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SCIENTIFIC OPINION

Scientific Opinion on bovine lactoferrin[1]

EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA)[2, 3]

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

Following a request from the European Commission, the EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA) was asked to carry out the additional assessment of 'lactoferrin' as a food ingredient in the context of Regulation (EC) No 258/97 taking into account the comments and objections of a scientific nature raised by Member States. Bovine lactoferrin (bLF) is a protein that occurs naturally in cow's milk. The applicant intends to market bLF as an ingredient for food supplements, infant and follow-on formulae, dietetic food for special medical purposes and sports nutrition, and for a variety of foods. For infants with an age of 0 - 6 months, the applicant has estimated an intake of approximately 200 mg per kg bodyweight and 1.2 g bLF per day at the proposed use level. For adults, the mean and 95th percentile daily intakes were calculated to be about 1.4 g and 3.4 g for an adult person. The toxicological information provided by the applicant included information from an *in vitro* genotoxicity study, a single dose study, a four week and a thirteen week oral repeated dose study in rats. The Panel notes that the estimated intake of "lactoferrin" for infants up to the age of one year of approximately 210 mg/kg bw per day would be around ten times lower than the highest dose (2,000 mg/kg bw per day) tested in the subchronic thirteen week rat study, which did not show adverse effects related to bLF. For adults above 19 years of age the proposed intake is approximately 100 times lower. This level of anticipated intake is considered a high intake scenario as opposed to a worst-case situation. The data provided suggest the absence of adverse effects of lactoferrin at the proposed levels of consumption. The Panel concludes that the novel food ingredient bLF is safe under the proposed uses and use levels. © European Food Safety Authority, 2012

KEY WORDS

Bovine lactoferrin, novel food, ingredient

[1] On request from the European Commission, Question No EFSA-Q-2010-01269 adopted on 27 April 2012.

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[3] Acknowledgement: The Panel wishes to thank the members of the Working Group on Novel Foods: Karl-Heinz Engel, Ines Golly, Marina Heinonen, Pagona Lagiou, Rosangela Marchelli, Bevan Moseley, Monika Neuhäuser-Berthold, Annette Pöting, Seppo Salminen, Hendrik Van Loveren, Hans Verhagen and EFSA's staff member Wolfgang Gelbmann for the preparatory work on this scientific opinion.

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SUMMARY

Following a request from the European Commission, the EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA) was asked to carry out the additional assessment for 'lactoferrin' as a food ingredient in the context of Regulation (EC) No 258/97 taking into account the comments and objections of a scientific nature raised by Member States.

Bovine lactoferrin (bLF) is a protein that occurs naturally in cow's milk. The applicant intends to market bLF in isolated and purified form. bLF is an iron-binding glycoprotein of approximately 77 kDa and consists of a single polypeptide chain of about 700 amino acids. The sequence homology between human and bLF is about 70 %. The tertiary structure of this glycoprotein has two iron-binding sites, giving it the capability to bind two Fe^{3+} ions per molecule of protein. Batch testing confirmed that the product complies with the given specifications. bLF from skimmed milk is concentrated via ion exchange and is subsequently subjected to filtration steps. Ultimately the bLF is dried by means of spray drying. The applicant provided sufficient information regarding the specification, manufacture, composition and stability of bLF.

The applicant intends to market bLF as an ingredient for food supplements, infant and follow-on formulae, dietetic food for special medical purposes and sports nutrition, and for foods such as non-alcoholic beverages, cakes and pastries, products derived from cheese, milk-based products, cold snacks and sweets. The intended maximum levels of bLF for foods in solid form vary from 667 mg/100 g for baby foods and foods intended for children aged 1 - 3 years to 4000 mg/100 g for energy bars for sportsmen and women. The proposed concentrations of bLF in mg/100 g for the various liquid products are for example: 100 mg/100 g for infant formulae, 120 mg/100 g for non-alcoholic beverages, 125 mg/100 g for food for special medical purposes, 200 mg/100 g for dairy products foods intended for children up to 3 years and 300 mg/100 g for beverages for sports nutrition.

For infants with an age of 0 - 6 months, the applicant has estimated an intake of approximately 200 mg per kg bodyweight and 1.2 g bLF per day assuming that the mean intake is 1.2 litres of infant formula per day. The mean estimated intake of bLF by infants of 8 - 10 months of age would amount to 1.9 g per day. For adults, the applicant's calculation estimates a mean and 95th percentile intake of 19 and 39 mg/kg bodyweight per day, respectively, and a mean and 95th percentile daily intake of about 1.4 g and 3.4 g, respectively.

The toxicological information provided by the applicant included information from an *in vitro* genotoxicity study, a single dose study, and a four week and a thirteen week oral repeated dose study in rats. The Panel considers that bLF up to the highest dose (2,000 mg/kg bw per day) tested in this subchronic rat study did not show adverse effects which could be attributed to the test substance.

In an overall evaluation, the Panel considered that the novel food ingredient, bLF, is essentially a protein, a constituent of cow milk. According to the information provided by the applicant, bLF is present in the novel food ingredient (NFI) mostly as non-denatured lactoferrin. The Panel notes that lactoferrin is a normal constituent of human milk, and that the intended consumption of the bLF as specified in the application is within the levels of human lactoferrin consumed in breast milk by infants; human lactoferrin is also non-denatured.

The Panel notes that the mean estimated intake of bLF for infants up to the age of one year of approximately 210 mg/kg bw per day would be around ten times lower than the highest dose (2,000 mg/kg bw per day) tested in a subchronic thirteen week rat study, which did not show adverse effects related to bLF. For adults above 19 years of age the estimated intake is approximately 100 times lower. This maximum level of anticipated intake is considered a high intake scenario as opposed to a worst-case situation. The data provided suggest the absence of adverse effects of bLF at the proposed levels of consumption.

The Panel concludes that the novel food ingredient bLF is safe under the proposed uses and use levels.

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BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION

On 2 March 2009, TNO on behalf of the company FrieslandCampina (formerly DMV International) submitted a request under Article 4 of the Novel Food Regulation (EC) N° 258/97 to place on the market 'BLF' as a novel food ingredient.

On 1 April 2010, the competent authorities of The Netherlands forwarded to the Commission their initial assessment report, which came to the conclusion that "although the information concerning safety is limited, according to the Committee, there is no reason for concern."

