Considerations on the safety of amino acids from human hair hydrolysate used in cosmetic products for topical application, with regard to Transmissible Spongiform Encephalopathy risks: adopted by the Scientific Steering Committee at its meeting of 25-26 May 2000

Opinion

Amino acids obtained from the hydrolysis of human hair sourced from hairdressers and barbers are used by the cosmetics industry for incorporation into hair- and skin-care products. These cosmetic products that may contain human hair hydrolysates are used and applied topically on humans and animals. The Scientific Steering Committee (SSC) was requested to prepare an opinion on the safety of these amino acids with regard to the risk of contamination with TSE infectivity. It addressed the following specific questions:

- Can amino acids obtained by hydrolysis of human hair be considered safe with regard to possible TSE infectivity if they were produced by hydrolysis with hydrochloric acid (maintained at a concentration of >20% throughout the whole process which is carried out at 100 ^{o}C over a period of six hours)?

- Are there other conditions or restrictions that might need to be considered such as a) the geographical source of the raw material, b) the molecular size of the end-product, and c) the impurity content of the end-product?

- Is it possible to define other processing conditions that are more effective with regard to the inactivation of TSE infectivity?

Given the fact that the cosmetic products that may contain human hair hydrolysates are used and applied topically, awaiting the results of the inactivation experiments with spiked material which are in progress, awaiting information from the industry on the composition of the end product and therefore on the condition that the end product is only composed of amino-acids, the Scientific Steering Committee shares the conclusions of the Working Group which was established to address these questions in detail. The report of the Working Group is attached. The conclusions can be summarised as follows:

- There is no evidence that hair or skin become intrinsically infected with TSE agents but the evidence is based on a limited number of bioassays;

- There is no likely means by which they could become contaminated with TSE agents if collected from healthy, living individuals whose scalp skin was healthy;

- The amino acid manufacturing process has a capacity for inactivating TSE infectivity;

- Topical application excludes, according the current knowledge, any possible transmission of prions.

- There would be no justification in sourcing hair from selected geographic areas;

- There is no justification for requiring the molecular weights of the end product to be monitored.

- There is no need to identify manufacturing procedures that more efficiently inactivate TSE agents during the production of the amino acids by acid hydrolysis.

- Crystallisation gives additional purification.

More generally it may be concluded that human hair obtained from living individuals in hairdresser's and barber's shops that are incubating TSEs is unlikely to be inherently infected. The same conclusion has to be drawn for the skin-tissue that is likely to be a contaminant of the hair. Thus, the risk resulting from the use of human hair to provide amino acids

for incorporation into human hair- and skin-care products would appear to be negligible, particularly since the manufacturing methods discussed above have the capacity to inactivate significantly more infectivity than might ever be present in such tissues.

However, the above opinion may need to be reconsidered should the end product be a mixture containing also impurities and longer polypeptides.

Considerations on the safety of amino acids from human hair hydrolysate used in cosmetic products for topical application, with regard to Transmissible Spongiform Encephalopathy risks:

WORKING GROUP REPORT

I. Background

Amino acids obtained from the hydrolysis of human hair sourced from barbers are used by the cosmetics industry for incorporation into hair- and skin-care products. The cosmetic products that may contain human hair hydrolysates are used and applied topically. They may also be used in animals. In any event consumers may have them applied to them e.g. in the hairdressers but they also apply them themselves. Furthermore amino acids from this source could be used also in medicines and biologicals. It is quite possible that the eyes can be exposed to cosmetic products and this could be a more effective route of transmission of TSE infectivity as could broken skin that might exist in users. The classical process for the complete hydrolysis of proteins is a treatment with 6M hydrochloric acid during 22 hours and at 105°C. This however results also in the destruction of part of the sulfur containing amino-acids (e.g., cystine and methionin) which are used in cosmetic products.

The Scientific Steering Committee (SSC) was requested to prepare an opinion on the safety of these amino acids with regard to the risk of contamination with TSE infectivity. It addressed the following specific questions:

- Can amino acids (e.g., L-cystine and L-cysteine in the crystal form) obtained by hydrolysis of human hair be considered safe with regard to possible TSE infectivity if they were produced by hydrolysis with hydrochloric acid (maintained at a concentration of >20% throughout the whole process which is carried out at 100 ^{o}C over a period of six hours)?

