EUROPEAN COMMISSION
HEALTH & CONSUMER PROTECTION DIRECTORATE-GENERAL

Scientific Steering Committee

UPDATED OPINION AND REPORT ON :

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

INITIALLY ADOPTED BY

THE SCIENTIFIC STEERING COMMITTEE

AT ITS MEETING OF 16 MAY 2002

AND REVISED AT ITS MEETING OF 7-8 NOVEMBER 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 hydrolysis. 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 in regard to possible human health and environmental risks arising from possible exposure to BSE / prion proteins. The opinion does not address practical issues such as economics and potential throughput of carcasses/tissues.

An opinion was initially adopted on 16 May 2002. Subsequently, comments, substantial additional data including analytical results, a risk assessment as well as a number of proposals for the safe recycling or disposal of the residues were submitted to the SSC secretariat by the company in June 2002. These were analysed by the rapporteurs, the TSE/BSE *ad hoc* Group (at its meeting of 5 September 2002) and the SSC (at its meeting of 7-8 November 2002). The amended opinion and report follow hereafter.

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 high pressure alkaline hydrolysis has been shown to reduce the infectivity of TSE/BSE by a factor of $10^{3.5}$ – $10^{4.5}$. Due to constraints specific to this experiment, further studies on the combination of heat, pH and time in clearance studies are needed before any final assurance could be given regarding the safety of the process with respect to TSE risks.

   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 not permit the quantification of 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; further data would be needed in order to make a definitive statement.

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 and/or environmental contamination risks 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, dilution of the possible residual infectivity, and environmental protection measures. The consideration whether or not the inactivation capacity of a process is effective must take fully into account such factors, but the SSC nevertheless considers, as a principle, that the release into the environment of residual TSE infectivity should be avoided.

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. Detailed analyses provided recently[1] by the company shows that dioxins in the air could not be detected nor chlorophenols and other polychlorinated hydrocarbons in the residual fluid of the alkaline process, but high biological oxygen demand (BOD) and chemical oxygen demand (COD) values were identified in the residual fluid. Most of the volatile organic compounds (VOCs) were reported to be below the given chemical detection limits. For the compounds detected most concentrations were below their respective odour thresholds. Emissions of some reduced sulphur compounds and amines require special attention in order to avoid potential odour impacts. Appropriate emission-reduction measures and air ventilation exhaust systems are recommended.

   The SSC is nevertheless concerned about the fact that, if formed in a liquid medium, dioxin is most unlikely to pass into air, instead it will adsorb to solids or to the sides of the processing chamber. The analyses of air emissions are therefore not a sufficient method to exclude the possible presence of dioxins in the effluent (either as “natural” environmental background contamination or as newly formed substances during the alkaline treatment).

   As the air emission analysis is not sufficient to confirm the possible presence of dioxins in the effluent, additional data/ analyses are needed to verify whether during the alkaline hydrolysis process described in the attached report, dioxins are formed in *addition* to background levels that may already have been present. For that purpose samples taken both before and after the process would need to be analyzed using a method of sufficient sensitivity. Such results would also provide the basis to decide whether or not the levels of possibly newly produced dioxins and dibenzofurans (if any) would exceed pre-set safety margins.

   The solidification of the digestate is reported by the company to be uncommon. However, from the test experiments that exclusively used materials of sheep carcass (with a high fat content) it 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

[1] On 18 July 2002, following a recommendation in the initial opinion adopted on 16 May 2002.

residual BSE/TSE material could co-precipitate and hence be accessible to sewer vermin.