On 13 April 2010, the Commission forwarded the initial assessment report to the other Member States. Several of the Member States submitted comments or raised objections.

The concerns of a scientific nature raised by the Member States can be summarized as follows:

- The heterogeneous composition raises concerns (lactoferrin content 91 – 98 %).
- The applicant does not justify the claim that the production process used does not denature bovine lactoferrin (bLF). A recent study on this subject (Schwarcz et al., 2008) shows in particular that the pasteurisation of milk is likely to bring about changes to the tertiary structure of bLF, and thus to the properties linked thereto.
- Test reports are not available and it is not clear what body carried out the analyses for the specification. It should therefore be noted that only accredited test laboratories should be used to carry out analyses with a view to authorising and/or establishing the substantial equivalence of novel foods.
- Given the product's sensitivity to changes of pH values and higher temperatures, the downstream manufacturer should however check, with a view to his manufacturing conditions, whether the specific properties (iron-binding capacity) remain in the end product.
- Concern was expressed with regards to an additional iron intake.
- Significant variations occur in food consumption between the different European countries in particular for dairy products and non-alcoholic beverages. The average consumption data for different categories of food in European countries (EFSA, 2009) show that consumption of dairy products is much higher in Finland, Iceland and Norway (437, 442 and 522 g/d respectively) than in the Netherlands (388 g/d) or France (206 g/d). Consumption of non-alcoholic beverages is also much higher in Belgium (346 g/d), Norway (416 g/d) and Iceland (426g/d) than in the Netherlands (254 g/d) or France (158 g/d). Estimates of lactoferrin intake for the populations of certain countries are thus likely to be higher than the intakes estimated by the applicant for the Netherlands.
- The average total intake of lactoferrin by an adult can vary from 20 to 50 mg/d, according to the type of dairy products consumed. The intended uses would lead to considerably higher intakes. By consuming products to which "lactoferrin" is added, the mean intake for infants aged approx. 9 months would be around 30 times higher than the intake from natural sources (approx. 63 mg/day). For adults and the group with the highest average intake (boys, 10–18 years) the intake would be, respectively, around 35 times and 58 times higher than the intake from natural sources of the general population (approx. 40 mg/day). Considering the estimated intake for infants and heavy users, e.g. boys aged 10-18 years old, the safety margin in relation to the No Adverse Effect Level (NOAEL) is too narrow (<10).

- A 13-week study in rats showed an increased incidence of fibrosis of the pancreatic islets in all treated groups. It is not possible to set a clear NOAEL from this study. Historical control data for the specific rat strain are required for the evaluation and should be requested from the testing facility. Fibrosis could be due to interaction with normal pancreatic lactoferrin production. It would be useful to review additional data on the chronic consequences. Thyroid weight and its weight relative to body weight were reduced in both sexes. This was viewed to be within the historical control values, but additional confirmation is required.

- The mesenteric lymph nodes should have been sampled in the 13-week rat study considering the potential allergenic and immunological effects of lactoferrin.

- Lactoferrin is involved in various aspects of reproduction and this justifies a more thorough investigation of this aspect of toxicity.

- In humans approximately 60 % of bLF survives the passage through the stomach. A study should be provided to demonstrate that a similar proportion survives native in the rat to validate the rat as a suitable test species.

- There are no human data on the kinetics.

- There was one dead rat in the high dose group of the 13-week study due to a malignant lymphoma. According to a reference provided by the applicant such tumours do occur in this strain but information on the current incidence in the animals from the specific source used could be reassuring.

- The full study reports on toxicity have not been provided.

- It is unclear whether the test materials for the toxicity studies corresponded to the proposed novel food ingredient.

- The use of native bLF as a food ingredient poses a potential risk as a result of long-term exposure and the possible absorption of native protein. There is no information available on the degree of absorption. The information on the metabolism of bLF is insufficient.

- Human trials were aimed at assessing the efficacy of lactoferrin, not its safety. Particularly with regard to children, further clinical studies should be carried out, with a larger number of subjects and over a sufficient period of time, in order to exclude possible health risks.

- There is a potential risk as a result of long-term exposure and the possible absorption of native protein since baby food based on cow's milk promotes the occurrence of the autoimmune disease type-1 diabetes in genetically predisposed children.

- The relevance of the reported high prevalence of auto-antibodies against lactoferrin among Diabetes mellitus type-1 patients (Taniguchi et al., 2003) should be addressed.

- The presence of lactoferrin and lactoferrin receptors has been found in increased concentrations in lesions in a number of neurodegenerative disorders. In addition to an age-related increase in the expression of lactoferrin in brain cells, an increased exogenous supply could also be of relevance. In animal experiments, orally administered bLF has been found as native protein in the brain, *inter alia* (Fischer et al., 2007; Kamemori et al., 2008). The possibility that lactoferrin is actively involved in neurodegenerative processes cannot be ruled out.

TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION

In accordance with Article 29 (1) (a) of Regulation (EC) No 178/2002, the European Food Safety Authority is asked to carry out the additional assessment of 'bovine lactoferrin' as a food ingredient in the context of Regulation (EC) N° 258/97.

EFSA is asked to carry out the additional assessment and to consider the elements of scientific nature in the comments raised by the other Member States.

ASSESSMENT

In accordance with Commission Recommendation 97/618/EC 'bovine lactoferrin (bLF)' is allocated to Class 2.1 'a complex (non-GM derived) novel food ingredient, the source of the novel food having a history of food use in the community'. The assessment of the safety of this novel food ingredient is based on data supplied in the original application, the initial assessment by the competent authority of The Netherlands, the concerns and objections of the other Member States and the responses of the applicant to these questions and those of The Netherlands. The data are required to comply with the information required for novel foods of Class 2.1 i.e. structured schemes I, II, III, IX, X, XI, XII and XIII. It is noted that the novel food ingredient (NFI) is intended by the applicant to be added to various foods, including foods for particular nutritional purposes (such as infant formulae, follow-on formulae, medical, and sports foods), to improve protection against pathogens, oral health, immune health and improvement of intestinal micro flora. This assessment concerns only risk that might be associated with consumption and is not an assessment of the efficacy of 'bLF' with regard to any claimed benefit.

