



**EUROPEAN COMMISSION**

HEALTH & CONSUMER PROTECTION DIRECTORATE-GENERAL

Directorate C - Scientific Opinions

**C2 - Management of scientific committees; scientific co-operation and networks**

**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)

## 1. SUMMARY OF PREVIOUS OPINIONS

1.1. In 1993, the SCF and SCAN were first asked their opinion about the safety of a protein-rich product "BioProtein<sup>®</sup>" which consisted of the heat-killed cells of *Methyloccus capsulatus* (Bath), *Aliccaligenes acidovorans* and two species of *Bacillus* (*Bacillus brevis* and *Bacillus firmus*) grown using natural gas as carbon source. In the Opinion published in 1995, SCAN and the SCF concluded that BioProtein<sup>®</sup> had an acceptable, but not exceptional, value as a protein source in animal nutrition. The product was considered to carry no microbiological risks or any appreciable risk to livestock, provided that the maximum level of incorporation in rations judged prudent by the Committees was not exceeded. No risk to individuals consuming the products of animals fed the protein source was identified and there were no adverse effects on the organoleptic quality of animal products. It was recognised that, in common with most other protein products, there was a risk to the health of workers of sensitisation by inhalation and the possibility of respiratory allergic reactions in susceptible individuals. In the view of the committee, this risk could be minimised if normal precautions were taken when handling the product.

However, a growth depression was noted in some target species fed high concentrations of BioProtein<sup>®</sup>. Primarily because of this, the Committees recommended that the conditions of usage should be restricted in pigs to growing pigs from 25 kg up to a 100 kg live weight and that the quantity of inclusion in complete feedingstuffs should not exceed:

- 8% for piglets starting at 25 kg
- 8% for veal calves starting at 80 kg
- 19% for salmon fish in fresh water
- 33% for salmon fish in seawater

1.2. A supplementary Dossier on BioProtein<sup>®</sup> produced in support of an extension of approval to include chickens for fattening and pigs from piglets to slaughter weight was examined in 1999. Notification was also included of small changes in the chemical composition of the final product thought to result from an increase in steady-state growth rate during fermentation and the exclusion of *B. brevis* from the inoculum.

SCAN, in an Opinion published in October 1999, concluded that omitting the strain of *B. brevis* from the fermentation was a sensible precaution and one that has no adverse implications for any previous assessment of product safety. Similarly, the minor changes to the composition of the product did not introduce any previously unrecognised hazards.

It was also noted that although the product at low concentration was well tolerated by pigs and poultry, at the higher inclusion levels tested BioProtein<sup>®</sup> resulted in the depression of growth and reduction in feed conversion efficiency noted previously. SCAN therefore recommended an upper limit of inclusion of BioProtein<sup>®</sup> in the total feed of:

- 8% for piglets and fattening pigs to slaughter weight
- 6% for chickens for fattening.