On the basis of the above evidence, the SSC considers that for the time being the direct discharge of the liquid residues to the sewer without further treatment is not appropriate. The SSC opinion of 24-25 June 1999 on "*Fallen Stock*" [2]provides some further guidance on the disposal or possible recycling for certain uses of the residues. As a summary this opinion implies:

- If the risk of TSE infectivity can be excluded, recycling of residues from the treatment of animal waste by means of high temperature (150°C, 3 Hours) and high pressure alkaline hydrolysis could be acceptable for various purposes provided the other risks discussed in the SSC opinion of 24-25 June 1999 are excluded (e.g., presence of heavy metals, toxic substances, etc.). If analyses of the various types of residues (air, effluent, sludge) from the field equipment, carried out as part of a risk analysis prior to its installation, show that such is the case[3], the use of the sludge as fertiliser, as soil additive or as feed is acceptable provided the standards[4] for these uses are met.
- If the presence of residual TSE infectivity cannot be excluded, residues should be disposed of as described in the SSC opinion of 24-25 June 1999. This opinion of 1999 did not consider anaerobic digestion as safe because the TSE clearance level by anaerobic digestion process is unknown. However, should reliable published data show that the levels of residual TSE infectivity in biogas are likely to be very low and would be eliminated during the burning of the gas, the recovery of bio-energy from anaerobically produced biogas could be acceptable if sufficient precautions (e.g., filters) are taken to exclude contamination of the gas. As the anaerobic process for the production of biogas may result in sludge containing infectious activity, burning, incineration or controlled landfill should be the methods of disposal for the residual sludge, filter contents, etc.

  Note: The formation of chlorophenols, dioxins and polychlorinated hydrocarbons in a combustion process can use any form of chlorine including chloride ions. Combustion of the effluent could thus give rise to dioxin formation. However, the SSC considers that this risk is minimized/excluded if the standards set for that purpose are respected, i.e., a rapid combustion at 900°C or above followed by rapid chilling (quenching) and with appropriate filtration by adding charcoal to the emissions or other air pollution devices.

4. Like for all waste disposal and recycling processes, a site-specific risk assessment of the process as a whole (from animal collection to final disposal of the residues) is required when considering the installation of the equipment in a particular situation, so as to minimise workers exposure, environmental impacts, etc.

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[2] 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.

[3] The dossier and the additional effluent analysis data submitted for this opinion, and taking into account the scientific report attached to its opinion of 24-25 June 1999, indicate that these other risks are probably minimal or excluded. But this would need to be confirmed for each installation and for each type and source of waste to be processed.

[4] For example: Na or K concentrations and pH of the fertiliser.

UPDATED REPORT ON :

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

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 derived 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 high pressure alkaline hydrolysis[5].

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?

A report was initially prepared for the SSC, who discussed it at its meeting of 16 May 2002. Subsequently, comments, substantial additional data including analytical results, a risk assessment as well as a number of proposals for the safe recycling or disposal of the residues were submitted to the SSC secretariat by the company at various dates between June and November 2002 (see list of references at the end of the report). At the request of the SSC, the secretariat also organised additional external expertise. All this information was analysed by the rapporteurs Prof.Dr.J.Bridges and Prof.Dr.Em.M.Vanbelle, the TSE/BSE *ad hoc* Group (at its meeting of 5 September 2002) and the SSC (at its meeting of 7-8 November 2002). The amended opinion and report follow hereafter.

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 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

[5] This process is currently applied in the USA on high volumes of several carcasses of deer and elk carcasses with CWD.

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 or 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. <u>General</u>

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 conditions proposed here 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 in this publication.

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*[6] are not completely effective *per se* for inactivating TSE agents, adequate inactivation 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 funded a validation study to test (at pilot scale) the effectiveness of the ”pressurised steam with alkaline hydrolysis as a means of inactivating TSE agents[7]“.

This investigation examined three alkali digestion conditions each at 150^{o}C: <u>(1)</u> sodium hydroxide for 3 hours, <u>(2)</u> sodium hydroxide for 6 hours and <u>(3)</u> 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

[6] 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 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.

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

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 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).

From the experiments it can thus be concluded that there is no 100% confidence that TSE infectivity can be eliminated totally by any of the options (1), (2)or (3).