1. Specification of the Novel Food (NF)

Bovine lactoferrin is a protein that occurs naturally in cow's milk. The applicant intends to market bLF in isolated and purified form. It is an iron-binding glycoprotein of approximately 77 kDa and consists of a single polypeptide chain of 689 amino acids; the sequence homology with human lactoferrin is 69 % (Pierce et al., 1991).

The protein does not contain free sulphydryl groups but it has intramolecular disulphide bonds. It is glycosylated at two different sites by N-linked glycans of the N-acetyllactosamine type. These glycans are characterized by α-1,3-linked galactose residues at the terminal non-reducing position. Unlike human lactoferrin, bLF also contains glycans of the oligomannosidic type.

The tertiary structure of this glycoprotein has two iron-binding sites, giving it the capability to bind two Fe^{3+} ions per molecule of protein.

The applicant specifies the novel product as follows, the contents being expressed as percentage by weight. The protein content is at least 93 %, at least 95 % of which is bLF (Table 1).

Table 1: Specification for the novel food ingredient

Parameter	Specification	Analytical Method
Moisture	Max. 4.5 %	ISO 5550:2006*
Protein (N x 6.38)	Min. 93 %	ISO 14891:2002
bLF (on protein basis)	Min. 95 %	DMV CM0503
Ash (550 °C)	Max. 1.0 %	NEN 6810:1998
pH (2 % solution, 20°C)	5.5-6.5	DMV CM0977
Solubility (2 % solution, 20°C)	Complete	DMV CM0056
Transmittance, 2 % sol., 600 nm	80-100 %	DMV CM0056
Total heavy metals (Cadmium, Lead, Arsenic, Mercury, Copper; expressed as Lead)	Max. 1 mg/kg	DMV CM0581 (ICP)
Iron	100-160 mg/kg	ISO 8070:2007
Microbiological		
Standard plate count	< 1000 cfu/g	ISO 4833:2003*
Enterobacteriaceae	< 10 cfu/g	ISO 21528-1:2004*
Yeasts & Moulds	< 10 cfu/g	ISO 6611 / IDF 94
Staphylococcus (coagulase positive)	Neg. in 1 g	ISO 6888-3

Salmonella	Neg. in 10 g	ISO 6579:2002*

According to the applicant, all methods are validated. *These methods were slightly modified to enhance reliability or to adapt to specific product characteristics.

The information on the physical and chemical properties of the ingredient are given below in Table 2 and were adopted by the applicant from Naidu (2000).

Table 2: Physical-chemical properties of bovine lactoferrin

Property	Bovine lactoferrin	Reference
Molecular mass (kDa)		
Sedimentation co-efficient	72,200 ± 1,300	Castellino et al., 1970
SDS-Page	76,000 ± 2,400	Querinjean et al., 1971
Iron titration	78,500	Aisen et Leibman, 1972
Isoelectric point		
Chromato focusing	8.2 – 8.9	Shimazaki et al., 1993
Isoelectric focusing	9.5 – 10.0	Yoshida et Xiuyun, 1991
Absorption spectra		Aisen et Leibman, 1972
Apo-form at 280 nm	12.7	
Holo-form at 470 nm	0.400	
Protease sensitivity	Relatively low*	Brines et Brock, 1983
Iron-binding		Aisen et Leibman, 1972
Equilibrium dialysis (K1 x 10^{-4})	3.73	
Thermal denaturation		Paulsson et al., 1993
Apo-LF denaturation (T_{max}: °C)	71 ± 0.3 and 90 ± 0.3	
Apo-LF enthalpy (ΔH_{cal}: J/g)	12 ± 0.4 and 2 ± 0.5	
Holo-LF denaturation (T_{max}: °C)	65 ± 0.3 and 93 ± 0.3	
Holo-LF enthalpy (ΔH_{cal}: J/g)	2 ± 1 and 37 ± 1	

*The original table by Naidu (2000) indicated a "high" protease sensitivity, but the original reference (Brines and Brock, 1983) points to a low protease sensitivity of bLF towards intestinal proteases. The applicant suggested amending the table accordingly.

Chemical properties vary with the amount of bound iron. The analytical data from six production lots, produced between December 2007 and August 2008 (Table 3), show that the protein content varies between 96.6 and 97.8 %. The moisture content fluctuates around 3 %.

Table 3: Analytical data from testing of six batches

Parameter	Batch. No						Specification
	10371335	**10388040**	**10390371**	**10391587**	**10393723**	**10409699**	
Protein (micro Kjeldahl) (%)*	97.4	97.7	97.7	97.8	96.6	97.3	≥ 93 %
Lactoferrin purity (%)	> 95	> 95	> 95	> 95	> 95	> 95	≥ 95 %
Ash 550 °C (%)	0.20	0.21	0.32	0.25	0.30	0.12	Max 1.0 %
Moisture (%)	3.04	3.39	3.25	3.29	3.53	2.95	Max. 4.5 %
Solubility (Transmission) (%)	91	88	91	90	90	93	80 - 100 %
pH (2 % solution)	5.8	5.8	5.8	5.8	5.7	5.8	5.5 – 6.5
Aerobic count number 30 °C (cfu/g)	< 1,000	< 1,000	< 1,000	< 1,000	< 1,000	< 1,000	< 1,000
Enterobacteriaceae MPN (cfu/g)	< 10	< 10	< 10	< 10	< 10	< 10	< 10

Yeasts and Moulds (cfu/g)	< 10	< 10	< 10	< 10	< 10	< 10	< 10
Salmonella in 50g	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
CP Staphylococcus in 1g	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.

*A Kjeldahl factor of 6.38 is used to determine the protein level in dairy practice of information exchange (Bulletin of the International Dairy Federation 405/2006).

The protein in the novel product contains a maximum of 5 % of proteins other than bLF. No analytical data were provided on the identity of this protein fraction. The applicant states, however, that small quantities of other milk proteins such as casein, beta-lactoglobulin and alpha-lactalbumin may be present.

The applicant provided data on the iron content of individual production lots and from an annual monitoring program. The values calculated from the batch data are: mean: 140 mg/kg; standard deviation: 25 mg/kg; range: 101 - 216 mg/kg. On the basis of the data collected the applicant proposes a limit of 100-160 ppm for the iron content of bLF manufactured at FrieslandCampina.

