

SCIENTIFIC OPINION

Scientific Opinion on bovine lactoferrin¹

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 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 that is isolated from cheese whey and skimmed milk, and purified. The applicant intends to add bLF to foods for particular nutritional uses, i.e. infant and follow-on formulae, dietary food for special medical purposes, dairy products, yoghurts and yoghurt drinks, and chewing gums. According to the applicant, the high intake estimate for infants would be 1.1 g bLF per day. For adults, the applicant's calculation estimates a mean and 97.5th percentile intake of 0.6 and 2.1 mg/kg bodyweight per day, respectively, and a mean and 97.5th percentile daily intake of about 45 mg and 150 mg, respectively. The Panel notes that the safety of bLF as a novel food ingredient has already been assessed with a favourable outcome. That evaluation was to a significant extent based on safety data on bLF produced by Morinaga. The Panel also notes that the applicant intends maximum use levels of bLF in foods which are equivalent or lower than those intended by the applicant of the previous Opinion, and that the range of foods to which it is intended to add bLF is smaller. Consequently, the estimated intake levels described for the present application are comparable for infants and lower for all other population groups. 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-2011-00974, adopted on 28 June 2012.

² Panel members: Carlo V. Agostoni, Jean-Louis Bresson, Susan Fairweather-Tait, Albert Flynn, Ines Golly, Hannu Korhonen, Pagona Lagiou, Martinus Løvik, Rosangela Marchelli, Ambroise Martin, Bevan Moseley, Monika Neuhäuser-Berthold, Hildegard Przyrembel, Seppo Salminen, Yolanda Sanz, Sean (J.J.) Strain, Stephan Strobel, Inge Tetens, Daniel Tomé, Hendrik van Loveren and Hans Verhagen. Correspondence: nda@efsa.europa.eu

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 Pötting, Seppo Salminen, Hendrik Van Loveren, Hans Verhagen and EFSA's staff member Wolfgang Gelbmann for the preparatory work on this scientific opinion

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. Batch testing confirmed that the product complies with the given specifications. Bovine lactoferrin from whey cheese or skimmed milk is concentrated via ion exchange and is subsequently subjected to filtration steps. Ultimately the bLF is dried by means of freeze-drying, milled, or sieved. The applicant provided sufficient information regarding the specification, manufacture, composition and stability of bLF.

The applicant intends to add bLF to foods for particular nutritional uses, i.e. infant and follow-on formulae, dietary food for special medical purposes, and foods such as fermented milks (e.g. buttermilk and fermented milk drinks) and non fermented milk drinks, ice cream, yoghurts and yoghurt drinks, and chewing gums. The intended maximum levels are 1,000 mg/L for infant formulae, 3000 mg/100 g for chewing gums and 133 mg/100g for dairy products.

According to the applicant, the high intake estimate for infants would be 1.1 g bLF per day. For infants of 6 to 12 months consuming follow-on formula, the applicant anticipated that the intakes on an absolute and body weight basis would be less than those for infants consuming solely formula milk, since the consumption of infant formulae per kg bodyweight decreases with the age of the infants. For male adults, the applicant's calculation estimates a mean and 97.5th percentile intake of 0.6 and 2.1 mg/kg bodyweight per day, respectively, and a mean and 97.5th percentile daily intake of about 45 mg and 150 mg, respectively.

The toxicological studies conducted were an Ames test study, a single dose oral toxicity study in rats and a four-week and a thirteen-week repeated dose oral toxicity study in rats. The Panel considers that the evidence provided does not raise safety concerns.

The Panel notes that the safety of bLF as a novel food ingredient has already been assessed with a favourable outcome (EFSA, 2012). That evaluation was to a significant extent based on safety data on bLF produced by Morinaga. The Panel also notes that the applicant intends maximum use levels of bLF in foods which are equivalent (infant formula) or lower than those intended by the applicant of the previous Opinion, and that the range of foods to which it is intended to add bLF is smaller. Consequently, the estimated intake levels described for the present application are comparable for infants and lower for all other population groups.

