised by company experts). It may well be the case that, in the field situation, less substantial reductions in infectivity would be achieved using alkaline hydrolysis.

2. Pirnie (2000) states that the levels of 68 priority pollutant semi-volatiles was low and that odour emission was moderate. However, his report gives no details of what was looked for, nor of sensitivity limits.

   Assessment is required of the nature of any products produced during the process other than protein, lipid, carbohydrate and nucleic acid degradation products.

3. Before the process can be recommended in a substantially scaled up form, measurement of appropriate parameters under actual practical plant conditions is essential. An effective monitoring regimen for the day-to-day performance of the equipment must also be devised.

4. Associated with the process, safe procedures for storage and handling of carcasses to be processed, which may be contaminated with TSE/BSE will need to be identified and implemented. These procedures should include consideration of the issues identified in the Notes of 27 October 2000 of the Scientific Steering Committee on the safe handling, transport and temporary storage of meat-and-bone meal which may be contaminated with a BSE agent or other pathogens.

5. On the basis of the evidence available, the liquid residues after a process duration of 3 hours retain a significant BSE/TSE infectivity risk [if the starting material contained high levels of infectivity]. They could also contain chemicals of toxicological concern. It is therefore not appropriate to permit their direct discharge to the sewer. Moreover, from a practical viewpoint the solidification of the hydrolysate on cooling is of concern in respect of the disposal. If hydrolysate is released on a large scale to a sewer in a warm condition without extensive dilution it is likely to precipate. In the absence of data to the contrary it must be assumed that any residual BSE/TSE material could co-precipitate and thence be accessible to sewer vermin.

   If it is proposed that the residue is incinerated, hydrochloric acid should not be used as a neutralizing agent since it will facilitate dioxin formation.

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WR^2, 2002c. Amino-acid composition of alkali-treated and control brain / spinal cord tissue previously submitted to the WR2 heat-alkaline treatment for 3 hours at 150°C and with either 7.5% or 5¨% of its weight with NaOH. Submitted to the SSC secretariat on 22 March 2002 and updated on 29 April 2002.

WR^2, 2002d. Basic strengths of alkali hydroxides. Submitted to the SSC secretariat on 29 April 2002.

WR^2, 2002e. Alkaline hydrolysis at elevated temperature. Executive Summary of submissions 2002-2002 and concluding remarks. Submitted by Waste Reduction Europe Ltd to the SSC secretariat on 29 April 2002.

Annex: published evidence on the inactivation capacity of alkaline treatment in combination with temperature.

Author(s)	Treatment	Strain	Source and initial infectivity level	Infectivity reduction Survival of infectivity
Prusiner *et al,* 1984;	Exposure to 1M NaOH at 121°C for 90 minutes followed by sequential gravity displacement (GD) autoclaving.	263 K	Hamster passaged scrapie 9.4	No infectivity detectable
Taguchi *et al,* 1991	Exposure to 1M NaOH during 1 hour, followed by autoclaving at 121°C for 30 minutes	Kitasoto 1	10% homogenate of CJD-infected mouse brain tissue containing $10^{5.8}$ mouse intracerebral ID_{50}/0.1 ml	No infectivity detectable
Ernst and Race, 1993	exposure to 0.09M or 0.9M sodium hydroxide for two hours, followed by autoclaving at 121°C for an hour.	263 K	a 10% homogenate of scrapie-infected hamster brain that contained $10^{9.4}$ ID_{50}/0.5ml.	No infectivity detectable in either sample
Taylor *et al* 1997	autoclaving in 2M sodium hydroxide at 121° C either immediately after adding the hydroxide or after a prior holding period in the hydroxide of one hour.	22 A	10% homogenate of scrapie-infected mouse brain tissue that contained around 10^7 ID_{50} /ml.	No infectivity detectable in either sample
Taylor *et al*, 1999	boiling in 1M sodium hydroxide for one minute.	301 V	The starting material was a 10% homogenate of BSE-infected mouse brain tissue that contained around 10^8 ID_{50}/ml.	No infectivity detectable
Grobben *et al*, 2002	(0,3 M NaOH, during 2 hours at pH ≥ 13 at room t°) after the demineralisation step in the manufacturing process of bone gelatine.	301 V	Bone spiked with BSE-infected mouse brain tissue (Titre $10^{8.7}$ ID_{50})	No detectable infectivity at the end of the overall gelatine production process.

Annex 2: Summary of the known characteristics of the residue after treatment. of animal waste by alkaline hydrolysis under pressure at 150°C during 3 Hours".

