

ADVISORY COMMITTEE ON NOVEL FOODS AND PROCESSES

BOVINE LACTOFERRIN

Issue

The Belgian Competent Authority has prepared an initial opinion on an application for the authorisation of lactoferrin derived from cows milk as a novel food ingredient (NI) under the Novel Food Regulation (EC) No. 258/97. The Committee is asked whether it agrees with the conclusions of the Belgian CA and whether it has any further comments or objections to make on the application. The Committee's advice will form the basis for the UK's formal response.

Introduction

1. On 7 July 2008, the European Commission forwarded the Belgian Competent Authority's (CA) initial assessment report on an application made by Bipole SA under Article 4(1) of Regulation (EC) 258/97, for the authorisation of bovine lactoferrin, extracted from cows milk, as a novel food ingredient. The Belgian report concludes that the proposed uses do not appear to pose a risk to consumer health. However, the report rejects its use as a preservative and highlights uncertainties in relation to the effect of heat processing when bovine lactoferrin is incorporated into infant formula.
2. Under the time scales set out in the regulation, the UK and other Member States have until 5 September 2008 to provide comments and/or reasoned objections to the initial opinion.
3. The Initial Assessment Report was prepared in French and the Commission's English translation is attached as **Appendix 1** (restricted). The main part of the dossier provided by the applicant is attached as **Appendix 2** (restricted).

Background

4. This application from the Belgian company Bipole SA is for the placing on the market of lactoferrin extracted from cows milk. This product has been previously

consumed in the EU as a food supplement but its use in other food products is considered novel and requires a pre-market safety assessment.

5. Lactoferrin is a mammalian iron-binding glycoprotein of about 80 kDa that occurs naturally in milk and other secretions. Bovine lactoferrin is isolated from cows milk and is a basic protein (isoelectric point pH 8.7) consisting of a single polypeptide chain of 689 amino acids.
6. In accordance with Commission Regulation 258/97 and Commission Recommendation 97/518/EC, which sets out the guidelines for novel food applications, the applicant considers that the NI falls within Class 6 i.e. foods produced using a novel process. The requirements for a submission for this class are as follows:

I	Specification of the NF	X
II	Effect of the production process applied to the NF	X
III	History of the organism used as the source of the NF	X
IV	Effect of the genetic modification on the properties of the host organism	-
V	Genetic stability of the GMO	-
VI	Specificity of expression of novel genetic material	-
VII	Transfer of genetic material from GM microorganisms	-

VIII	Ability to survive in and colonise the human gut	-
IX	Anticipated intake/extent of use of the NF	X
X	Information from previous human exposure to the NF or its source	X
XI	Nutritional information on the NF	X
XII	Microbiological information on the NF	X
XIII	Toxicological information on the NF	X

7. Section 3 of the assessment report is structured accordingly and the key issues for consideration are presented below under these headings.

I. Specification of the novel food

Appendix 1 Section p.4-5; Appendix 2 p.131-135 and Annex 11

8. The NI is a protein extract derived from cows milk (see section II below). A detailed specification is set out in Table 2 (Appendix 2, p141). The extract

contains 97% protein (on the basis of Kjeldahl analysis, N x 6.38) and a minimum of 95% lactoferrin (by hplc). Other proteins have been identified as milk immunoglobulins. The specifications also cover heavy metals (<10 ppm), arsenic (<2 ppm) and iron (<36 ppm). Taking account of the iron binding capacity of lactoferrin, it is estimated that the extract has an iron saturation rate of approximately 20%. The specifications include tests for antibacterial properties and endotoxins. (See Appendix 2 'Annex 11').

9. No results have been presented showing whether different production batches comply with the parameters set out in the specification.
10. The Belgian report concludes that the product is highly purified, relative to other protein isolates and highlights the importance of the iron content. The potential for lactoferrin to bind to milk allergens and its heat stability were noted and these factors were taken into account elsewhere in the evaluation.