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

TABLE OF CONTENTS

Abstract	1
Summary	2
Table of contents	3
Background as provided by the European Commission	4
Terms of reference as provided by the European Commission	4
Assessment	5
1. Specification of the Novel Food (NF)	5
2. Effect of the production process applied to the NF	7
3. History of the organism used as a source	8
4. Anticipated intake/extent of the use of the NF	8
5. Information from previous exposure to the NF or its source	11
6. Nutritional information on the NF	11
7. Microbiological information on the NF	11
8. Toxicological information on the NF	11
8.1. Human studies	11
8.2. Allergenicity	11
Documentation provided to EFSA	12
References	12
Glossary / Abbreviations	14

BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION

On 2 March 2011, the company Morinaga Milk Industry Co., Ltd. submitted a request under Article 4 of Novel Food Regulation (EC) N° 258/97 to place on the market 'lactoferrin' as a novel food ingredient.

On 22 June 2011, the competent authorities of Ireland forwarded to the Commission their initial assessment report, which came to the conclusion that for 'lactoferrin' an additional assessment was required in order to consider this application in conjunction with an earlier application concerning 'lactoferrin'.

In consequence, a Community Decision is now required under Article 7, paragraph 1 of Regulation (EC) No 258/97.

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 for 'lactoferrin' as a food ingredient in the context of Regulation (EC) N° 258/97.

EFSA is asked to carry out the additional assessment.

ASSESSMENT

In accordance with Commission Recommendation 97/618/EC, 'bovine lactoferrin (bLF)' is allocated to Class 2.1 as '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 and the initial assessment by the competent authority of Ireland. 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 as indicated by Regulation (EC) No 258/97. It is noted that the novel food ingredient (NFI) is intended by the applicant to be added to foods for particular nutritional uses (PARNUTS), i.e. infant and follow-on formulae, dietary food for special medical purposes, and foods such as fermented milks (e.g. buttermilk and fermented milk drinks) and non fermented milk drinks, ice cream, yoghurts and yoghurt drinks, and chewing gums, to improve immune health. 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.

The Panel notes that the safety of bLF as a novel food ingredient has already been assessed with a favourable outcome (EFSA, 2012). That evaluation was to a significant extent based on safety data on bLF produced by Morinaga. Where applicable the present opinion makes reference to that assessment.

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 sulphhydryl 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 food ingredient as follows, and the contents are expressed as percentage by weight. The pink, odourless powder contains at least 94.5 % protein, at least 96 % of which is bFL (Table 1).

Table 1: Specification for the novel food ingredient

Parameter	Units	Specification	Method of Analysis
Appearance	Visual	Pink and odourless powder	Visual Inspection
Total Protein	% (dry weight basis, N x 6.38)	Min. 94.5	Kjeldahl method (N x 6.38)
Purity (lactoferrin)	% w/w of total protein	Min. 96.0	HPLC (per protein)
Total lactoferrin	% w/w	Min. 90.7	Calculation
Ash	% (dry weight basis)	Max. 1.3	Heat to >550°C
Loss on Drying	% w/w	Max. 4.2	Heat to 150°C

pH (2% solution w/w)	pH units	5.2 to 7.2	pH meter
Iron Content	mg/ 100 g	Max. 35	ICP
Arsenic	mg/kg	Max. 2	ICP-MS
Heavy metals as Lead	mg/kg	Max. 20*	ICP-MS
Total bacteria	cfu/g	Less than 1,000	Standard agar
Coliform bacteria	per 0.1 g	Negative	Desoxicholate agar
Coagulase positive staphylococci	per 0.01 g	Negative	Mannitol salt agar with egg yolk
Yeast	cfu/g	Less than 30	Potato dextrose agar
Moulds	cfu/g	Less than 30	Potato dextrose agar

* Maximum limit in accordance with Regulation (EC) No 1881/2006

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 bLF

Property	bLF	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		
Apo-form at 280 nm	12.7	Aisen et Leibman, 1972
Holo-form at 470 nm	0.400	
Protease sensitivity		
	Relatively low*	Brines et Brock, 1983
Iron-binding		
Equilibrium dialysis ($K_1 \times 10^{-4}$)	3.73	Aisen et Leibman, 1972
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 three production lots produced in November 2005 and in January and July 2008 (Table 3) show that the protein content varied from 98.3 to 99.6 %.