- Are there other conditions or restrictions that might need to be considered such as a) the geographical source of the raw material, b) the molecular size of the end-product, and c) the impurity content of the end-product?

- Is it possible to define other processing conditions that are more effective with regard to the inactivation of TSE infectivity?

Hereafter follows the report of the working group which was established to address these questions in detail.

II. On the first question*:*

Can amino acids obtained by hydrolysis of human hair be considered safe with regard to possible TSE infectivity if they were produced by hydrolysis with hydrochloric acid (maintained at a concentration of >20% throughout the whole process which is carried out at 100 ^{o}C over a period of six hours)?

The human hair that is used as a source of these amino acids is collected from barbers following normal haircutting. The first point that needs to be addressed is whether or not there is any evidence that the normal form of the PrP ^{C} is expressed in hair. This is important because there is clear evidence that TSE infectivity cannot replicate in tissues that are devoid of PrP ^{C} (Büeler *et al*, 1993). However, it is noted that all cells in the body contain the PrP gene and therefore have the potential to express PrP ^{C} even if they do not normally do so. Weak expression of the PrP ^{C} was immunohistochemically demonstrated in normal human epidermis. But it was strongly upregulated in inflammatory

skin diseases, warts and tumours (Pammer *et al*, 1998). Although Pammer *et al* (1998) do not specifically mention or illustrate scalp skin or hair follicles, it could be assumed that both behave in the same way as the epidermis in other parts of the body. Thus, at least theoretically, hair follicles would have the prerequisite for PrP^{Sc} production and deposition in hairs. Also, in a trangenic mouse model, PrP^{c} does appear to be expressed in some of the cellular populations of skin (Lemaire-Vielle, 2000) [1].

On the other hand, PrP^{C} expression in a tissue does not *per se* dictate that it will support the replication of infectivity. There are tissues that express PrP^{C} but do not replicate infectivity. The capacity of any given tissue to replicate infectivity probably depends partly upon the level of expression of PrP^{C} but also on a number of other factors. Thus the presence of PrP^{C} is not a predictor of the presence of PrP^{Sc} in that tissue even if the patient was clinically affected with CJD. However, it is presently not known what level is needed to support replication of infectivity.

The cutting of hair does not essentially involve trauma to tissue other than hair, but haircutting implements such as scissors can be expected to frequently cause minor trauma to the scalp, resulting in the contamination of hair with skin-tissue. Also, scales of skin tissue in the form of dandruff would be expected to commonly contaminate hair. Thus, in assessing the TSE risk from hair, one is forced to consider also the TSE risk from skin, especially (Lemaire-Vielle, 2000) from epidermis cells or keratinocytes. If infectivity was actually contained in hair and skin, which are shed naturally, and the infectivity titre was sufficiently high the incidence of the human TSEs amongst hairdressers and others occupationally involved with hair would be expected to be detectable given the widespread nature of the resulting contamination. It is important to note that the products that contain the amino acids derived from hair are used for topical application to skin and hair. There is evidence that even high levels of TSE infectivity do not establish infection when brought into contact with intact skin, but infection does takes place when the skin surface has been broken by scarification (Taylor *et al*, 1996). In that case, impure products may constitute a risk if there had been PrP^{Sc} replication, but the WG recognises that the risk is extremely small. It is also noted that cosmetic products could come in contact with the eye (particularly of children) or with broken skin and thus provide a potentially more efficient route of infection if any was present.

The absence of any evidence for the presence of TSE infectivity in hair or skin has been acknowledged by Masters *et al* (1980) [2] , the SSC (EC, 1998, 1999a), the SC-MPMD [3] (EC, 1999b) and EMEA (1992). Similarly, no infectivity was detected in bioassays carried out on the skin of sheep with scrapie (Stamp *et al*, 1959) and cattle with BSE (SEAC, 1994). These skin samples would, of course, also carry hair even if they had been shaved. Bovine skin/hair is also reported on in the BSE Progress report of the UK Ministry of Agriculture, Food and Fisheries (MAFF, 1999) and is based on the work of the Neuropathogenesis Unit (NPU) reported in the EC Consultation on TSE in 1993, (Fraser and Foster, 1994).

