Safety of Whey basic protein isolates as a novel food pursuant to Regulation (EU) 2015/2283

EFSA Panel on Dietetic Products, Nutrition and Allergies (EFSA NDA Panel); Turck, Dominique; Bresson, Jean Louis; Burlingame, Barbara; Dean, Tara; Fairweather-Tait, Susan

Published in:
EFSA Journal

DOI:
10.2903/j.efsa.2018.5360

Publication date:
2018

SCIENTIFIC OPINION

EFSA Journal

ADOPTED: 27 June 2018

doi: 10.2903/j.efsa.2018.5360

Safety of Whey basic protein isolates as a novel food pursuant to Regulation (EU) 2015/2283

EFSA Panel on Dietetic Products, Nutrition and Allergies (EFSA NDA Panel), Dominique Turck, Jean-Louis Bresson, Barbara Burlingame, Tara Dean, Susan Fairweather-Tait, Marina Heinonen, Karen Ildico Hirsch-Ernst, Inge Mangelsdorf, Harry J McArdle, Androniki Naska, Monika Neuhäuser-Berthold, Grażyna Nowicka, Kristina Pentieva, Yolanda Sanz, Alfonso Siani, Anders Sjödin, Martin Stern, Daniel Tomé, Marco Vinceti, Peter Willatts, Karl–Heinz Engel, Rosangela Marchelli, Annette Pöting, Morten Poulsen, Josef Rudolf Schlatter, Mathias Amundsen and Henk van Loveren

Abstract

Following a request from the European Commission, the EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA) was asked to deliver an opinion on whey basic protein isolate as a novel food (NF) pursuant to Regulation (EU) 2015/2283. The NF is obtained by ion exchange chromatography of skimmed cow's milk. The applicant intends to market the NF in infant and follow-on formulae and meal replacement beverages, dietary foods for special medical purposes and as food supplements. The highest estimated intake of the NF based on the proposed uses and use levels would be 24.8 mg/kg body weight (bw) per day in infants and 27.8 in toddlers. The information provided on composition, specifications, production process and stability of the NF do not raise safety concerns. Taking into account the composition of the NF and the intended use levels, the Panel considers that the consumption of the NF is not nutritionally disadvantageous. The Panel considers that there is no concern with respect to genotoxicity. The no observed adverse effect level (NOAEL) of a subchronic 13-week rat study was 2000 mg/kg bw per day. Considering the source, the production process and nature of the NF, the Panel considers the margin of exposure (MOE) of 154 to be sufficient for the adult population (on a high-estimated intake of 13 mg/kg bw). For infants and toddlers, the MOE would be at least 81 and 72, respectively. Taking into account the composition of the NF, its source, the history of consumption of the main components of the NF, the production process and that the NOAEL in a subchronic rat study was the highest dose tested the Panel considers that also the MOE for infants and toddlers are sufficient. The Panel concludes that the novel food ingredient, whey basic protein isolate, is safe under the proposed uses and use levels.

Keywords: basic whey protein isolate, novel food, ingredient, safety

Requestor: European Commission following an application by Armor Protéines S.A.S.

Question number: EFSA-Q-2018-00104

Correspondence: nda@efsa.europa.eu

Panel members: Jean-Louis Bresson, Barbara Burlingame, Tara Dean, Susan Fairweather-Tait, Marina Heinonen, Karen Ildico Hirsch-Ernst, Inge Mangelsdorf, Harry J McArdle, Androniki Naska, Monika Neuhäuser-Berthold, Grażyna Nowicka, Kristina Pentieva, Yolanda Sanz, Alfonso Siani, Anders Sjödin, Martin Stern, Daniel Tomé, Dominique Turck, Henk van Loveren, Marco Vinceti and Peter Willatts.

Suggested citation: EFSA NDA Panel (EFSA Panel on Dietetic Products, Nutrition and Allergies), Turck D, Bresson J-L, Burlingame B, Dean T, Fairweather-Tait S, Heinonen M, Hirsch-Ernst KI, Mangelsdorf I, McArdle HJ, Naska A, Neuhäuser-Berthold M, Nowicka G, Pentieva K, Sanz Y, Siani A, Sjödin A, Stern M, Tomé D, Vinceti M, Willatts P, Engel K–H, Marchelli R, Pöting A, Poulsen M, Schlatter JR, Amundsen M and van Loveren H, 2018. Scientific Opinion on the safety of Whey basic protein isolates as a novel food pursuant to Regulation (EU) 2015/2283. EFSA Journal 2018;16(7):5360, 18 pp. https://doi.org/10.2903/j.efsa.2018.5360

ISSN: 1831-4732

The EFSA Journal is a publication of the European Food Safety Authority, an agency of the European Union.