Table 3: Analytical data from the testing of three batches each from cheese whey and skimmed milk.

Parameter	Units	Analytical Results						Methods of Analysis
		bLF from Cheese Whey			bLF from Skimmed Milk			
		Batch 1	Batch 2	Batch 3	Batch 1	Batch 2	Batch 3	
Protein	% w/w	99.4	98.3	99.0	98.7	99.5	99.6	Kjeldahl method (N x 6.38)
Lactoferrin (purity)	% w/w of total protein	97.1	97.1	96.3	97.5	97.6	97.2	HPLC
Total lactoferrin	% w/w	96.5	95.4	95.3	96.2	97.1	96.8	Calculation ¹
Fat	% w/w	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	Rose Gottlieb method
Ash	% w/w (dry weight)	0.26	0.26	0.23	0.80	0.33	0.45	Heat to >550°C
Loss on drying	% w/w	0.44	0.58	0.14	1.85	0.85	0.46	Heat to 150°C
Iron	mg/100 g	23.3	23.4	20.5	10.9	11.1	10.6	ICP
pH	pH units	5.58	5.86	5.80	6.92	5.82	6.10	pH meter
Inorganics and Minerals								
Sodium	mg/100 g	55.5	28.9	46.7	178	71.5	118	ICP
Potassium	mg/100 g	18.20	2.13	3.60	11.5	2.53	4.95	ICP
Magnesium	mg/100 g	1.14	0.63	1.47	7.08	1.65	2.04	ICP
Phosphate	mg/100 g	4.5	3.13	6.97	3.35	11.5	6.21	ICP
Calcium	mg/100 g	15.5	7.66	18.6	75.6	18.1	23.0	ICP
Chloride	mg/100 g	813	805	779	914	799	845	Potentiometric titration method
Copper	mg/100 g	0.09	0.12	0.91	nd (<0.05)	nd (<0.05)	nd (<0.05)	ICP
Zinc	mg/100 g	0.17	0.12	0.94	0.28	0.22	0.22	ICP
Manganese	mg/100 g	0.008	nd (<0.003)	nd (<0.003)	0.013	0.006	0.007	ICP

HPLC = High Performance Liquid Chromatography; ICP = inductively coupled plasma; nd = not detected

¹ Total lactoferrin content = lactoferrin (purity of total protein content, %) x protein content (%).

Minor compounds [lactogenin-like protein, angiogenin, insulin-like growth factor-binding protein (IGFbp)] of the residual protein fraction were detected with gel electrophoresis tests.

According to the dossier, the iron saturation is less than 25 % and the iron content varied from 10.6 to 23.4 mg/100 g.

The applicant provided satisfactory analytical data on levels of contaminants. The analyses included heavy metals, mycotoxins, dioxins and dioxin-like PCBs, pesticides and chlorinated hydrocarbons. All foods, and hence also novel foods, have to comply with the 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

The applicant provided information on the production process of bLF derived from cheese whey or skimmed milk. After the selective isolation of lactoferrin from these sources, the subsequent purification processes are identical.

Initially, skimmed milk is cooled before undergoing filtration. Lactoferrin is isolated by ion exchange chromatography from cheese whey or skimmed milk, respectively, before undergoing a series of demineralisation stages and pasteurisation to yield the novel ingredient. After selective isolation of lactoferrin from either cheese whey or skimmed milk, the subsequent purification process are identical. All processes employed are physical separation and pasteurisation techniques common to the dairy industry. In particular, the isolation process is analogous to that employed in whey protein concentrate production, except that the conditions are optimised for selective purification of lactoferrin.