## **2. NEW DATA RELATING TO THE SAFETY OF THE PRODUCT**

- 2.1. Some months after the publication of the SCAN Opinion in 1999, the Company made available the results of additional toxicological studies. These had been initiated in support of an experimental product intended for possible use as a human food. The approximately 10% of nucleic acids present in BioProtein<sup>®</sup> are not considered a problem for livestock. However, unlike most livestock, humans lack the enzyme urate oxidase that breaks down the uric acid derived from purines to allantoin. Consequently, for food use WHO/FAO recommends that no more than 2g/day RNA should come from single cell protein and that the intake of nucleic acid from all sources should not exceed 4g/day. In order to meet this requirement the Company developed an autolytic method using endogenous nucleases to degrade and remove RNA/DNA from BioProtein<sup>®</sup>. The resulting new product, nucleic acid reduced BioProtein<sup>®</sup> (NA-BioProtein<sup>®</sup>), contains less than 2% nucleic acid.
- 2.2. The Company commissioned a thirteen-week oral feeding and toxicity study in the rat using various doses of NA-BioProtein<sup>®</sup> to a maximum of 24% of diet (other dietary components were equalled). This study was initiated in July 1997 and the full results provided to the Company in November 1999, although some preliminary results were made available earlier. A number of adverse effects were noted at all doses, the most important of which related to the immune system. All dosed animals had a higher titre of NA-BioProtein<sup>®</sup> reactive IgM than controls and females had also a higher IgG titre. Macroscopic examination revealed a substantial (2-3-fold) enlargement of the mesenteric lymph nodes at all doses and a general enlargement of lymph nodes in approximately half of the dosed animals; also spleen weight was increased and liver weight decreased. In hematology there was neutrophilia, eosinophilia and lymphopenia. The most pronounced histopathological changes induced by NA-BioProtein<sup>®</sup> were a complex of inflammatory changes in the mesenteric lymph nodes and, predominately in females, in the liver. Some of these effects were observed even at the lowest concentration used and consequently a no-effect level for NA-BioProtein<sup>®</sup> could not be set.
- 2.3. A reassessment by the Company of data from an earlier thirteen week toxicity study made with BioProtein<sup>®</sup> failed to reveal similar differences in treated groups. However, while mesenteric lymph nodes had been fixed for microscopic examination, they had not been weighed prior to fixation.
- 2.4. Although the reassessment did not indicate a similar immune response to that seen with NA-BioProtein<sup>®</sup>, a four-week “palatability” study with rats established to determine potential effects of BioProtein<sup>®</sup> on some aspects of the immune system did show adverse effects. In this study 40 rats were divided into four groups, one control and the three experimental groups fed 6.5%, 13% and 26% BioProtein<sup>®</sup> respectively. The weight of the mesenteric lymph nodes was significantly increased in males fed the two highest doses and a positive trend towards increased weight was seen in males fed the

lowest dose and in females at all doses. Microscopic examination indicated only minimal changes to mesenteric lymph nodes in the highest dose group, although these were consistent with the observations seen in NA-BioProtein<sup>®</sup>-fed rats.

- 2.5. Finally, the Company initiated late in 1998, an eight-week “lymph node toxicity study” in the male rat. A total of 30 rats were allocated to one of six groups given 1) a standard rat chow (Altromin), 2) Altromin mixed with 15% BioProtein<sup>®</sup>, 3) a semi-synthetic diet, 4) the semi-synthetic diet with 22% BioProtein<sup>®</sup>, 5) a synthetic diet and 6) the synthetic diet with 15% BioProtein<sup>®</sup>; control groups 3) and 5) were fed casein instead of BioProtein<sup>®</sup>. Inclusion of BioProtein<sup>®</sup> caused a significant increase in numbers of neutrophils and monocytes and an increase in organ weight of the mesenteric lymph nodes and spleen with all diets. However, these increases, while significant, were not of the same magnitude as seen with NA-BioProtein<sup>®</sup>. Changes to mesenteric lymph nodes determined under microscopic examination were also observed in all three treated groups and included increased focal necrosis, granuloma formation and an increased degree of dilated sinusoids. It was also noted that the synthetic diet fed in combination with BioProtein<sup>®</sup> appeared to aggravate the microscopic findings.
- 2.6. In the Opinion of the Company, the results observed with NA-BioProtein<sup>®</sup> can be ascribed either to the formation of protein antigens during autolysis or to the formation of particulate material of a size suitable for phagocytosis by M cells. Since neither of these effects was considered transferable through livestock, the Company considers that there are no down-stream consequences for consumers of products from livestock fed BioProtein<sup>®</sup>. In addition, although the Company recognises that limited effects were seen on the immune system of rats fed BioProtein<sup>®</sup>, this is ascribed to a “normal” immune response shown to any dietary protein fed as a large proportion of the total diet. The Company considers that this view is supported by histological examination of tissues from minipigs and salmon fed BioProtein<sup>®</sup>, which failed to demonstrate any adverse effects that could be ascribed to BioProtein<sup>®</sup>. The committee realises, however, that fish do not have lymph nodes and therefore the absence of the challenged effects in salmon is not surprising and thus this statement is of no value.