Summary

Following a request from the European Commission, the European Food Safety Authority (EFSA) Panel on Dietetic Products, Nutrition and Allergies (NDA) was asked to deliver a scientific opinion on whey basic protein isolate as a novel food (NF) pursuant to Regulation (EU) 2015/2283. The assessment of the safety of this NF, which follows the methodology set out in the EFSA Guidance on the preparation and presentation of an application for authorisation of a novel food Regulation (EU) 2015/2283 and in the Commission Implementing Regulation (EU) 2017/2469, is based on the data supplied in the application, the initial assessment by the competent authority of Ireland, the concerns and objections of a scientific nature raised by the other Member States and the responses of the applicant, the information submitted by the applicant following EFSA requests for supplementary information and additional data identified by the Panel.

The NF in subject of the application is a whey basic protein isolate, obtained by ion exchange chromatography of skimmed cow's milk.

The applicant intends to market the NF in infant and follow-on formulae and meal replacement beverages, dietary foods for special medical purposes and as food supplements.

The NF is a mixture of basic whey proteins obtained by ion exchange chromatography from skimmed cow's milk. The main constituents are bovine lactoferrin (bLF), lactoperoxidase (bLP) and transforming growth factor β2 (TGF β2), which share an amino acid sequence homology of approximately 70, 83 and 99%, respectively, with their analogues present in human milk. The highest estimated intake of the NF based on the proposed uses and use levels would be 24.8 mg/kg bw per day in infants and 27.8 in toddlers.

The information provided on composition, specifications, production process and stability of the NF do not raise safety concerns. Taking into account the composition of the NF and the intended use levels, the Panel considers that the consumption of the NF is not nutritionally disadvantageous.

The Panel considers that there is no concern with respect to genotoxicity of the NF. The no observed adverse effect level (NOAEL) of a subchronic 13-week rat study was 2000 mg/kg bw per day (the highest dose tested). The human studies did not raise safety concerns.

Considering the source, the production process and the nature of the NF, the Panel considers the margin of exposure (MOE) of 154 to be sufficient for the adult population (on a high-estimated intake of 13 mg/kg bw).

For infants and toddlers, the MOE would be at least 81 and 72, respectively. Taking into account the composition of the NF, its source, the history of consumption of the main components of the NF, the production process and that the NOAEL in a subchronic rat study was the highest dose tested the Panel considers that also the MOE for infants and toddlers are sufficient.

The Panel concludes that the NF ingredient, whey basic protein isolate, is safe under the proposed uses and use levels.

Table of contents

1. Introduction

1.1. Background and Terms of Reference as provided by the European Commission

On 22nd August 2016, the company S.A.S. submitted a request in accordance with Article 4 of the Novel Food Regulation (EU) 258/1997[1] to place on the market whey basic protein isolate as a novel food (ingredient) (NF).

On 27 June 2017, the Food Safety Authority of Ireland forwarded to the Commission its initial assessment report, which came to the conclusion that the whey protein isolate meets the criteria for acceptance of a novel food defined in Article (3)1 of the regulation (EC) No 258/97.

On 4 July 2017, the Commission forwarded the initial assessment report to the other Member States. Several Member States raised objections or submitted comments.

The concerns of a scientific nature raised by the MS can be summarised as follows:

- Qualitative and quantitative data on the proteins contained in the NF were requested to understand the profile of the food.
- Proof of accreditation of the laboratories used to perform the relevant tests was requested.
- The description of the method of determination for bovine lactoferrin (bLF) and lactoperoxidase (bLP) was requested.
- Analytical results for content of arsenic in the NF were requested.
- The use of gluten or wheat was used as a processing aid in spray drying to prevent clumping.
- The enzymatic activity of bLP, and the potential effect that the NF may have on the food matrixes to which it will be added. As this may affect the storage stability.
- Additional data on the storage stability of the product in other packaging and intended areas of application was requested.
- A safety study involving infant and follow on formula was requested.
- The toxicological relevance of the results shown in a 6-week developmental toxicology study in juvenile rats. The effect of the NF on body weight and increased thymus weight.