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.

c. Comments on the above experiments:

- It is sometimes stated that all TSE infectivity should be removed if a process or product is to be considered effective at destroying or removing TSE infectivity. However it is not valid to state that infectivity has been destroyed in any of these experiments. All that can be stated is that no infectivity can be detected to the limits of detection of the assay used. All experiments measuring destruction or removal of infectivity are indeed constrained by the starting titre of the material to be treated and the sensitivity of detection of the assay to be used. It must be assumed in the absence of other evidence that infectivity at levels below the limits of detection is present even if none is detected. Inactivation experiments measure "clearance", the difference between input and output titres (assumed to be the limit of detection if no infectivity is detected). It is more effective to demonstrate a high clearance than to demonstrate that no infectivity has been detected but with a lower clearance because the input titre is lower or the sensitivity of the assay is poorer. Accordingly when evaluating an inactivation process it is necessary to decide what clearance levels should be demonstrated for the process to be acceptable.
- 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; WR^2, 2002f; 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 reported that none of the peptides is of more than 25 residues[8]. 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 (Kjeldahl method).

In additional analyses performed by Newtec Lab. (S.Fryas 2002) using matrix assisted laser desorption /Ionisation time of flight mass spectrometre (MaldiTof S.M.) on samples derived from a digestion of an entire sheep animal carried out for 3 hours, 150°C, 5 bars in a standard 130 kg capacity commercial digester loaded with fresh sheep carcass and several large bovine bones, demonstrate in all samples low molecular weight signals of amino acids, but no signals consistent with the possible presence of peptides.

Finally another laboratory (S.M Kelly University of Glasgow) analysed aliquots of the same samples with a technique optimised to detect small peptides and reported that no peptides with a M.W. higher than 800 (i.e. 7-8 amino acid residues) were present.

Note: Comment from the SSC:

The question is what level of prions would be detected by this methodology? One could assume that prion degradation is proportional to the degradation of other proteins/peptides. Data to date suggest this assumption is not necessarily justified. Only if the analysis of the material at the end of the Sommerville experiments had shown that such longer chain peptides were present in the test positive samples but not in the test

[8] WR^2 (2002, f) also reports on analyses of the sizes and size distribution of the peptides produced by the alkaline hydrolysis show that more than 91% of the peptides produced are in the 1-5 residue range (M.W. of 110 to 550 Daltons) and less than 1% could larger as 19 amino acid residues (M.W .of ~2190 Daltons)

negative samples, could one conclude that the peptide chain length is a criterion for exclusion of TSE infectivity

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%

f. Notes:

1) 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 were 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 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 (i.e.: between 1/100 and 1/1000th of the original strength)[9]. The *ad hoc* Group notes that this may be what will happen in practice.

On the other hand, there may exist other speculative 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)...

Like for most similar experiments, little is also known as to how the results would apply under 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.

2) Risk assessment calculations (Taylor, 2002) indicate that even if small amounts of BSE infectivity survive the hot alkaline process discussed in this report, this may not have any real direct implications for the safety of animal or human health when the effluent is released to the sewerage system. However the TSE/BSE *ad hoc* Group considers, as a principle, that the release into the environment of residual TSE infectivity should be avoided, because of their unknown fate, the long survival time and the possible risk of concentration and the unknown minimal infectious doses.

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 each of the pathogens investigated is destroyed after the alkaline high temperature and pressure treatment process (see for example Kaye *et al,* 1998). A number of chemical agents are highly likely to remain intact however, for example metals.

III.2.3. Chemicals created by the process

General:

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 other digestate analyses (WR[2], 2002b), BOD levels of 100.000 – 149.000 mg/l and COD levels of 58.000-151.000 mg/l were reported. 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.

[9]. 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:

- *chlorophenols, dioxins and other polychlorinated hydrocarbons*
- *Generation of fumes and gases at the end of the process*

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 WR^2 process might also generate toxic chemicals. Research has shown 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. (*for example*: Wikström & Marklund, 2001)

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, furans, SO_2 or NO_x.