In relation with the mesenteric lymph node effects, the company has also supplied the following information (Supplement VI and VII – June 2001):

- (1) Target animal (tolerance) studies in cats, pigs and foxes in a request for extension of use.
- (2) One-generation study in rats
- (3) Expert evaluation of the histopathological findings by external experts
- (4) Study on microbiological safety.
- (5) Several scientific papers on the mucosal immunity

Later the firm supplied a “Memorandum BioProtein<sup>®</sup>”, on the safety assessment aspects, drafted by three experts, one of which also participated in the former expert group.

### **3. OPINION OF SCAN ON THE CONTINUED USE OF THE PRODUCT AS A SOURCE OF PROTEIN IN ANIMAL FEEDS.**

#### **3.1. Target animal safety studies**

Target animals safety studies included pigs, cats and foxes. The pig study, using 0, 6 and 12 % BioProtein<sup>®</sup> in the diet of growing pigs (>25 kg) showed initial growth depression and no histological changes in the mesenteric lymph nodes.

The cat study (0, 5, 10, 20 % BioProtein<sup>®</sup>, for 8 week) showed body weight depression in females fed 20% BioProtein<sup>®</sup>, increased weight of mesenteric lymph node in which also erythrophagocytosis was seen (note: histology data not present in study report). The study used three animals per group, and in view of the variation in the data, conclusions on statistics (*i.e.* absence of differences) are doubtful.

In the fox study (0, 4, 8, 12 %, for 120 days) the mesenteric lymph node showed increased weight, and lymphoid hyperplasia and sinus histiocytosis (no histology data in study report).

Note: As a tolerance test it is questionable if foxes are acceptable substitutes for dogs as intended target animal.

In a target animal safety study (tolerance study) a limited set of parameters is used than in general safety studies, as it is intended for general health and production parameters. Doses are normally higher than under practical feeding conditions (e.g. up to 10 x) to determine the margin of safety, although this is difficult to achieve with bulk feed ingredient. Therefore these studies have limited value for general safety assessment and should be judged in conjunction with the general toxicological profile.

In any case, the observations in the cat and fox confirm the findings in the rat studies in that the immune system is a target system. Moreover it shows it to be persistent in character (120 days in foxes!). In addition, body weight depression seems a common finding at higher doses; although this may be due to dietary imbalance or poor palatability, the possibility of a toxic factor cannot be excluded in regard of the accompanying effects and the compensation in dietary composition.

#### **3.2. The one-generation study in rats**

In this study using 0, 5.5, 11 and 22 % BioProtein<sup>®</sup>, no effects on reproductive performance was seen, but the increase weight of the mesenteric lymph nodes was confirmed in the parents, accompanied with increased pathology in those lymph nodes. These observations were reportedly absent in the offspring (no pathology data given). However, only male offspring was examined post weaning, while females appear to be more sensitive to these BioProtein<sup>®</sup>-related effects.

### **3.3. Expert report.**

- The expert report includes an overview of mucosal immunology, a presentation of the lymph node findings in the target animals (cat and fox) and the one-generation rat study, as well as an evaluation of the findings, where it is concluded that the observed findings can be considered as a normal immune response.
- The report on histopathological evaluation of mesenteric lymph node by the experts presents a narrative description as in clinical pathology. However, without quantitative data on severity and incidences per group – as customary in GLP-compliant toxicological pathology – the conclusions on differences between the groups are difficult to evaluate. Moreover, the data in the report of the experts and in that from the test facility (SCANTOX test report (Lab 259995, p. 24) are not in agreement.