II. Effect of the production process applied to the novel food

Appendix 1 p.5-6; Appendix 2 p.125-130

11. The flow chart on page 126 of Annex 2 applicant provides an overview of the production process. Milk whey is obtained from pasteurised milk and lactoferrin is extracted by ion exchange resins prior to concentration, microfiltration and freeze drying. The process is controlled by HACCP.
12. The Belgian CA is satisfied with the process controls and also concludes that the process is and the relevant control parameters are described in Figure 3 and Table 13 (Appendix 2, p170 and pp157-9).

III. History of the source organism

Appendix 1 p.6; Appendix 2 p. 136-138

13. Cows milk is widely consumed but heat treatment will convert lactoferrin into a denatured, less active, form. The average daily consumption of active bovine lactoferrin in France, based on the consumption of cheese made from raw cows milk, is estimated to be 5.4 mg.

IX. Anticipated intake/extent of use of the NI

Appendix 1 p.6-8 Appendix 2 p.186-193

14. The applicant proposed the following uses: foods for children and pregnant women; functional foods; nutritional foods such as yoghurt; foods for athletes; chewing gum and as a natural preservative. (Note: preservatives in food are

generally subject to food additive legislation. However, the EU definition of "food additive" excludes milk proteins).

15. The level of addition to infant formula would be 750mg per kg powder or c.95 mg per litre. Special diets for premature infants and children with diarrhoea would provide a daily intake of lactoferrin of 13 and 15 mg per kg body weight respectively.
16. Addition to foods intended for other specific population groups would be controlled to give a daily intake ranging from 40 to 900 mg/day (Annex 1, p.7).
17. The Belgian report noted that it is difficult to determine current levels of active bovine lactoferrin and that the proposed use levels were based on a range of different considerations. Also, the applicant had been unable to provide intake estimates for bovine lactoferrin used as a preservative, which appear to require much higher levels of addition in order to be effective.

X. Information from previous human exposure to the novel food or its source

Appendix 1 p.8-9; Appendix 2 p.203-219

18. The applicant reports that infant formulae with added lactoferrin have been on sale in Japan since 1986 and similar products can also be found in Korea and Taiwan. Lactoferrin is also used in Japan and other Asian countries in other products such as yoghurts and food supplements.
19. In the USA, bovine lactoferrin has been classified as GRAS (generally regarded as safe) since 2001, for use in food supplements and sports foods providing 100mg per serving. (Note: the dossier also refers to its use for decontaminating meat carcasses but this is not mentioned in the FDA letter at pages 210-214 of Appendix 2). It can also be used in food supplements in Australia & New Zealand and in the EU. The applicant states that infant formulae containing lactoferrin were launched in Romania in 2005, although they do not explain how these products could be marketed without approval under the novel food regulation.
20. Other than reporting the legal status of lactoferrin in different regions and highlighting some of the current products, the applicant has not provided any information on reports of adverse reactions or on the levels of intake in relation to

their current proposal. The Belgian report does not comment on this aspect of the application dossier.

XI. Nutritional information on the novel food

Appendix 1 p.9-12; Appendix 2 p.139-185 & 220-226

21. Lactoferrin has a structure very similar to the transferrin family of proteins and, largely due to its iron-binding properties, has a number of biological activities including promotion of iron absorption, inhibition of the pro-inflammatory action of cytokines, stimulation of the reticulo-endothelial system and antimicrobial effects.
22. The Belgian report notes that the value of lactoferrin supplementation would appear to be more evident in infants switching from human milk to cows milk-based infant formula. Addition of bovine lactoferrin to infant formula results in alterations in the faecal flora, notably an increase in *Bifidobacteria* and a reduction in anaerobic organisms.
23. Lactoferrin is present in human milk (around 1-2 mg per ml) and the Belgian report notes that the human lactoferrin and bovine lactoferrin are not identical. For example, the bovine form appears to be more resistant to digestion and may retain its activity during passage through the GI tract. Also, the bovine form appears to have no impact on the absorption of iron when incorporated into infant formula, possibly because it does not bind to the specific human receptor and/or because its binding properties are affected by the processes used to manufacture the formula.
24. Although there is evidence that bovine lactoferrin retains biological activity when dissolved in dairy products (milk and yoghurt), the Belgian report notes that there is no evidence of its stability in more complex matrices. It is reported that the applicant is to study factors that may inactivate bovine lactoferrin, in the context of non-food products such as toothpaste and mouthwashes.