The applicant provides no information on heavy metals in the test lots; however, the Panel notes that the novel food has to comply with existing legislation.

The Panel considers that the information provided on the composition, specification and data from batch testing do not raise concerns.

2. Effect of the production process applied to the NF

According to FrieslandCampina, skimmed cow's milk intended for human consumption is the exclusive source for manufacturing bLF. According to the applicant, the cow's milk complies with European hygiene regulations. The applicant provided a description of the sequential steps in the production process. Raw milk is heated to 50 °C, separated into skimmed milk and cream, and then pasteurised at the equivalent of 72 °C for at least 15 seconds. Pasteurised skimmed milk is subjected to microfiltration in order to reduce microbial load and fat content. In the first phase of the production process the protein is isolated via ion exchange and subsequently subjected to ultra-filtration steps. Ultimately the bLF is dried by means of spraying. Excessively large powder particles are sieved out before the bLF is packaged in special bags. Metal detection is performed on the final product to prevent metal parts in the packaged product. Regarding technical details, the applicant refers to US Patent 5596082.

Stability

The applicant analysed the chemical and microbiological quality during long-term storage of seven lots from the production period June 2004 to March 2007 (DMV, 2008). The bLF content of the starting material was at least 95 % of the total quantity of protein, and after 8 to 41 months this content was between 91 and 98 %. According to the applicant, the native (natural) form is retained, but this is not further substantiated. Other results from this stability study relating to important properties such as solubility, moisture content and ash content are provided. The applicant concludes that the product in the prescribed packaging remains stable for a storage period of at least 3.5 years when refrigerated. A lot from 2001, which was stored in an ordinary plastic bag at room temperature, still contained 95.3 % bLF (of the total protein) after six years. This product did contain relatively more moisture.

The information in the dossier on the stability of bLF in the proposed foods (end products) is limited to laboratory research into the optimum conditions for preventing denaturation as a result of heat

treatment. Upon explicit request by EFSA, the applicant indicated that the degree of denaturation in FrieslandCampina bLF is 'low'. Based on the applicant's own results for some of the intended applications, and data from third parties, the applicant concludes that the native form of the incorporated bLF can be substantially retained during the production of, for example, milk, yoghurt and infant formulae. However, lactoferrin is subject to denaturation upon heating and the degree of denaturation is dependent on several factors, such as pH, heating time and level of iron saturation. Although the applicant did not specify the degree of iron-binding capability of the NFI, the Panel considers that it will not be essentially different from native bLF.

BLF is produced with techniques commonly used in the dairy industry. According to the applicant, certified procedures (ISO 9000:2000 and HACCP) are used to monitor the quality of production. According to the initial assessment report of the Netherlands, the production plant has also been approved for the production of dairy products in conformity with Regulation (EC) no. 853/2004 which lays down specific hygiene rules for food of animal origin.

The Panel concludes that the production process is sufficiently described by the applicant and does not raise concerns.

3. History of the organism used as a source

The source of the novel food is milk from cows. The applicant indicates that the milk meets all the relevant applicable criteria of Dutch and European legislation.

4. Anticipated intake/extent of the use of the NF

The applicant intends to add bLF to foods for particular nutritional uses (PARNUTS) i.e. infant and follow-on formulae, dietetic food for special medical purposes and sports nutrition and to foods such as non-alcoholic beverages, cakes and pastries, products derived from cheese, milk-based products, cold snacks and sweets.

Table 4 shows a list of the proposed product groups with the maximum levels of bLF, expressed in mg per 100 g end product. For the foods in solid form, this varies from 667 mg/100 g for baby foods and foods intended for children aged 1 - 3 years to 4000 mg/100 g for energy bars for sportsmen and women. The proposed concentrations of bLF in mg/100 g for the various liquid products are, for example: 100 mg/100 g for infant formulae, 120 mg/100 g for non-alcoholic beverages, 125 mg/100 g for food for special medical purposes, 200 mg/100 g for dairy products and 300 mg/100 g for beverages for sports nutrition.

Table 4: Intended use categories of the NFI in foods and beverages and the maximum intended usage levels per category as proposed by the applicant

Food group	Concentration bLF (mg/100g)
infant nutrition – liquid and powdered formula (for 0 – 6 and 6-12 months)	100
infant nutrition – "growing up" milk	200
follow-on formulae and foods intended for children aged 1 - 3 years	667
medical nutrition – liquid and powdered formula	125
medical nutrition -tube feeds	50
medical nutrition – other	800

sports nutrition – liquid and powdered formula	303
sports nutrition – bars and other food like applications	4,000
non-alcoholic drinks	120
cakes and pastries[2]	1,000
cheese products	2,000
milk and milk products	200
cold snacks	1,200
small candy and candy bars	750

The applicant has estimated the intake of the novel food ingredient for infants with an age of 0 - 6 months. The applicant assumed a high intake of 1.2 litres of infant formula per day, which would result in an intake estimate of 1.2 g bLF. For older babies, the applicant made use of Dutch data on the intake of foods in young children performed in 2002. The mean estimated intake of bLF by babies of 8 - 10 months of age would amount to 1.9 g per day according to the applicant.

In order to estimate the intake of other groups in the population (older than one year) the applicant has made use of individual consumption data collected in the databank of the Dutch national food consumption surveys. The study was carried out amongst the general Dutch population from 1997 - 1998 and is based on records in food diaries over a two-day period.

The figures provided in Tables 5 and 6 originate from the entire group (*all persons)*, because everyone had consumed at least one of the foods examined. A negligibly small number of non-users in the adult group (< 1 %) was left out of consideration. The estimate for the mean intake is lowest in adults (≥ 19 years of age), i.e. approximately 1.4 g/person/day and highest in boys of 10 - 18 years of age, i.e. 2.3 g/person/day. In pregnant women (around 1 % of all adults) the mean consumption of bLF is 1.8 g/person/day. The applicant also gives the calculated figures for the 50^{th}, 95^{th} and 97.5^{th} percentile of the intake. Young children (aged 1 - 3 years), girls (aged 4 - 9 years), and adult women have the lowest intake at the 95^{th} percentile, which varies from 2.7 to 2.8 g/person/day. Boys of 10-18 years of age again score the highest, with an intake at the 95^{th} percentile of 4.2 g/person/day (TNO, 2009).