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 organic chorine compounds or inorganic chloride. According to G. Russell (WR^2, personal communication, 30.04.02), because of the aqueous nature and low temperature (150°C) in the WR^2 process, no 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

Detailed analytical testing results of bovine carcass treated in a commercial 35 litre Digester (150°C, 3 Hours, 5 bars) with NaOH or KOH did not detect dioxins in airborne emissions or chlorophenols, and other polychlorinated hydrocarbons in the residue fluid (WR^2, 2002f). Detection limits are given and vary between 20 and 50 microgr./l for chlorophenols, phthalates and benzene derivatives and 200 microgr/ l. for PCBs.

Comments from the SSC:

1. Dioxins appear only to have been measured in air, but the exact sampling method used to measure it is not specified (e.g., direct sampling of emissions or of steam distillate). Assuming that direct emissions were analyzed, one could accept that no

dioxins were present and because the detection limits were sufficiently low to verify this (Prof.Em.Dr.C.Rappe, 15 October 2002, pers.comm.). The SSC is nevertheless concerned about the fact that, if formed in a liquid medium, dioxin is most unlikely to pass into air, instead it will adsorb to solids or to the sides of the processing chamber; the analyses of air emissions is therefore not a sufficient method to exclude the possible presence of dioxines in the effluent (either as "natural" environmental background contamination or as newly formed substances during the alkaline treatment).

The SSC secretariat therefore invited WR^2 to provide additional data to assess the risk of dioxin formation during the alkaline process. This information was provided on 2 October, 1 and 5 November 2002 (WR^2, 2002g, 2002h, 2002i). It basically supports the company's hypothesis that the alkaline process as described in this report does not create suitable conditions for the new creation of dioxins, at least not in addition to background levels that may already be present in the animal carcass for example as a result of environmental contamination. The information also states that an extensive literature search failed to find any scientific publication pointing at such risk.

WR^2 (2002g, 2002h, 2002i) was, together with all the other analytical data listed in section IV-Literature, further analyzed in detail. Taking also into account external expert advice (Rappe, 2002a, 2002b), the SSC concludes as follows:

a) There does exist literature that provides grounds for a concern that the alkaline hydrolysis treatment as described in this report, may create conditions that are suitable for the formation of dioxins from chlorophenols present in the carcass (For example: EPA, 1980; UK Department of the Environment, 1995). The alkaline environment, the process temperatures at or above 150°C and the presence of chloride and/or organochlorine compounds create such possibly suitable conditions. Without additional analyses, it can however not be stated what the level of such formations would be (if any).

b) As air emission analysis is not sufficient to verify the possible presence of dioxins in the effluent, additional data/ analyses are needed to verify whether during the alkaline hydrolysis process described in this report, dioxins are formed in addition to background levels that may already have been present. For that purpose a sufficient number of samples taken both before and after the process would need to be analyzed. Such results would also provide the basis to decide whether or not the levels of possibly newly produced dioxins and dibenzofurans (if any) would exceed the internationally accepted safety margins.

c) WR^2 is therefore invited to obtain the results such additional analyses, which could be carried out by a number of laboratories across Europe. (Sensitivity of currently available laboratory methods: picogram/gram).

2) The formation of chlorophenols, dioxins and polychlorinated hydrocarbons in a combustion process can use any form of chlorine including chloride ions. Combustion of the effluent could thus give rise to dioxin formation. However, the

SSC considers that this risk is minimized/excluded if the standards set for that purpose are respected, i.e., a rapid combustion at 900°C or above followed by rapid chilling (quenching) and with appropriate filtration by adding charcoal to the emissions or other air pollution devices.

Fumes and gases at the end of the process

Information was further made available 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.

Annex 2 provides an overview of this information. Most of the numerous volatile organic compounds (VOCs) were reported to be below the given chemical detection limits. For the compounds detected, most concentrations were below their respective odour thresholds. Emissions of some reduced sulphur compounds and amines require special attention in order to avoid potential odour impacts. Appropriate trapping devices or failing this, air ventilation exhaust systems are recommended.