According to the applicant, the production of bLF is conducted consistent with current good manufacturing practices (GMP) and the principles of Hazard Analysis Critical Control Point (HACCP), and all practices employed are in compliance with the relevant hygiene legislation.

Stability

The applicant provided analytical data (using an ELISA) on three batches of lactoferrin isolated from cheese whey and one batch from skimmed milk when stored in aluminium bags at room temperature for at least 36 months. In addition, data were provided on the stability of three batches of lactoferrin added to yoghurt and stored for 16 days under refrigerated conditions, of one batch added to dried skimmed milk stored for 46 months, and of three batches added to a commercial infant formula and stored for 24 or 36 months. Most of the results showed a recovery rate of close to 100 % (90 to 103 %).

The Panel concludes that the production process is sufficiently described by the applicant and does not raise safety 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 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, dietary food for special medical purposes, and foods such as fermented milks (e.g. buttermilk and fermented milk drinks) and non fermented milk drinks, ice cream, yoghurts and yoghurt drinks, and chewing gums.

Table 4 shows a list of the proposed product groups with the maximum levels of bLF, expressed in mg per 100 g end product.

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

Food Category	Proposed Food Use	Maximum Use-Level (mg/serving)	Serving Size ¹ (g)	Maximum Use-Level mg/100 g
Milk and milk products	Fermented milks ²	100	200	50
	Ice-cream	100	75	133
	Milk beverages ³	100	200	50
	Milk powders and milk-based	60 ⁴	200 (RTD)	30 (RTD)

	powdered drink mixes		20 (Powder)	300 (Powder)
	Yoghurts	100	125	80
	Yoghurt drinks	100	200	50
Sugar confectionery	Chewing gums	60	2	3,000
Foods for Particular Nutritional Uses (PARNUTS)	Foods for special medical purposes (FSMP)	Depending on the nutritional needs of the individual up to a maximum level of 3 g/day		
	Infant formulae and follow-on formulae	Formulae (powder):	30 - 770 mg/100 g	
		Prepared (liquid) formulae:	40 - 1,000 mg/L	

RTD = Ready to drink;

¹ Serving sizes are based on food portion sizes established by the Food Standards Agency (FSA, 2002), unless otherwise specified;

² The NDNS databases (Office for National Statistics, 2005; UKDA, 1995, 2001); do not include food codes under the group "fermented milks" specifically, and food codes for "buttermilk" and "yakult" were used as surrogates to represent these types of products on the UK market;

³ The applicant indicated that the target products are "fortified-type milk beverages" either provided as one or multiple servings, and food code selection was therefore limited to specific milk drink products or fortified milks as surrogates for the intended use, and did not include plain milks;

⁴ Use-levels based on reconstituted dried milk beverages (i.e. providing 60 mg lactoferrin per 200 g serving).

The applicant provided an assessment of the consumption of bLF measured as the total intake of the ingredient maximally dosed in products, which was conducted based on the proposed food uses and use-levels summarised in Table 4.

Calculations for the mean and high-level (97.5th percentile) all-person and all-user intakes, and percent consuming were performed for each of the individual proposed food-uses for bLF and for all food-uses combined. The estimated per person and per kilogram body weight intakes were calculated for various population groups based on consumption data from the United Kingdom [(NDNS 2000-2001, Office for National Statistics, 2005); (NDNS 1992-1993, UKDA, 1995); (NDNS 1997, UKDA, 2001)].

The use in foods for special medical purposes (FSMPs) was excluded from the intake assessment on the basis that these products would be consumed as partial or complete replacements for conventional foods, rather than in combination with them, and because the dietary needs of the individual would be different to those of the general population. For the exposure assessment of bLF from chewing gums, the applicant conservatively assumed that the ingredient maximally dosed is consumed either by leaching during the chewing process or by swallowing the gums.