Expressed per kg body weight, the applicant's calculation shows that exposure to bLF is lowest in adults (Table 6). The mean value is around 19 mg/kg/day, and for the 95^{th} percentile around 39 mg/kg bw per day. For children aged 1 - 3, the mean estimate was 124 mg/kg bw per day and for the 95^{th} percentile 203 mg/kg bw per day. The applicant calculates the mean exposure for infants (0 - 12 months) to be around 210 mg/kg bw per day.

Table 5: Estimated daily intake of bLF (mg/d) in Dutch population groups from food products intended to be fortified with bLF (DNFCS-3)[a] as provided by the applicant.

	All-person intake (g bLF per day)					
	N	**Mean**	**SD**	**P50**	**P95**	**P97.5**
Total population	6250	1.53	0.98	1.41	3.04	3.57
Children, 1-3 years	254	1.67	0.55	1.63	2.71	2.90
Boys, 4-9 years	242	1.88	0.93	1.74	3.11	3.60
Girls, 4-9 years	272	1.72	0.62	1.70	2.76	3.00
Boys, 10-18 years	391	2.33	1.19	2.17	4.20	4.87
Girls, 10-18 years	381	2.00	0.89	1.89	3.38	3.77
Males, ≥ 19 years	2117	1.42	1.04	1.25	2.93	3.57
Females ≥ 19 years	2544	1.38	0.87	1.26	2.80	3.30
Pregnant women	50	1.84	0.83	1.87	n. r.	n. r.

[a] Assuming the maximum usage level is applied in the products and the consumers use only the enriched products;
n. r.: not reported due to low sample size.

Table 6: Estimated daily intake of BLF (mg/kg bw per day) in Dutch population groups from food products intended to be fortified with BLF (DNFCS-3)[a] as provided by the applicant

	All-person intake (mg bLF/kg bw per day)					
	N[b]	**Mean**	**SD**	**P50**	**P95**	**P97.5**
Total population	6241	32	34	21	131	135
Children, 1-3 years	254	124	51	117	203	225
Boys, 4-9 years	241	80	41	73	139	165
Girls, 4-9 years	271	76	30	70	125	152
Boys, 10-18 years	390	46	25	40	88	123
Girls, 10-18 years	379	41	19	38	75	93
Males, ≥ 19 years	2116	18	14	16	37	43
Females ≥ 19 years	2540	20	13	18	41	48
Pregnant women	50	25	11	23	n. r.	n. r.

[a] Assuming the maximum usage level is applied in the products and the consumers use only the enriched products;
[b] Bodyweight was not available from all individuals; these individuals were not taken into account when estimating the intake of bLF per kilogram bodyweight per day.
n. r.: not reported due to low sample size.

According to the applicant half the bLF consumption would come from the consumption of milk and milk products. The applicant concludes that these products, in combination with the categories 'non-alcoholic beverages' and 'cakes and pastries', would contribute about 70 % of the daily intake of bLF from the range of foods investigated.

The Panel notes that this type of intake methodology for fortified foods is generally considered to be a "high intake" assessment as a result of several conservative assumptions made in the consumption estimates assuming that all food products within a food category contain the ingredient at the maximum specified level of use. However, with regard to infants, the intake estimate is considered to be realistic.

5. Information from previous exposure to the NF or its source

Bovine lactoferrin has been consumed by humans for a long time through its natural occurrence in cow's milk. According to the applicant, the mean concentration is around 100 mg per litre of bovine milk and can vary between 20 to 200 mg/L (King et al., 2007). Consumption of bLF through dairy sources has been estimated to be 73, 75 and 50 mg/day at the 90th percentile of intake by children, young adults and adults, respectively. Based on consumption data of milk and milk products, the applicant concludes that the mean daily consumption for Dutch people (above the age of 1) is around 40 mg bLF. For Scandinavian countries, this figure is higher, but there are no data available for most Southern European countries. The mean intake in Dutch infants aged 9 months is 63 mg bLF per day. Considering this intake of bLF of Dutch 9 months old infants, an estimated intake of 1.9 g of bLF would be approximately 30 times higher. Assuming that Dutch people above 1 year of age would exclusively consume products with added bLF as intended by the applicant, the mean intake of bLF would be almost 60 times higher than the intake from foods with no bLF added. AFSSA indicated that in France, consumption data suggest a mean intake of native bLF by adults varying from 20 – 50 mg per day depending on the type of consumed dairy products and that approximately 5.3 mg per day of native bLF is consumed which originates from raw milk and cheeses produced from raw milk (AFSSA, 2008).

The Panel considers that the relevance of baseline consumption data for bLF is limited, since most of the consumed products are expected to have undergone heat treatment while bLF intended as a novel food ingredient might be consumed as a native protein. The Panel considers that the intake of native bLF is only a small fraction of bLF consumed in dairy products.

Infants who are breast-fed consume human lactoferrin, a natural compound of human milk. According to references provided by the applicant, human milk contains from 1 to 4.2 mg lactoferrin per ml (Hennart et al., 1991; Masson and Heremans, 1971; Rudloff and Kunz, 1997). The content of human lactoferrin in colostrum was reported to be 10.6 mg/mL. The intended consumption of bLF via infant formula is within the levels of human lactoferrin consumed via breast milk.

bLF is currently marketed in the US and Europe as a dietary supplement. According to the applicant, US consumption of bLF from dietary supplements ranges from 10 to 1200 mg/day. AFSSA (2010) notes in its comments on the initial assessment report that lactoferrin is authorised in food supplements within the European Union. According to the applicant, a small part of the population in the EU consumes a commercial product containing bLF that claims to promote skin health which may provide an additional intake for some individuals of the target group (teenagers aged 10-18) of 3.2 mg bFL/kg bw per day.