XII. Microbiological information on the novel food

Appendix 1 p.12

25. The dossier contains no specific information on this point but the Belgian report notes that the company uses a HACCP approach and concludes that there is no particular risk to human health.

XIII. Toxicological information on the novel food

Appendix 1 p.12-13; Appendix 2 p.221-226

26. The applicant describes a series of studies carried out with bovine lactoferrin in Japan, including a 4-week subacute toxicity study (Annex 8 to Appendix 2) and a 13-week subchronic study (Annex 7 to Appendix 2) which both found no adverse effects in rats at doses up to 2000 mg/kg bw/day. A mutagenicity test (Ames test) was also negative.
27. No side-effects were noted in four clinical trials with bovine lactoferrin, including a 14-day trial in infants and a 6-month trial with cancer patients.
28. **Allergenicity:** (Appendix 1, p.6; Appendix 2, p.227-228). The applicant notes that recognised milk allergens are acidic proteins and that these are not present in significant amounts in the bovine lactoferrin preparation. In this context the applicant refers to a study of basic milk proteins (lactoferrin (56%) and lactoperoxidase (41%) and smaller amounts of cystatin C and kininogen, none of which has been identified as an allergenic component of milk) which concluded that it is most unlikely that the components of this extract will cause allergic reactions in susceptible individuals (Goodman et al, 2007). This analysis was accepted by the Belgian assessment body.
29. The FDA letter concerning the GRAS status of bovine lactoferrin mentions that there is extensive literature documenting the presence of anti-lactoferrin antibodies in various autoimmune diseases, although there is no evidence that such antibodies play a role in the pathology of these diseases. The development of a harmful autoimmune response as a result of bovine lactoferrin's use as a food ingredient was considered unlikely. (see Appendix 2, p.212-213)

Labelling

30. Neither the application dossier nor the Belgian report addresses the labelling of the novel ingredient. In 2001 the FDA responded favourably to a GRAS notification for bovine lactoferrin (Appendix 2, p.210-214) but included the proviso that the ingredient statement of food products containing this ingredient should indicate its source (e.g. milk or cows milk), and suggested that "milk-derived lactoferrin" is a more appropriate title for the ingredient than "bovine lactoferrin".

Committee Action Required

31. The Committee is asked:

- whether it agrees with the initial opinion from the Belgian CA that bovine lactoferrin produced by Biopole SA does not appear to pose a risk to the health of consumers;
- whether it agrees with the Belgian CA that further studies are needed to demonstrate benefits from its use in infant nutrition;
- whether it agrees that the source of the ingredient ("milk" or "cows milk") should be indicated on ingredient lists;
- whether it wishes to make any additional comments on the application.

Secretariat
August 2008

Appendices attached:

Appendix 1 – Belgian Competent Authority's initial assessment report on bovine lactoferrin
(RESTRICTED).

Appendix 2 - Application dossier submitted by Bipole SA for the approval of bovine lactoferrin as a novel food ingredient. Also 'Annex 11: Specification.
(RESTRICTED)
Detailed Annexes are available on request

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Belgian Competent Authority's Initial Assessment Report

Translation provided by the European Commission

**Secretariat
August 2008**

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RESTRICTED

Application dossier submitted by Biopole SA for the approval of bovine lactoferrin as a novel food ingredient.

Annex 11 : Specification

**Secretariat
August 2008**