III.3. DISPOSAL OF THE RESIDUES.

In its letter of 18.07.02 to European Commission Services, WR2 proposes a number of ways of safe disposal and/or recycling of the process residues (See **Annex 3**).

The TSE/BSE ad hoc Group considers that the SSC opinion of 24-25 June 1999 on "*Fallen Stock"* [10] provides guidance on the disposal or possible recycling for certain uses of the residues. As a summary this opinion implies:

- If the risk of TSE infectivity can be excluded, recycling of residues from the treatment of animal waste by means of high temperature (150°C, 3 Hours) and high pressure alkaline hydrolysis could be acceptable for various purposes provided the other risks discussed in the SSC opinion of 24-25 June 1999 are excluded (e.g., presence of heavy metals, toxic substances, etc.). If analyses of the various types of residues (air, effluent, sludge) from the field equipment, carried out as part of a risk analysis prior to its installation, show that such is the case, the use of the sludge as fertiliser, as soil additive or as feed is acceptable provided the standards[11] for these uses are met.

  Note (see also section III.2.3.):

  There remains concern about the fact that, if formed in a liquid medium, dioxin is most unlikely to pass into air, instead it will adsorb to solids or to the sides of the processing chamber; the analyses of air emissions is therefore not a sufficient method to exclude the possible presence of dioxins in the effluent (either as

[10] 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.

[11] For example: Na or K concentrations and pH of the fertiliser.

"natural" environmental background contamination or as newly formed substances during the alkaline treatment). Additional analyses are therefore required. As air emission analysis is not sufficient to verify the possible presence of dioxins in the effluent, additional data/ analyses are needed to verify whether during the alkaline hydrolysis process described in the attached report, dioxins are formed in addition to background levels that may already have been present. (See above).

- If the presence of residual TSE infectivity cannot be excluded, residues should be disposed of as described in the SSC opinion of 24-25 June 1999. This opinion of 1999 did not consider anaerobic digestion as safe because the TSE clearance level by anaerobic digestion process is unknown. However, should reliable published data show that the levels of residual TSE infectivity in biogas are likely to be very low and would be eliminated during the burning of the gas, the recovery of bio-energy from anaerobically produced biogas could be acceptable if sufficient precautions (e.g., filters) are taken to exclude contamination of the gas. As the anaerobic process for the production of biogas may result in sludge containing infectious activity, burning, incineration or controlled landfill should be the methods of disposal for the residual sludge, filter contents, etc.

  Note (see also section III.2.3.):

  The formation of chlorophenols, dioxins and polychlorinated hydrocarbons in a combustion process can use any form of chlorine including chloride ions. Combustion of the effluent could thus give rise to dioxin formation. However, this risk is likely to be minimized/excluded if the standards set for that purpose are respected, i.e., a rapid combustion at 900°C or above followed by rapid chilling (quenching) and with appropriate filtration by adding charcoal to the emissions or other air pollution devices.

IV. CONCLUSIONS

1. The process appears to cause a reduction of BSE/TSE infectivity after 3 hours but does not result in complete destruction of TSE/BSE infectivity. 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 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 were low and that odour emission was moderate.

   Recent detailed analysis provided by the company (WR^2, 25 June 2002, i.e., after the adoption on 16 May 2002 of the SSC's previous opinion) have shown that dioxin in the air could not be detected nor chlorophenols and other polychlorinated hydrocarbons in the residual fluid. Most of the numerous analysed VOCs were reported to be below the given chemical detection limits. For the compounds detected, most concentrations were below their respective odour thresholds. Nevertheless, the alkaline hydrolysis system could produce air emissions with potential to cause odour impact, especially for some reduced sulphur compounds, some VOCs and amines. Odours should be controlled via appropriate trapping devices or failing this via air ventilation exhaust system. The treatment goal for the waste management facilities exhaust air would be to produce a treated air stream with the concentration of all compounds below their odour threshold concentration prior to discharge to environmental atmosphere.