According to the initial assessment report of the Netherlands, there are food supplements containing native bLF manufactured by various firms available in the EU. The dossier also provides intake estimates for the US population (above two years of age) which show that bLF intake can amount to 100 mg per day (95^{th} percentile). This has been calculated on the basis of consumption data from 1994 – 1998 (USDA, 2000. 1994-96. 1998), in which dairy products and whey proteins, which are added to many of foods as an ingredient, were combined as a source of bLF. Estimating the intake of bLF consumed with a daily intake of 2 – 3 servings of milk products as recommended by the food guide pyramid of the US Department of Agriculture (USDA, 1996), together with bLF from whey protein sources, also amounts to approximately 100 mg per day. Although the applicant does not discuss this, it can be assumed that most of the bLF is denatured during the sterilization and pasteurization of dairy products. According to the applicant, FrieslandCampina sells bLF to business partners to be included in end products manufactured by these food business operators, the primary markets for which are in Asia. In the US, bLF of FrieslandCampina holds a notified GRAS status and may be used in sports and conventional foods in amounts up to 100 mg per product serving. Lactoferrin is approved for use in foods, including infant food, in Japan and South-Korea. There are a number of suppliers of bLF in

the various markets, but their sales data are confidential so the applicant could not provide verified consumption data for bLF.

6. Nutritional information on the NF

One Member State commented on an unfavourable increase in iron intake which may result from consumption of foods with added bLF. Considering the specified maximum levels of iron (250 mg per kg novel food ingredient) and the intended uses and use levels, the Panel considers that the additional iron intake would be very low and is not of health concern.

Based on the information provided on the protein nature and the proposed use levels, the Panel considers that the intake of bLF is not nutritionally disadvantageous.

7. Microbiological information on the NF

The Panel has no safety concerns with regard to the microbiological specifications (Table 3) of bLF.

8. Toxicological information on the NF

The Panel notes that no toxicological investigation has been carried out with the bLF product of the applicant. The applicant provided publications and toxicological study reports on an Ames test study (Kawai, 1997; Yamauchi et al., 2000a), a single dose oral toxicity study in rats (Nishimura, 1991) and a four-week (Nishimura, 1997) and a thirteen-week (Nishimura, 1998; Yamauchi et al., 2000b) repeated dose oral toxicity study in rats. These studies were conducted with a bLF provided by the Japanese enterprise Morinaga Milk Industry Co., Ltd.

Following a request by EFSA to provide information which demonstrates that the test substance (Morinaga's bLF) can be considered representative for the bLF produced by FrieslandCampina, particularly with respect to its status of denaturation and iron content, the applicant responded that it had collected detailed information on the production process from Milei, which produced bLF for Morinaga in Europe. The applicant argued that the source of the NFI is identical, namely European cow's milk, and also that the production process, particularly the use of an ion exchange column to concentrate bLF, and compositional data including iron content (200 ppm vs. 100 - 160 ppm), are largely similar or equivalent. The drying process (freeze drying vs. spray drying) should guarantee low levels of denatured lactoferrin in both cases. The applicant also conducted and provided a chromatographic (HPLC) comparison between the bLF produced by Morinaga and the bLF produced by FrieslandCampina.

The Panel considers that the data provided by the applicant provide sufficient information to accept toxicological studies conducted with the Morinaga bLF preparation for the submitted application.

8.1. Genotoxicity

In tests on gene mutations in bacteria (Ames test) using *Salmonella typhimurium* strains TA98, TA100, TA1535 and TA1537 and the *Escherichia coli* strain WP2uvrA, no mutagenic activity was detected, either with or without metabolic activation (S9 mix), at the highest dose tested of 5 mg bLF/plate (Yamauchi et al., 2000a).

The Panel considers from the nature of the NFI as a protein and the negative result in the Ames test that there are no safety concerns regarding genotoxicity.

8.2. Animal toxicity studies

8.2.1. Absorption, distribution, metabolism, excretion

In a study by Schmitz et al. (1988), the degradation of bLF was examined in six adult minipigs and three piglets; ingestion resulted in up to 20 % undegraded bLF in faeces.

Fischer et al. (2007) studied the uptake of ingested bLF and its pattern of tissue accumulation in adult mice; immunoreactive bLF is absorbed by the mature mouse intestine. Bovine lactoferrin is transferred from the intestine into the blood circulation and subsequently distributed over various tissues of the mouse such as liver, kidney and spleen. Bovine lactoferrin resists major proteolytic degradation in the intestinal lumen and can be transported across the intestinal lumen as the native molecule (Fischer et al., 2007). Continuous ingestion of bLF seems to reduce its intestinal absorption.

With regard to the suitability of rats to assess human safety, the applicant considered rats as a suitable species since rats have been shown to absorb native bLF (Kitagawa et al., 2003). Moreover, orally administered bLF was found in the liver, kidneys, gall bladder, spleen and brain of adult mice (Fischer et al., 2007) detected using an ELISA. The Panel considers that the provided studies show absorption of bLF in the intestine in rats, and that rats, therefore, are suitable to study bLF toxicity.

8.2.2. Acute and sub-acute toxicity studies

An acute toxicity study in rats was provided by the applicant. According to the applicant, administration of a single oral dose of 1,000 or 2,000 mg/kg bLF or iron-saturated bLF resulted in no adverse effects (Nishimura, 1991).

In a four-week study in Sprague-Dawley rats, doses of 0 (water), 200, 600 or 2,000 mg bLF/kg bw per day were administered by oral intubation. According to the applicant, no adverse effects were observed up to the highest administered dose (Nishimura, 1997).

8.2.3. Sub-chronic toxicity

In a thirteen week study on subchronic toxicity in Sprague-Dawley rats, doses of 0 (water), 200, 600 or 2,000 mg/kg bw per day were administered by gavage once daily to groups of 12 male and 12 female rats (Nishimura, 1998; Yamauchi et al., 2000b).

No clinically relevant effects were observed in any of the groups. Feed intake and body weight were comparable. In the haematological, clinical biochemical and urine analyses, no toxicologically relevant differences were found between the groups. Ophthalmological examination revealed no abnormalities in any animal. Determination of the weights of selected organs, and macroscopic and histopathological examinations during necropsy, did not reveal toxicologically relevant effects.

During the treatment period one male in the 2,000 mg/kg body weight group died because of an error in intubation and a female in the 2,000 mg/kg body weight group died because of a malignant lymphoma. There were no findings in the histopathology or peripheral blood profile, which were indicative of lymphoma in other animals. The authors of the study report commented that the appearance of lymphoma in Sprague-Dawley rats at an early age has been observed in many laboratories, and provided references on the incidence of spontaneous lymphoma in this rat strain. The Panel considers that the lymphoma is not related to the test substance.