The basic reaction of hot alkali (NaOH or KOH 1M) solution with tissue is first to solubilise and then to hydrolyse the different components of the carcasses. At the same time, the treatment destroys all representative classes of potentially infectious micro organisms (Kaye *et al*, 1998). The undiluted effluent (pH = 11-12) has a BOD value averaging 70.000 mg/ L, if the ratio of tissue to fluid (water + alkali) is 4 parts tissue to 6 parts liquid.

The proteins of the carcasses are hydrolysed and degraded, yielding a sterile solution of amino acids and peptides. Information on the exact nature and the molecular weights of the peptides was not available. From the available documentation (WR2, 2002c), the following can be derived:

> A pure protein like casein submitted to an alkaline hydrolysis under the WR^2 conditions results, after 3 hrs at 150°C in the cleavage of approximately 35 % of all peptide bonds. The bulk of the resulting mixture consists of free amino acids together with di-tri-tetra-and penta peptides. The available analysis results (WR2, 2002c) indicate the breakdown of proteins primarily into free amino acids and peptides up to 12 residues and not larger than 25 residues. Some amino acids such as arginine, asparagine, glutamine and serine are destroyed. Cross-linking of generated peptides following β-elimination reaction at serine, cysteine and threonine residues occurs along with racemisation from the original L-configuration into a mixture of DL amino acids. These compounds are not normally found in casein. A comparative amino acid analysis of the sheep brain samples from the repeat of the Edingburgh digestion procedure shows that after 3 hours or 6 hours alkali treatment versus control, the following amino acids are strongly (30-50 %) affected: aspartic acid, glutamic acid, serine, cystine, histidine and arginine. This is, as said above, expected because of the β- elimination of water, phosphate and thiol groups. Lysine is partially transformed into lysinoalanine and arginine is hydrolysed into ornithine. Ammonia content is increased due to the destruction of serine, arginine, glutamine and asparagine. Under the hot alkaline conditions of the WR^2 process, the amino-acid sequence of the prion neuro-toxic synthetic peptide 106-126 PrP(res) would be expected to be affected. Especially lysine, threonine, asparagine and histidine should be involved. But there is no precise information as to what part of the sequence may initiate the neuro-toxic effect and whether it is specific or not, particularly as the data are derived from cell culture studies. *It is believed that infectious prions (PrP(res)), with their unique three dimensional architecture and internal cross-linking, resisting to the normal proteolytic enzymes, even one as strong as proteinase K should be attacked by a 1 molar solution of alkali such as NaOH or KOH at the level of any exposed peptide bound, liberating amino acids and peptides and causing other bounds to be exposed that can then be attacked leading to the destruction of the protein tertiary structure and, hence its normal biological activity*.

Nucleic acids. There is a rapid depolymerisation of RNA, and a slower destruction of DNA. Both RNA and DNA viruses are destroyed by the pressurised hot alkali solution.

The lipids. The ester bonds of triglycerides are hydrolysed by the alkali solution as well as the sterol esters and phospholipids, producing the salts of fatty acids, i.e. soaps, aside the other components. As shown in the Edinburgh experiment there is an increase in saponification with time of hydrolysis. Amide groups in glycolipids are also hydrolysed. Poly-unsaturated fatty acids and carotenoïds undergo molecular rearrangements and are thus lose their biological activity.

The carbohydrates. Glycogen of muscles and liver, as well as starch of the gut content are solubilised and hydrolysed to monosaccharides, but more slowly than proteins or fats. The monosaccharides are also rapidly destroyed. It is well known that the β(1-4)- linked glycans

as cellulose or some hemi-celluloses are quite resistant to the alkali treatment. Glycoproteins, glycolipids and glycosaminoglycans of connective tissues as well as chitins of insects, lose critical groups from their molecules. Also the 1-3 linked glycans of chondroitin sulfate are slowly degraded.

Solid residues. The composition of the solid residues(< 3%) is related to the bone content of the digested carcasses . The bone residues are so fragile that they can easily be crushed by virtually any compaction or crushing means, including bare hands. According to Edinburgh City Council (2002), the composition of of 4 kg of semi-solid mush of deintegrated bones, discrete fragements of bones and a brown coloured liquid resulting from the high temperature (150°C, 3 Hours) and corresponding high pressure alkaline hydrolysis under pressure of a 46 kg sheep carcass. is as follows:

Water:	51.0%
Mineral matter:	47.6%
Organic and volatile matter:	1.4%
Calcium (as calcium phosphate):	47.6%
pH value:	12.9
Ammonia (as ammonium hydroxide):	0.06%