The applicant provided high-intake data for infant formulae of 1,060 mL (Kersting et al., 1998) and 1,200 g per day (IOM, 1991). Based on these two references, EFSA has estimated a mean intake for infant formula of 800 g per day and a rounded high value of 1100 g per day (EFSA, 2010). Considering the proposed maximum concentration of 1000 mg/L, the resulting high intake estimate for infants would be 1.1 g bLF per day. For infants of 6 to 12 months consuming follow-on formula, the applicant anticipated that the intakes on an absolute and body weight basis would be less than those for infants consuming solely formula milk, since the consumption of infant formulae per kg bodyweight decreases with the age of the infants.

Table 5 summarises the estimated total intake of bLF (g/person/day) from all proposed food-uses. Table 5 presents this data on a per kilogram body weight basis (mg/kg body weight/day). Consistent with bLF being intended for use as a food ingredient in only a limited range of foods for the general population (i.e. dairy products only, with the exception of chewing gums), the percentage of users was

moderate among all the population groups evaluated in the current assessment. According to the application, each population group had 45.5 % or more identified users of food products in which bLF is proposed for use; Children of 4-10 years were identified as the population group containing the greatest percentage of users at 74.6 %, followed by female teenagers at 63.2 % and male teenagers at 60.3 %. Moderate user percentages within a population group typically lead to dissimilar results for the all-person and all-user consumption estimates, and therefore it is the all-user estimates that are more applicable to the assessment of safety as they are more likely to represent exposure in the target populations.

Of the individual population groups, male adults were determined to have the greatest mean and 97.5th percentile all-user intakes of 47 and 150 mg/person/day (0.6 and 2 mg/kg body weight/day), respectively. Female teenagers had the lowest mean and 97.5th percentile all-user intakes of 30 and 112 mg/person/day (0.6 and 2.4 mg/kg body weight/day), respectively (Tables 5 and 6).

Table 5: Estimated daily intake of bLF (mg) in UK population groups from food products intended to be fortified, as provided by the applicant based on NDNS consumption data (1992-1993, 1997, 2000-2001).

Population Group	Age Group (Years)	% User	Actual # of Total Users	All-Person Consumption				All-Users Consumption			
				Mean (mg)	Percentile (mg)			Mean (mg)	Percentile (mg)		
					90 th	95 th	97.5 th		90 th	95 th	97.5 th
Children	1½ - 4½	57.9	955	23	68	88	110	40	85	103	124
Young People	4-10	74.6	624	28	72	93	111	37	81	100	118
Female Teenagers	11-18	63.2	282	19	57	74	97	30	68	83	112
Male Teenagers	11-18	60.3	251	24	73	107	125	39	95	123	136
Female Adults	16-64	51.6	494	24	72	100	124	44	97	120	143
Male Adults	16-64	45.6	349	23	69	92	114	47	98	120	150

Table 6: Estimated daily intake of bLF (mg/kg bw per day) in UK population groups from food products intended to be fortified, as provided by the applicant based on NDNS consumption data (1992-1993, 1997, 2000-2001).

Population Group	Age Group (Years)	% User	Actual # of Total Users	All-Person Consumption				All-Users Consumption			
				Mean (mg/kg)	Percentile (mg/kg bw)			Mean (mg/kg)	Percentile (mg/kg bw)		
					90 th	95 th	97.5 th		90 th	95 th	97.5 th
Children	1½ - 4½	57.9	955	1.6	4.8	6.6	7.7	2.8	6.2	7.4	8.6
Young People	4-10	74.6	624	1.1	2.7	3.6	4.5	1.5	3.1	4.4	5.1
Female Teenagers	11-18	63.2	282	0.4	1.1	1.4	2.0	0.6	1.3	1.7	2.4
Male Teenagers	11-18	60.3	251	0.5	1.5	1.9	2.5	0.8	1.8	2.3	2.7
Female Adults	16-64	51.6	494	0.3	1.1	1.4	1.9	0.6	1.4	1.9	2.1
Male Adults	16-64	45.6	349	0.3	0.8	1.1	1.5	0.6	1.2	1.5	2.0

The Panel notes that this type of intake methodology for fortified foods is generally considered to be “high intake”, as a result of several conservative assumptions made in the consumption estimates where it is assumed 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 also in real life.