Islet fibrosis in the pancreas was observed in male rats and the incidence and severity of the finding in each bLF administration group were slightly higher than in the control group (slight islet fibrosis in the pancreas: two males in the control group, two males in the 200 mg/kg group; mild islet fibrosis in

the pancreas: one male in the control group, two males in the 200 mg/kg group, six males in the 600 mg/kg group, and six males in the 2,000 mg/kg group). The authors of the study report commented that islet fibrosis in the pancreas is known to be a lesion which occurs at a relatively high frequency as a phenomenon accompanying ageing in this strain of rat, that there were no morphological differences in the fibrosis of islets between the control group and any bLF administration group. They considered that the islet fibrosis was not due to the lactoferrin administration and concluded that the highest administered dose of 2,000 mg/kg body weight per day can be considered as NOAEL. This conclusion was shared in the initial assessment report of The Netherlands. One Member State questioned this conclusion. In its response to Member States' comments, the applicant provided additional data on the incidence of pancreatic islet fibrosis in this strain of rat. According to Imaoka et al. (2007) and Molon-Noblet et al. (2001) the incidence of spontaneous pancreatic islet fibrosis was 14/20 (70 %) and 7/15 (47 %) in 18 and 20 week old rats which corresponds to the age (19 weeks) of the rats used in this sub-chronic study.

The Panel considers that the highest dose tested (2,000 mg/kg bw per day) did not result in effects which could be attributed to the test substance.

8.2.4. Chronic toxicity/carcinogenicity, reproductive and developmental toxicity

No studies on chronic toxicity/carcinogenicity and reproductive and developmental toxicity were provided.

8.2.5. Allergenicity

The applicant referred to labelling requirements (bovine milk derived). The Panel considers that the risk of allergic reactions is not dissimilar to other dairy products derived from bovine sources.

8.3. Human studies

The dossier refers to 33 published human studies conducted with bLF. The Panel notes, however, that all of these studies, with the exception of a study with infants (King et al., 2007), used lactoferrin products from companies other than that of the applicant. Almost all of the trials were designed to study presumed efficacy. No data were provided on the kinetics of bLF in humans. The initial assessment report of the Netherlands confirmed the applicant's statements that there was no mention of any adverse effects and no indications of subjects discontinuing consumption due to adverse reactions in any of these studies.

8.3.1. Studies in children

Four published studies with a duration of fifteen days to nine months investigated presumed antimicrobial effects of bLF at intakes between 100 and 1,000 mg per day alone or in co-administration with other substances (Ajello et al., 2002; Egashira et al., 2007; Ochoa et al., 2008; Zavaleta et al., 2007). Safety relevant endpoints were not studied. In two studies in Peruvian children which reported on adverse events the number of adverse events did not differ between the treatment and the placebo group and no adverse event could be attributed to the test substance (Ochoa et al., 2008; Zavaleta et al., 2007).

Six studies in infants with a duration from fourteen days to five months studied the effect of bovine lactoferrin at intakes from 100 to 2,900 mg per day on iron homeostasis and iron retention (Chierici et al., 1992; Davidsson et al., 1994; Fairweather-Tait et al., 1987; Hernell and Lonnerdal, 2002; Lonnerdal and Hernell, 1994; Schulz-Lell et al., 1991) and two trials studied the effects of lactoferrin on the microbiota (Balmer et al., 1989; Roberts et al., 1992). The studies did not include safety endpoints apart from body weight and height which were not affected by the treatment.

King et al. (2007) performed a one-year double-blind, placebo-controlled intervention study in 79 healthy infants (≤ 4 weeks of age, born at ≥ 34 weeks of gestation, ≥ 2,000 g, and strictly bottle fed). Exclusion criteria included intolerance to cow's milk formula, major congenital anomalies, known immunodeficiency, an HIV-infected mother, or parental inability to follow the protocol. The infant formula given to those in the experimental group contained added bLF produced by DMV International (now FrieslandCampina) giving a total concentration of 85 mg/100 mL. The infant formula of the control group contained 102 mg bLF per litre (a standard commercial product). During the intervention, the growth of the babies, body weight, length and head circumference, was determined regularly. The frequency and duration of diarrhoea, respiratory infections and acute middle ear infections *(otitis media)* were collected. When the babies were around 9 and 12 months old, blood was also taken. The following haematological characteristics were determined: haemoglobin, haematocrit value and the mean size of red blood cells (median corpuscular volume). Moreover, the concentrations of specific antibodies were measured, i.e. against diphtheria, tetanus, Hepatitis B and *Haemophilus influenzae b*., in order to evaluate the response to the usual vaccinations. Twenty-seven children (34 %) dropped out, nineteen because of parental perception of infants' intolerance to the randomized formula, 3 infants were withdrawn by their parents without explanation, and 5 were lost to follow-up. Fifty-two infants completed the study, 26 each in the treatment and control group. The baseline characteristics, number of dropouts, illnesses and adverse effects recorded in this study were statistically not different between the placebo and the bLF groups. The Panel notes the limitations of this study for safety assessment, i.e. the relatively low dose used (85 mg/100 mL), the lack of actual consumption data of bLF/milk, the small number of study subjects and the limited number of safety related endpoints. The Panel also notes that the full study report was not provided to EFSA. The applicant informed EFSA that although the study was paid for by DMV International, Delhi NY (now FrieslandCampina), it does not hold or own the original study data.

The Panel notes that no adverse effects related to bLF have been reported in eleven studies in children, but that ten of the studies did not include safety relevant endpoints other than body weight and height, which were not affected by bLF. Due to the limitations described, the Panel considers that the human studies provided are of limited relevance for the safety assessment of bLF.