An important point is that there is no means by which hair collected during haircutting from humans that are incubating TSEs could become contaminated by contact with high-risk tissues such as those of the central nervous system, provided the person was living at the time of collection or, if it is a deceased person, no autopsy with opening the skull was previously performed.

Mould *et al* (1965) observed a loss of less than one log of scrapie infectivity after exposure to a pH of 2.1 at 2 ^{o}C for 24 hours. Similarly, Brown *et al* (1986) observed a loss of only 1.8 logs after exposure to 1M hydrochloric acid for an hour at room temperature. However, hair is hydrolysed for six hours at 100 ^{o}C. Although Appel, Groschup & Riesner (1999) have reported that exposure to 1M hydrochloric acid for an hour at temperatures of 65 ^{o}C or higher leads to almost complete inactivation, the level of surviving infectivity in these experiments could well have been higher than that calculated. This is because these calculations were based upon incubation period assays, and these can considerably underestimate infectivity levels (compared with the true values obtained by bioassays) when infectivity has been exposed to partially-inactivating procedures (Taylor *et al*, 1999; 2000). Nevertheless, given that the infectivity level in hair and skin is undetectable and either nil or extremely low, the high titre losses of TSE agent after treatment with hot 1M hydrochloric acid at 65 ^{o}C reported by Appel, Groschup & Riesner (1999) suggest that the process used for the hydrolytic extraction of amino acids from human hair is likely to result in a safe product. This is because the actual

extraction process involves exposure of the hair to approximately 6M hydrochloric acid for six hours at a temperature of 100 ^{o}C. Bioassays are in progress from validation studies that involved exposing hair to a commercial hydrolytic process in which the process was spiked with hamster scrapie agent (Riesner & Appel, 1999). However, the conditions used in these studies were not identical to the ones that the SSC have defined. For example, the temperature at which the process was carried out was >105 ^{o}C, compared with the temperature of 100 ^{o}C defined by the SSC. Also, the final concentration of hydrochloric acid in the process was approximately 10%, which contrasts with the > 20% concentration that the SSC considered for hydrolysed proteins derived from bovine hides (see: Update of the SSC opinion of 22-23 October 1998 on the Safety of hydrolysed proteins, adopted on 25-26 May 2000). Also, it is evident that Riesner & Appel (1999) believe that the bioassays in hamsters need only take 150-200 days. Unfortunately, this is not the case; late positives are a common consequence of chemical and physical treatment of the agent. Incubation periods of more than 400 days have been observed frequently (Taylor, 1993), the maximum being 618 days (Taylor, 1999b). It has been recommended that such bioassays should be maintained for eighteen months (Pocchiari *et al*, 1991) or two years (Brown *et al*, 1986) to detect late positives.

III. On the second question*:*

Are there other conditions or restrictions that might need to be considered such as a) the geographical source of the raw material, b) the molecular size of the end-product, and c) the impurity content of the end-product?

As discussed above, there is no evidence that hair or skin are inherently infected or are likely to become adventitiously contaminated with TSE infectivity under the conditions of collection specified. Furthermore, as discussed above, the hydrolytic process used to manufacture the amino acids appears to have the capacity to inactivate any low level of TSE infectivity that might theoretically be present in the raw material. Also, since CJD occurs world-wide (though vCJD is currently geographically restricted), *any restrictions on the geographical sourcing of raw materials would appear to be unwarranted*. It is noted that neither PrP studies nor bioassays of skin and hair have been reported from patients with vCJD. If it was necessary to reduce any theoretical risk from such patients, then geographical sourcing advice could be given.