   There remains nevertheless concern about the fact that, if formed in a liquid medium, dioxin is most unlikely to pass into air, instead it will adsorb to solids or to the sides of the processing chamber; the analyses of air emissions is therefore not a sufficient method to exclude the possible presence of dioxins in the effluent (either as "natural" environmental background contamination or as newly formed substances during the alkaline treatment). Additional analyses are therefore required.

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 process duration of 3 hours retain a significant BSE/TSE infectivity risk [if the starting material contained high levels of infectivity]. They contain also very high BOD and COD values. 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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- An Executive Summary
- A Brief Analysis of and Commentary on the SSC Opinion and Report of May 16, 2002
- Submissions of Additional Scientific Evidence and Analysis Related to the SSC Opinion and Report:
- A Risk Assessment Relating to the Use of the Hot Alkaline Hydrolysis Process for Disposing of the Carcases of Confirmed Cases of BSE, David M. Taylor PhD MBE, SEDECON 2000, 23 May 2002
- Correction of Erroneous Analysis Method Used to Determine Presence of Protein (SSC Report Reference WR2, 2002 b) Two Confirming Independent Laboratories' Analyses of Hydrolysate Samples taken from a routine 3-Hour Digestion (of pig bones and head March 2002) at Prosper de Mulder, Doncaster, England:
  a) Report of Dr Sharon M. Kelly, Senior Protein Scientist, Protein Characterisation Facility/Post Genomic Research Centre, Institute of Biomedical and Life Sciences, University of Glasgow, Scotland UK.
  b) Report of Stephanie Fryars, Analytical Chemist and Dr. AJ Reason, Managing Director, M-Scan, Ltd., Edinburgh, Scotland, UK.

- Manufacturer's Recommendations for Specific Operating Protocols for each category of TSE/BSE Wastes and Animal By-Products Based on the SSC Opinion and Report of May 16, 2002
- Manufacturer's Recommendations for Disposal or Recovery of Value by Recycling of the Sterile Hydrolysate produced by the WR² Process.
- Alkaline Hydrolysis Is Green, Environmentally Responsible Technology
- *Submission of Additional Analysis Sensitivity Data Referenced as Needed by SSC Report, Supplied by Malcolm Pirnie, Inc., Environmental Engineers and Consultants retained by the New York State University Construction Fund for Cornell University College of Veterinary Medicine:*
  a) Pilot Test Plan: Alkaline Hydrolysis at the Cornell Vet College, Updated October 2000
  b) List of Contents, Appendix N, University of Florida Air Emissions Evaluation, Draft Environmental Impact Statement, Cornell College of Veterinary Medicine, Waste Management Facility, State University Construction Fund, August 2001
  c) Odor Evaluation Report, St. Croix Sensory, Inc. Stillwater, Minnesota USA, Deb Mathis Laboratory Manager and Charles McGinley Laboratory Technical Director, August 2001
- Introduction and Description Of Development Of The WR² Process And Its Evolution Through Significant Commercial Use And Experience
- Comparisons between Alkaline Hydrolysis And Incineration

WR^2, 2002g. Responses to questions raised by the secretariat of the Scientific Steering Committee (SSC), on behalf of the SSC and as a follow-up to its meeting of 12-13 September 2002. Including 3 annexes:

a) Alkaline hydrolysis and the destruction of various organic molecules, including drugs, dyes, chemotherapeutic agents and various toxins. 6 pages.

b) **Saegerman, C., Berkvens, D., Boelaert, F., Speybroeck, N., Van Vlaenderen, I., Lomba, M., Ermens, A., Bironf, P., Broeckaert, F., De Cock, A., Mohimont, L., Demont, S., De Poorter, G., Torfs, B., Robijns, J.M., Monfort, V., Vermeersch, J.P., Lengele, L., Bernard, A., 2002.** Detection of polychlorinated biphenyls and dioxins in Belgian cattle and estimation of the maximal potential exposure in humans through diets of bovine origin. J Toxicol Environ Health A 2002 Sep 27;65(18):1289-305