The Panel notes that the intake estimates are, with the exception of the intake estimates for infants, lower than those of a previous application for bLF (EFSA 2012). The difference can be explained by lower levels of intended maximum concentrations, less food categories to which it is intended to add bLF, and different food consumption data used (UK data versus data from the Netherlands used a previous application for bLF (EFSA, 2012).

5. INFORMATION FROM PREVIOUS EXPOSURE TO THE NF OR ITS SOURCE

The Panel refers to its previous assessment of bLF as a novel food ingredient (EFSA, 2012).

6. NUTRITIONAL INFORMATION ON THE NF

The Panel refers to its previous assessment of bLF as a novel food ingredient (EFSA, 2012). 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 applicant provided information on the microbiological analysis of each of three batches of bLF derived from cheese whey and skimmed milk. The Panel has no safety concerns with regard to the microbiological specifications of bLF.

8. TOXICOLOGICAL INFORMATION ON THE NF

The Panel notes that the toxicological data on bLF produced by Morinaga Milk Industry Co., Ltd. have been evaluated in a previous Opinion on the safety of bLF as a novel food ingredient (EFSA, 2012). The toxicological studies conducted were 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. The Panel considers that the evidence provided does not raise safety concerns.

8.1. Human studies

The Panel refers to its previous assessment of bLF as a novel food ingredient (EFSA, 2012). 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.

8.2. Allergenicity

The Panel refers to its previous assessment of bLF as a novel food ingredient (EFSA, 2012). The Panel considers that the risk of allergic reactions is not dissimilar to other dairy products derived from bovine sources.

DISCUSSION

The Panel notes that the safety of bLF as a novel food ingredient has already been assessed with a favourable outcome (EFSA, 2012). That evaluation was to a significant extent based on safety data on bLF produced by Morinaga. The Panel also notes that the applicant intends maximum use levels of bLF in foods which are equivalent (infant formula) or lower than those intended by the applicant of the previous Opinion, and that the range of foods to which it is intended to add bLF is smaller. Consequently, the estimated intake levels described for the present application are comparable for infants and lower for all other population groups.

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' by Morinaga, received on 6 September 2011. Additional information was provided on 28 May 2012.
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'. Ref. Ares (2011)944545 – 06/09/2011.
3. Initial assessment report carried out by the competent authority of Ireland which concluded that an additional assessment was required (22 June, 2011).

REFERENCES

- Aisen P and Leibman A, 1972. Lactoferrin and transferrin: a comparative study. *Biochim Biophys Acta*, 257, 314-323.
- Brines RD and Brock JH, 1983. The effect of trypsin and chymotrypsin on the in vitro antimicrobial and iron-binding properties of lactoferrin in human milk and bovine colostrum. Unusual resistance of human apolactoferrin to proteolytic digestion. *Biochim Biophys Acta*, 759, 229-235.
- Castellino FJ, Fish WW and Mann KG, 1970. Structural studies on bovine lactoferrin. *J Biol Chem*, 245, 4269-4275.
- EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA); Scientific Opinion on bovine lactoferrin. *EFSA Journal* 2012;10(5):2701. [26 pp.]. doi:10.2903/j.efsa.2012.2701. Available online: www.efsa.europa.eu/efsajournal
- FSA. Food Portion Sizes, 3rd ed. Food Standards Agency (FSA), Her Majesty's Stationery Office (HMSO), London, Engl.; 2002.
- Kawai A, 1997. Final Report. Reverse mutation test of MONL-01 using bacteria. (Study Number 9701A). August 28, 1997.
- Nishimura N, 1991. Single dose oral toxicity study of monl-01 and monl-02 in rats (Study Number B-1969). Gotemba Laboratory, Bozo Research Center Inc., Setagaya-ku, Tokyo, Japan.
- Nishimura N, 1997. Four-week oral repeated dose toxicity study of monl-01 in rats (Study Number B-3578). Bozo Research Center Inc., Setagaya-ku, Tokyo, Japan.
- Nishimura N, 1998. Thirteen-week oral repeated dose toxicity study of monl-01 in rats (Study Number B-3579). Bozo Research Center Inc., Setagaya-ku, Tokyo, Japan.

