



**UPDATE OF THE REPORT OF THE SCIENTIFIC COMMITTEE ON ANIMAL NUTRITION ON  
THE SAFETY OF PROTEIN-RICH BIOMASS  
DERIVED LARGELY FROM CELLS OF METHANOTROPHIC BACTERIA  
GROWN USING NATURAL GAS AS CARBON SOURCE  
(BIOPROTEIN®)**

(Adopted on 14 February 2003)

**1. BACKGROUND**

Following the revision of the SCAN opinion of 3 December 2001, on the use in animal feed of protein-rich biomass derived largely from cells of methanotrophic bacteria grown using natural gas as carbon source (BioProtein®), the Company owning product BioProtein® submitted the following documents to the European Commission:

- 14-02-02 Information of bacterial strains used in animal studies.
- 6-05-02 Brewers yeast comparison to BioProtein® (enclosed to this letter).
- 16-08-02 Approval of BioProtein® – effects on Lymph nodes.

**2. TERMS OF REFERENCE**

The Scientific Committee on Animal Nutrition is asked to consider the above data in the light of the concerns it expressed in its last opinion on the effects of BioProtein® on the mesenteric lymph nodes.

**3. OPINION OF SCAN**

**3.1. Brief history**

BioProtein® is a heat-inactivated bacterial single-cell protein derived primarily from *Methylococcus* and two other species grown on natural gas as carbon source. In a toxicity study in rats, amongst others, initially no significant adverse effects were reported, and the company was granted approval for feeding this product to livestock. However, work with a modified product (NA BioProtein®) that had been heat-shocked and allowed to autolyse to reduce the RNA content for possible human food application, toxicity tests revealed significant effects in body weight, mesenteric lymph

node weight and pathology, liver pathology, white blood cell profile, bone marrow and spleen weight.

These results triggered a review of the earlier BioProtein® study, which also showed some histological changes in the mesenteric lymph nodes. Additional studies with BioProtein® confirmed increased mesenteric lymph node weights in cats and foxes, but not in pigs. Thus, these effects occurred in various species and were not satisfactorily explained with regard to product safety. This caused the SCAN to reconsider its original Opinion concerning the safety of the BioProtein® product as the heat shock / autolysis treatment apparently revealed effects that were less prominent or escaped the attention during earlier studies with BioProtein®. Explanations offered by the Company suggesting that the observations could be considered as “normal tolerance” or “normal reactions” were considered speculative and not satisfactorily supported in the context of risk assessment.

The firm has addressed the outstanding concerns of the SCAN by providing new studies to support their safety claims:

### 3.2. Antibody studies

3.2.1. An antibody study was provided that showed that BioProtein® produced BioProtein® specific antibodies in BioProtein® fed parents but not in their offspring. The conclusion of the firm is that this demonstrates that feeding sufficiently early during life prevents antibody production.

**Comment i:** the data presented raise several questions. In view of the methodology and the relatively high background level of antibodies in the control group compared to the exposed groups it is questionable whether the serum binding in this study can be concluded to be “BioProtein®-specific”. In addition the high background levels in the controls needs explanation. The increase in serum concentration was only observed in females in a dose-related manner.

**Comment ii:** The fact that serum binding to BioProtein® in offspring does occur to a lesser degree remains unexplained. In the absence of an explanation for the nature of this BioProtein® binding phenomenon, any interpretation of differences in binding remains speculative. Moreover, a need for BioProtein® feeding before weaning to avoid antibody response is not realistic in livestock production.

3.2.2. An equivalent **antibody study** was also provided that showed that substitution of Brewers Yeast for BioProtein® also produces a non-dose related increase in BY specific antibodies, which is similar but higher after prolonged feeding. The company concludes that antibody production increases with age.

**Comment i:** as regards to antibody specificity, the same argument applies as for BioProtein® antibodies, see above.

**Comment ii:** the fact that antibody production is age dependent does not allow a conclusion on safety. Furthermore, the increase in antibody production over a two-month period indicates a persistent stimulation of antibody production, and raises questions as to the suitability of the feed material.

**Comment iii:** it is not clear what the intention / conclusion of this study is with respect to the safety of BioProtein®.