8.3.2. Studies in adults

Fifteen studies with adult patients measured the presumed efficacy of 20 mg bLF per day for 5 days in post-surgical patients (Zimecki et al., 2001) or at bLF intakes between 400 and 7,200 mg per day for up to 12 months in patients suffering from chronic hepatitis C (Ishii et al., 2003; Kaito et al., 2007; Okada et al., 2002; Tanaka et al., 1999; Ueno et al., 2006), *Helicobacter pylori* infections (de Bortoli et al., 2007; Di Mario et al., 2003; 2006; Okuda et al., 2005; Tursi et al., 2007; Zullo et al., 2005; 2007), Sjögren's syndrome (Dogru et al., 2007) or tinea pedis (Yamauchi et al., 2000c). The authors of five of these studies in adults reported that there were no statistically significant differences in the number of recorded adverse events between the treatment and the control group (de Bortoli et al., 2007; Kaito et al., 2007) or that none of the adverse effects were related to bLF, respectively (Di Mario et al., 2003; Ueno et al., 2006; Yamauchi et al., 2000c).

Four human studies were conducted with healthy volunteers, three of which studied immunological endpoints. In an uncontrolled trial, 10 healthy male subjects received approximately 2 g bLF per day over 4 weeks to study the effect on a number of immunological parameters (Yamauchi et al., 1998). In another uncontrolled trial, 8 healthy males received 100 mg lactoferrin for 7 days followed by 200 mg per day for another 7 days to study the effect on a large number of cellular and humoral immunological parameters (Mulder et al., 2008). In one open controlled study with 300 women at different stages of pregnancy, 107 women received 100 mg bLF twice per day over 30 days to study its efficacy on haemoglobin and total serum iron levels (Paesano et al., 2006). The authors reported that no side effects were observed in women receiving lactoferrin. The Panel notes the low dose. One RCT with 16 healthy female long distance runners studied the effect of 1.8 g lactoferrin plus 6 mg

iron per day given over 4 weeks on iron haemostasis, red blood cell parameters and blood lactate concentration after intensive exercise, but did not include safety relevant endpoints (Koikawa et al., 2008). The authors did not report on the occurrence of adverse effects.

The Panel notes that no adverse effects related to bLF have been reported in nineteen studies in adults which were not designed to study the safety of bLF.

Immunotoxicology and neurotoxicolology

Concerns were expressed and cited by Member States that cow's milk baby food may promote the occurrence of the autoimmune disease type 1 diabetes in genetically predisposed children. The Panel considers that this discussion concerns bovine dairy products in general and not specifically the NFI of this application.

Other comments received from Member States were on the reported high prevalence of auto-antibodies against lactoferrin among diabetes mellitus type 1 patients (Taniguchi et al., 2003), that orally-administered bLF has been found as a native protein in the brain of mice (Fischer et al., 2007) and that dietary intake of bLF may interfere with the production and function of endogenous lactoferrin. Comments were also made on the finding of increased concentrations of lactoferrin and/or lactoferrin receptors in the characteristic lesions of patients with neurodegenerative disorders (Leveugle et al., 1994) and that lactoferrin, therefore, may be actively involved in neurodegenerative processes.

The applicant responded that the history of use of bLF and the presented evidence do not raise safety concerns with regard to interference of exogenous with endogenously produced lactoferrin. The applicant acknowledged the lack of information on absorption of bLF in humans. It also considered that bovine and human lactoferrin share about 70 % amino acid sequence homology, and that it was therefore theoretically possible that an immune response generated against exogeneous bLF could react with an identical or near-identical epitope of human lactoferrin. However, there are no data on a correlation between anti-bovine and anti-human lactoferrin antibodies in individual patients. A study by Brock et al. (1998) in infants and adults showed that antibodies to bLF were present in a substantial part of the population. The incidence in normal individuals was sufficiently high to suggest that these antibodies had no pathological effect, and that they were coincidental to the development of autoimmunity.

With regard to a Member State's concerns on a potential role of lactoferrin in neurodegenerative disorders, the applicant responded that Leveugle et a1. (1994) showed that lactoferrin accumulates in the characteristic lesions of different neuropathologic conditions. Wang et al. (2010) reported a strong up-regulation of lactoferrin in the brains of patients with Alzheimer's disease (AD) and in the brain of a transgenic mouse model for AD. The authors reported that the deposition of lactoferrin in the senile plaques of AD patients does not occur in early stages, but is mediated by pathologic processes that are common to both AD patients and APP-transgenic mice. This indicates that the accumulation of lactoferrin might be the result of the development of neuropathologic lesions in patients with certain neurodegenerative disorders.

The Panel considers that the evidence provided does not support the concerns voiced by Member States, and does not raise safety concerns.

DISCUSSION

In an overall evaluation, the Panel considered that the NFI is essentially a protein, a constituent of cow's milk. According to the information provided by the applicant, bLF is present in the NFI mostly as non-denatured lactoferrin. The Panel notes that lactoferrin is a normal constituent of human milk, and that the intended consumption of bLF as specified by the application is within the levels of human lactoferrin in breast milk consumed by infants; human lactoferrin is also non-denatured.

The Panel notes that the mean estimated intake of "lactoferrin" of approximately 210 mg/kg bw per day for infants up to the age of one year would be around ten times lower than the highest dose (2,000 mg/kg bw per day) tested in a subchronic thirteen week rat study, which did not show adverse effects related to bLF. For adults above 19 years of age the intake is approximately 100 times lower. This maximum level of anticipated intake is considered a high intake scenario as opposed to a worst-

case situation. No adverse effects have been reported in studies with humans which were not designed for safety, though. The data provided suggest the absence of adverse effects of bLF at the proposed levels of consumption.

CONCLUSIONS

The Panel concludes that the novel food ingredient, bLF, is safe under the proposed uses and use levels.

DOCUMENTATION PROVIDED TO EFSA

1. Dossier on 'bovine lactoferrin'. November 2010. Submitted by FrieslandCampina. Additional information was submitted on 17 and 25 November 2011.

2. Letter from the European Commission to the European Food Safety Authority with the request for an opinion on the safety of the safety of 'bovine lactoferrin'. SANCO E6/AK/bs Ares (2010)790871.

3. Initial assessment report carried out by The Netherlands: Bovine lactoferrin. Assessment of safety for the consumer, in accordance with European Regulation 258/97 concerning novel foods and novel food ingredients (31 March 2010).

4. Member States' comments and objections

5. Response by the applicant to the initial assessment report and the Member States' comments and objections

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GLOSSARY / ABBREVIATIONS

bw	bodyweight
bLF	bovine lactoferrin
NF(I)	novel food (ingredient)