- Office for National Statistics, 2005. The National Diet and Nutrition Survey: Adults Aged 19 to 64 Years. 2000-2001 [Computer File]. Office for National Statistics, Social and Vital Statistics Division & Food Standards Agency (FSA); Colchester, Essex, UK, UK Data Archive [distributor]. SN: 5140.
- Paulsson MA, Svensson U, Kishore AR and Naidu AS, 1993. Thermal behavior of bovine lactoferrin in water and its relation to bacterial interaction and antibacterial activity. *J Dairy Sci*, 76, 3711-3720.
- Pierce A, Colavizza D, Benaissa M, Maes P, Tartra A, Montreuil J and Spik G, 1991. Molecular cloning and sequence analysis of bovine lactoferrin. *European Journal of Biochemistry*, 196, 177-184.
- Querinjean P, Masson PL and Heremans JF, 1971. Molecular weight, single-chain structure and amino acid composition of human lactoferrin. *Eur J Biochem*, 20, 420-425.
- Shimazaki K, Kawaguchi A, Sato T, Ueda Y, Tomimura T and Shimamura S, 1993. Analysis of human and bovine milk lactoferrins by Rotofor and chromatofocusing. *Int J Biochem*, 25, 1653-1658.
- UKDA, 1995. National Diet, Nutrition and Dental Survey of Children Aged 1 ½ to 4 ½ Years, 1992-1993 [computer file]. Office of Population Censuses and Surveys, Social Survey Division, Medical Research Council Centre for Human Nutrition Research, Ministry of Agriculture, Fisheries and Food (MAFF), and U.K. Department of Health. Colchester, Essex; UK Data Archive (UKDA) [distributor], 13 December 1995. SN: 3481.
- UKDA, 2001. National Diet Nutrition Survey: Young People Aged 4 to 18 Years, 1997. Office for National Statistics Social Survey Division, Medical Research Council Centre for Human Nutrition Research, Ministry of Agriculture, Fisheries and Food (MAFF), and Department of Health. Colchester, Essex; UK Data Archive (UKDA) [distributor], 25 January 2001. SN: 4243.
- Yamauchi K, Toida T, Kawai A, Nishimura S, Teraguchi S and Hayasawa H, 2000a. Mutagenicity of bovine lactoferrin in reverse mutation test. *J Toxicol Sci*, 25, 63-66.
- Yamauchi K, Toida T, Nishimura S, Nagano E, Kusuoka O, Teraguchi S, Hayasawa H, Shimamura S and Tomita M, 2000b. 13-Week oral repeated administration toxicity study of bovine lactoferrin in rats. *Food Chem Toxicol*, 38, 503-512.
- Yoshida S and Ye X, 1991. Isolation of lactoperoxidase and lactoferrin from bovine milk rennet whey and acid whey by sulphopropyl cationexchange chromatography. *Netherlands Milk and Dairy Journal*, 45, 273-280.

GLOSSARY / ABBREVIATIONS

bw	bodyweight
bLF	bovine lactoferrin
NF(I)	novel food (ingredient)
PARNUTS	foods for particular nutritional uses
FSMP	Foods for special medical purposes