### 3.3. Feeding study with Brewers Yeast

A feeding study in rats was conducted with **Brewers Yeast** with emphasis on the lymph node effects as reported for diets containing NA BioProtein®. Briefly, 20 female rats per group, 6 weeks of age, were fed 11 or 22% Brewers Yeast in their diet, the control group receiving casein. Ten rats per group were examined after 4 and 8 weeks, the parameters were routine haematology, body weight, organ weights and histopathology, with emphasis on lymphoid organs. The results showed treatment (generally dose and time) related decreases in body weight and increases in the weights of mesenteric lymph node, spleen and liver. Histopathological changes were found in liver and mesenteric lymph node, and in haematology, white blood cell counts (mainly neutrophils) were increased. The firm concludes that these phenomena are not unique to BioProtein® and can occur in other commonly consumed protein sources.

**Comment i:** Feeding of Brewers yeast at concentrations of 11 or 22 % in the diet is beyond the practical use. The fact that the adverse effects seen with (NA) BioProtein® are similar as in this brewers yeast study is no evidence that it is harmless by itself.

**Comment ii:** the study demonstrates that the effects are not fortuitous, but are apparently consistently associated with some single cell proteins. The study seems only to confirm the effects observed with NA BioProtein® / BioProtein® as found previously in different studies.

### 3.4. Review of previous studies' results

The histological slides from several previous studies have been **reviewed by a second pathologist** at the request of the firm. In general, the conclusion of the second consultant pathologist is that the lesions are milder in severity than previously stated and, in some instance the initial conclusions are “unconvincing”. The reviewer also mentions that the white blood cell count changes were observed in relative (differential) counts, and no absolute counts were given.

**Comment i:** the reviewer concludes that some surrounding tissue was present in the histological slides of the lymph nodes and that this might account for the measured weight changes. It is recognised that this is not uncommon in such minute organs that require careful preparation, but this observation does not explain the observed differences, dose relationship and the repeatability

among the various groups and studies. The reviewer does not comment on the associated increased spleen weight.

**Comment ii:** It is correct the white blood cell counts were relative, not absolute, a fact that the SCAN also regretted. However, either scenario, namely decreased number of lymphocytes, suggestive for immune suppression, or increased number of neutrophils, indicating a mobilisation of non specific inflammatory cells, is to be regarded as adverse.

In the recently submitted Brewers Yeast study, the firm concluded that the latter explanation (increase in neutrophils) was the case.

**Comment iii:** it may be correct that the results in some studies taken in isolation are unconvincing. However, the consistency of the findings among several studies seems confirming. The possible absence of serious histological effects does not render the other observed effects as being irrelevant.

### 3.5. In summary

3.5.1. The challenged observations through various studies are rather consistent. Although the applicant suggests that the effects are not very serious, their significance in terms of hazard is unclear and not well characterised. In the domain of toxicological pathology the observed adverse effects are not a common and established non-relevant change, at least not for food / feed stuffs.

3.5.2. It is not clear why all the effects observed (changes in white blood cell counts, lymph node weights, spleen and liver changes, histology changes, body weight depression) can be considered as irrelevant. However, even if the histopathological change appears to be limited as suggested in the pathology review report, it does not disqualify the other effects observed, as well as their consistency.

3.5.3. It is not clear why it is concluded that the observed effects of NA BioProtein® are essentially different from those of BioProtein®. Indeed it is in the opinion of the SCAN likely that they are mechanistically / qualitatively similar, and differ only in grade / severity.

3.5.4. The reproduction of the adverse effects observed in the (NA) BioProtein® study by a study with Brewers Yeast further supports the present concern.

In conclusion, the applicant has provided inadequate scientific evidence on the nature of the observed effects on health parameters, and insufficient information to demonstrate the safety of BioProtein® as protein source in

feeding stuff. The new information does not alleviate the concerns expressed previously<sup>1</sup>.

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<sup>1</sup> Revision of the previous reports of the Scientific Committee on Animal Nutrition on the use in animal feed of protein-rich biomass derived largely from cells of methanotrophic bacteria grown using natural gas as a carbon source, adopted on 3 December 2001