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a) Letter of 1 November 2002 from G.I.Kaye, Chairman of the WR^2 board, to the SSC secretariat

b) Wikström,**E., Marklund S., 2001** The influence of level and chlorine source on the formation of mono- to octa-chlorinated dibenzo-p-dioxins, dibenzofurans and coplanar biphenyls during combustion of an artifical municipal waste. Chemosphere, **43**, 227-234.

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Annex 1: 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	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 (WR^2, 2002c; WR^2, 2002,f), 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 (WR^2, 2002c) indicate the breakdown of proteins primarily into free amino acids and peptides up to 12 residues and not larger than 25 residues[12]. 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.

In additional analyses performed by Newtec Lab. (S.Fryas 2002) using matrix assisted laser desorption /Ionisation time of flight mass spectrometry (MaldiTof S.M.) on samples derived from a digestion carried out for 3 hours, 150°C, 5 bars in a standard 130 kg capacity commercial digester loaded with fresh sheep carcass and several large bovine bones, demonstrate in all samples low molecular weight signals of amino acids, but no signals consistent with the possible presence of peptides.

Finally another laboratory ('S.M Kelly University of Glasgow) analysed aliquots of the same samples with a technique optimised to detect small peptides and reported that no peptides with a M.W. higher than 800 (i.e. 7-8 amino acid residues) were present.

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*

[12] WR^2 (2002, f) also reports on analyses of the sizes and size distribution of the peptides produced by the alkaline hydrolysis show that more than 91% of the peptides produced are in the 1-5 residue range (M.W. of 110 to 550 Daltons) and less than 1% could larger as 19 amino acid residues (M.W .of ~2190 Daltons)

that can then be attacked leading to the destruction of the protein tertiary structure and, hence its normal biological activity.

Air emissions

The possible formation of toxic air emissions from a 16OO kg tissues digester is reported in a recent publication (Malcolm Pirnie Inc., 2002) for ammonia, amines, methanol, phenol, total hydrocarbons, total fluoride, metals, dioxins and selenium:

- A maximum ammonia concentration of 4.2mg/m^3 (6.0 ppm)
- Amine concentrations (for diethanolamine, methylamine, p.phenylenediamine and triethylamine) were below the given detection limits .Ethylamine and trimethylamine were above the detection limits ,respectively 1.2 mg/m^3 (0.62 ppm) and 4.8 mg/m^3(2 ppm).2 samples of dimethylamine were also greater than the detection limit with the highest value as 5.3 mg/m^3 (2.9 ppm)
- No methanol and phenol were detected (detection limit respectively<1.4 mg/m^3 (1.1 ppm) and 0.18 mg/m^3 (0,0 48 ppm).
- No dioxins were detectable in the air emissions (detection limit between 0.85 and 4.5 pg/m^3.
- The maximum total hydrocarbons detected expressed as hexane was 43 mg/m^3 (12 ppm)
- Total fluorides was less than the detection limit of 0.33 mg/m^3 (0.43 ppm)
- From 10 heavy metals only ARSENIC was detected in the air emissions above its given detection limit at a concentration of O,OO28 mg/m^3 below the TWA (Time Weighted Average established by OSHA (Occupational Safety and Health Administration of the US).