### **3.4. Comments on the Conclusions of the experts:**

- It is recognised that the dietary proteins may interfere with the immune system in the development of normal tolerance and immunity. However, this argument does not exclude any other alternative or superimposed mechanism; in this context it is important to note that the effect concerns principally the non-specific inflammatory defence system – typically lymph node weight increase with accumulation of neutrophils and histiocytes, including granuloma and necrosis, as well as sinusoidal dilatation; this was associated with systemic effects such as increased blood levels of these phagocytic cells, and lesions seem persistent in character (up to 120 days in foxes), indicating a sustained effect which is uncommon in normal immune responses. It also seemed that the more pronounced effects were observed in the longer-term studies. The SCAN is of the opinion that this profile is not consistent with a development of a “normal” immune response or oral tolerance; also the literature data provided on mucosal immunity and tolerance do not show that these observed effects are archetypal part of this normal reaction. Also it is difficult to explain along these lines the wide range of effects seen in the (“purified”) NA – BioProtein<sup>®</sup> study in 13 weeks in rats. In the experiments with non-treated BioProtein<sup>®</sup> the effects were indeed milder and more limited, but not essentially different; moreover, as the source material is similar, it is reasonable to accept that they represent a similar process though possibly at a lower intensity.
- With respect to the induction of tolerance, this is not strictly limited to the very young animals. In addition, the animals used in the studies were sufficiently young (from 4 weeks) and still apparently failed to develop “normal tolerance”. Furthermore, the SCAN recognises that tolerance is a functionally defined entity without distinctive morphological endpoints. In the underlying case no evidence for functional tolerance is presented, while indeed distinctive histopathological and haematological changes were reported.
- Another aspect is the interpretation of adverse versus non-adverse, which is a pivotal aspect in the discussion on this product as a whole. The experts from the firm claim that the lymph node reaction is normal and not

adverse. However, the fact that a change fits into the normal programme of organ's responses does not render this response not-adverse *per se*. Criteria for adversity are not well defined and may be different in the perspective of a diagnostic clinician or a risk assessor. The expert's notion of *adverse* is a typical clinical one, as the experts seem to be concerned in particular for hypersensitivity, scarring and malignant lymphoma, and apparently other (reactive) processes are not adverse. For clinical diagnostic purposes this may indeed be valid, but in safety assessment other rules apply. A normal transient (and immunologically efficacious) defence reaction to an invading pathogen or a vaccine can obviously be considered non-adverse. However, in the opinion of the SCAN, for a feed ingredient, inflammatory responses, in particular those seen after BioProtein<sup>®</sup> feeding and over such a long period, must be considered adverse and unacceptable.

- Although the company claims that the BioProtein<sup>®</sup>-induced effects - long term enlargement of mesenteric lymph nodes with the morphological inflammatory features as described - is a normal response, in laboratory animal safety studies this is a very uncommon finding which, should it occur, will normally be considered abnormal as such.
- In conclusion, the arguments of the firm were not considered satisfactory by the SCAN, and in regard of the unusual reaction in terms of composition and persistence on a bulk food ingredient, the SCAN concluded that the firm has not adequately demonstrated the safety of the product

### **3.5. Memorandum by the experts**

The "Memorandum BioProtein<sup>®</sup>" drafted by three experts deals largely with principles of mucosal immunology. With respect to BioProtein<sup>®</sup> the authors state that the development of tolerance is likely the case and there is no indication of development of hypersensitivity. The SCAN can concur with at least the latter statement, but as explained earlier in this opinion, the observed pathological effects in the immune system of several mammalian species (see above) can in the opinion of the SCAN not satisfactorily be explained by the development of a normal functional tolerance.

## **4. CONCLUSION**

On the basis of the comments above SCAN concludes that the response of the firm has not satisfactorily alleviated the concerns of the SCAN as regards the safety of BioProtein<sup>®</sup>.

## **5. RECOMMENDATION**

Consequently, in the presence of an abnormal response and the lack of a satisfactory explanation for the observed effects, SCAN suspends its previous recommendations to allow the use of BioProtein<sup>®</sup> as a source of protein in feeds for pigs, calves, poultry and farmed salmon (fresh and sea water).