The question has been raised as to whether the end-products should be subjected to monitoring to determine whether the hydrolytic process has been efficient. The suggested criterion is to determine the average/maximum molecular weight of the end-product of the hydrolysis. The question is what molecular weight would represent the cut-off point between acceptable and non-acceptable products, should the end-product not consist of pure amino acids but of a mixture of amino acids and longer polypeptides. To some extent, this exercise has already been carried out with regard to the safety of polypeptides that are derived by alkaline hydrolysis from the "fleshings" obtained from bovine hides and are permitted to be incorporated into ruminant feed.

In its update of 25-26 May 2000 of the opinion of 22-23 October 1998 on the Safety of hydrolysed proteins, the SSC states:

"(...) it may be concluded that a molecular weight of <10.000D cannot be seen as an absolute guarantee for safety, per se. The criterion is indicative, and not exclusive for the quality of the hydrolysing process and of the safety regarding possible residual TSE infectivity of the final product. It seems theoretically possible that the infectious fraction (segment, part) of the BSE-agent could be smaller. However, a product with most of the molecules having a MW of < 10.000D has most likely been produced by means of production processes which, together with appropriate sourcing and respecting the other safety conditions given in the above cited SSC-opinion, guarantee a safe product. A limited range of molecular weights above the target value of less than 10.000D is therefore unlikely to affect the safety of the final product, provided, of course, all the other criteria of the opinion are complied with. Thus, a molecular weight below 10.000D may be used as an indicator but not as a safety guarantee per se."

As to the possible the impurity content of the end product, the working group sees no reason to consider possible impurities in the end product as a possible reason for concern, provided of course that it can be excluded that these impurities are due to, for example, contamination with possible TSE-infected materials.

IV. On the third question*:*

Is it possible to define other processing conditions that are more effective with regard to the inactivation of TSE infectivity?

It does not appear necessary to define conditions that are more effective with regard to the inactivation of TSE agents during the processing of human hair to provide amino acids for incorporation into hair- and skin-care products. If the above assessments had indicated that there was any significant degree of risk, the aim would have been to identify more rigorous manufacturing methods. However, the perceived level of risk is such that this is considered to be unnecessary.

V. Summary conclusions

The Working Group summarises its conclusions as follows, *provided only free amino acids are present in the product*:

- There is no evidence that hair or skin become infected with TSE agents;

- There are no likely means by which they could become contaminated with TSE agents if collected from healthy, living patients with a healthy scalp;

- The amino acid manufacturing process has a capacity for inactivating TSE infectivity;

- The cosmetic products that may contain human hair hydrolysates are used and applied only topically. It is noted that the eye could be exposed to cosmetics or that broken skin may exist and that these routes of exposure could be more efficient. Nevertheless this type of application excludes, according the current knowledge, transmission of prions.

- There would be no justification in sourcing hair from selected geographic areas unless a particular risk was perceived from patients incubating vCJD. There is currently however, no evidence of such a risk;

- There is no justification for requiring the molecular weights of the end products to be monitored; (however such monitoring may be indicated if the end product is a mixture containing also impurities and longer polypeptides.)

- There is no reason for concern over microbiological impurities [4] in the amino acid end-products;

- There is no need to identify manufacturing procedures that more efficiently inactivate TSE agents during the production of the amino acids by acid hydrolysis.

VI. Overall conclusions

The available evidence suggests that human hair obtained from living individuals in barber's shops that are incubating TSEs is unlikely to be inherently infected. The same conclusion has to be drawn for the skin-tissue that is likely to be a contaminant of the hair. Thus, the use of human hair to provide amino acids for incorporation into human hair- and skin-care products would appear to be safe, particularly since the manufacturing methods discussed above have the capacity to inactivate significantly more infectivity than might ever be present in such tissues.

VII. Acknowledgements:

The above report was prepared by a Working Group of the Scientific Steering Committee, composed as follows: Dr. D.Taylor (rapporteur), Dr.R.Bradley, Prof.Budka, Prof. J.-Y. Cesbron, Prof.D.Dormont, Prof.F.Kemper, Prof.M.Vanbelle, Prof.M.Vandevelde, Prof.J.Vives-Rego.