  Even more appropriate odour emissions analysis have been reported at the University of Florida (Performance Analytical Inc. 2002). 20 reduced sulphur compounds, aside 60 alkanes, alkenes, ketones, aromatic and halogenated hydrocarbon compounds (VOCs) as well as 8 aromatic amines were analysed using gas chromatography coupled with mass-spectroscopy and the NIOSH method for the amines. The main results may be summarised as following:

- 5 reduced sulphur compounds were detected (dimethylsulfide, n-propylmercaptan, dimethyl disulfide, carbonyl sulfide and carbon disulfide) at low concentrations but above their respective odour threshold values.
- Most of the VOCs were reported below their detection limit .For the detected compounds most of them were also below their respective odour thresholds, except for dimethylsulfide and dimethyldisulfide.
- Four aromatic amines were detected (diethylamine, dimethylamine, ethylamine and methylamine); dimethylamine was detected above its respective odour threshold but also diethylamine could present a potential odour concern.

As said before amines were also found to be present in emissions from the alkaline hydrolysis process at the University of Florida where three amines: diethylamine, ethylamine,

methylamine were found below their respective odour concentrations ,suggesting that amines present in the hydrolysate may vary with the carcasses being processed.

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%

Annex 3: Disposal of the residues.

In its letter of 18 July 2002 to European Commission Services, WR2 proposes a number of ways of safe disposal and/or recycling of the process residues. There are listed hereafter (quotations)

"a) For known BSE/TSE contaminated carcases and tissues:

Treatment of animal waste and by-products by alkaline hydrolysis for 3 hours at 150°C. [* Wherever "alkaline hydrolysis for 3 hours at 150°C" is referred to, it is understood to mean the alkaline hydrolysis process conducted in accordance with the manufacturer's recommended protocols for the use of the WR² Digestors as detailed in the submissions of 25 June.] The hydrolysate can then be cooled and processed through an anaerobic digestion system to recover energy as biogas. Because the hydrolysis of the animal tissue produces many single amino acids and small peptides, much of the nutritive value of this hydrolysate as feedstock is immediately available to the microorganisms of the anaerobic digestion system. The residual biomass from the anaerobic digester may be sent directly to a lined landfill or incinerated as a fuel additive or in a cogeneration system at appropriate temperatures.*

Alternatively, the hydrolysate can de dehydrated and the solid residue thus produced used as a fuel source for power generation or cogeneration or simply incinerated at appropriate temperature.

b) For specified risk material (SRM) and material from the over thirty month cull:

Treatment of animal waste and by-products by alkaline hydrolysis for 3 hours at 150°C. The hydrolysate can then be cooled and processed through an anaerobic digestion system to recover energy as biogas. Because the hydrolysis of the animal tissue produces many single amino acids and small peptides, much of the nutritive value of this hydrolysate as feedstock is immediately available to the microorganisms of the anaerobic digestion system. The residual biomass from the anaerobic digester may be sent directly to a lined landfill or incinerated as a fuel additive or in a cogeneration system at appropriate temperatures.

Based on the risk analysis provided by Dr. Taylor even for untreated Category 1 waste, we believe (…) the residual biomass from the anaerobic digestion of hydrolysate derived from SRM wastes may then be mixed with other biomass materials for composting or could also be land applied in wet form as a soil additive or dried and used as a fertilizer.

c) For all other biological waste material not related to BSE:

Treatment of animal waste and byproducts by alkaline hydrolysis for 3 hours at 150°C. The hydrolysate can then be cooled and used as feedstock for an anaerobic digestion system to recover energy as biogas. Because the hydrolysis of the animal tissue produces many single amino acids and small peptides, much of the nutritive value of this feedstock is immediately available to the microorganisms of the anaerobic digestion system. For the same reason, the sterile liquid hydrolysate could be pumped to a tanker truck or storage tank for subsequent dilution and spray or irrigation application as a carbon- and nitrogen-rich fertilizer.

If the hydrolysate is used as feedstock for an anaerobic digestion system, the residual biomass from the anaerobic digestion process can then be land applied in wet form as a soil additive or dried and used as a fertilizer. In this instance, where there is no history of exposure of the source tissue to TSE/BSE, it would be most conservative of the nutritive value of the biomass to return it to the soil and would complete a series of benign ecological steps that would constitute a true recycling system."