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***Attachment*: Short report to the SSC, adopted by the TSE/BSE *ad hoc* Group at its meeting of 15 April 1999 on the basis of a Working Group report drafted by Dr.D.M.Taylor, and attached as annex 3to the SSC minutes of 22-23 April 1999.**

***Subject*: Hydrolysed proteins: are they more safe if their molecular weight is less than 10kD?**

It has been argued that these products might be considered to be safe if the hydrolysed proteins have a MW of <10 kD. The basis for such a suggestion would be the knowledge that PrP^{sc} has a MW of 27-30kD.

The TSE/BSE ad hoc Group is of the opinion that the use of such a criterion could lead to a false sense of security because it is not known whether protein sub-components or peptides of low MW derived from PrP^{Sc} can trigger the conversion of PrP^{C} to PrP^{Sc}. However, one needs to bear in mind that the 27-30 kD PrP^{Sc} is itself a sub-component. It is the resistant 'core' that remains after proteolytic digestion of the full-length PrP^{Sc} protein that has a MW of 33-35kDa. Therefore, there is existing formal proof that a somewhat truncated form of PrP^{Sc} can convert PrP^{C} into PrP^{Sc}. It is known that more severely truncated forms of PrP^{C} can be converted to PrP^{Sc}. For example, a PrP^{C} peptide consisting of only 21 residues had properties akin to PrP^{Sc}. [6] It was neurotoxic, and had a tendency to form amyloid fibrils analogous to the scrapie-associated fibrils (SAF) found in brain extracts from TSE-infected individuals. It must be assumed that this truncated form of PrP^{Sc} might convert non-truncated PrP^{C} to PrP^{Sc}. Also, a form of PrP^{C} containing only 106 residues (MW approximately 10 kDa) was converted to PrP^{Sc} *in vitro*. [7] In addition, when this truncated PrP was expressed in transgenic mice that were deficient for wild-type PrP, these mice developed scrapie when challenged with the RML strain of mouse-passaged scrapie agent, demonstrating the convertibility of this 106 residue PrP^{C} to PrP^{Sc}. [8] More importantly, when brain-tissue from the scrapie-infected PrP106 mice was passaged into mice of the same genotype, they developed scrapie. [8] These data confirm that PrP^{C} peptides with a MW of approximately 10 kDa can not only be converted to PrP^{Sc}, but that they can also convert PrP^{C} to PrP^{Sc}. The above data tend to confirm the opinion that declaring hydrolysed proteins safe if the resulting peptides are <10 kDa could lead to a false sense of security.

The TSE/BSE *ad hoc* Group considers that only low levels of BSE infectivity could be present in the raw materials. The

two typical manufacturing processes described in the SSC opinion of 22-23 October 1998 are likely to completely inactivate even considerably higher levels of BSE infectivity than could ever be present under worst-case conditions. This is because of the combinations of heat (especially steam under pressure) and alkali that are used. A variety of data are available that show the effectiveness of heat combined with alkali on BSE and scrapie agents. [1-5]

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[1] *Lemaire-Vieille et al (2000) built transgenic mice expressing a green fluorescent protein reporter gene under the control of 6.9 kb of the bovine PrP gene regulatory sequences. The tg mice exposed under the UV light exhibited very clearly a fluorescent signal in the skin, the nails and the hair. Histological analysis of the skin showed a fluorescence signal in basal and spinocellular keratinocytes and in the external root sheet of the hair follicles and its associated sebaceous gland.*

[2] *Masters et al (1980) reported no infectivity in the hair of human patients with CJD but there is no indication of how many patients were tested.*

[3] *At its meeting of 24 March 1999, the Scientific Committee on Medicinal Products and Medical Devices (SC-MPMD) stated: "Pammer et al (1998) unequivocally demonstrate the presence of PrPc in the skin. By this finding, they extend the range of tissues known to express PrPc. The theoretical possibility that skin contains also PrPSc, the pathologic form of the prion, or TSE infectivity in significant amounts is not supported by the majority of observations. However, crucial experiments as a search for PrPSc in the skin have not yet been performed. The methods are easily available. Until those studies are completed there is no sound justification for a change in policy regarding the use of hides and skins for the preparation of gelatine."*

[4] *Toxics, heavy metals, radioelements, etc. not included.*