According to Article 35 (1) of Regulation (EU) 2015/2283[2], any request for placing a novel food on the market within the Union submitted to a Member State in accordance with Article 4 of Regulation (EU) 258/1997 and for which the final decision has not been taken before 1 January 2018 shall be treated as an application under this Regulation.

In accordance with Article 10 (3) of Regulation (EU) 2015/2283, EFSA shall give its opinion as to whether the update of the Union List referred to in Article 10 (1) is liable to have an effect on human health.

2. Data and methodologies

2.1. Data

The safety assessment of this NF is based on data supplied in the application and information submitted by the applicant following EFSA request for supplementary information. During the assessment, the Panel identified additional data which were not included (US FDA, 2017).

Administrative and scientific requirements for NF applications referred to in Article 10 of Regulation (EU) 2015/2283 are listed in the Commission Implementing Regulation (EU) 2017/2469[3].

[1] Regulation (EU) No 258/97 of the European Parliament and of the Council of 27 January 1997 concerning novel foods and novel food ingredients. OJ L 43, 14.2.1997, p. 1–6.

[2] Regulation (EU) 2015/2283 of the European Parliament and of the Council on novel foods, amending Regulation (EU) No 1169/2011 of the European Parliament and of the Council and repealing Regulation (EC) No 258/97 of the European Parliament and of the Council and Commission Regulation (EC) No 1852/2001 (2013/0435 (COD). OJ L 327, 11.12.2015, p. 1–22.

[3] Commission Implementing Regulation (EU) 2017/2469 of 20 December 2017 laying down administrative and scientific requirements for applications referred to in Article 10 of Regulation (EU) 2015/2283 of the European Parliament and of the Council on novel foods. OJ L 351, 30.12.2017, pp. 64-71.

A common and structured format on the presentation of NF applications is described in the EFSA guidance on the preparation and presentation of a NF application.[4] As indicated in this guidance, it is the duty of the applicant to provide all of the available (proprietary, confidential and published) scientific data, including both data in favour and not in favour to supporting the safety of the proposed NF.

This NF application includes a request for protection of proprietary data in accordance with Article 26 of Regulation (EU) 2015/2283. Data claimed to be proprietary by the applicant include: Armor Protéines (2013; unpublished), Armor Protéines (2017; unpublished), Schmitt and Mireaux (2008; unpublished), Silvano (2012; unpublished), Sire (2012a; unpublished, 2012b; unpublished) and Spézia (2013; unpublished).

2.2. Methodologies

The assessment follows the methodology set out in the EFSA guidance on NF applications and the principles described in the relevant existing guidance documents from the EFSA Scientific Committee. The legal provisions for the assessment are laid down in Article 11 of Regulation (EU) 2015/2283 and in Article 7 of the Commission Implementing Regulation (EU) 2017/2469.

This assessment concerns only risk that might be associated with consumption of the NF under the proposed conditions of use, and is not an assessment of the efficacy of basic whey protein isolate with regard to any claimed benefit.

3. Assessment

3.1. Introduction

The NF is a whey basic protein isolate obtained from skimmed cow's milk by chromatographic fractionation and has a protein content higher than 90%. The applicant intends to market the NF as an ingredient for infant and follow-on formulae, meal replacement beverages, dietary foods for special medical purposes and food supplements.

3.2. Identity of the NF

The whey basic protein isolate is produced from bovine skimmed milk. The protein fraction of the NF is composed mainly of two minor whey basic proteins, bLF and bLP which account for approximately 80% of the NF and contains only low quantities of the predominant proteins of bovine whey (i.e. β-lactoglobulin and α-lactalbumin). bLF and bLP constitute only 2–4% of the whey proteins from cow's milk.

3.3. Production process

The production of whey basic proteins starts with removal of fat by centrifugation of bovine milk. The whey basic protein fraction is isolated by ion-exchange chromatography using a cation resin. From the resin, the basic whey proteins are eluted with a NaCl solution, which again is largely removed by ultrafiltration. For reduction of the microbial load, the product is microfiltrated and pasteurised, before it is spray-dried. Following a question from a Member State (MS), the applicant clarified that no gluten or wheat products were used as a processing aid in spray-drying to prevent clumping. The NF is manufactured in accordance with good manufacturing practice (GMP) and the hazard analysis critical control point (HACCP) principles in a production plant certified under ISO 22000. The techniques used in the production process of the NF are commonly used in the dairy industry and are comparable to techniques used in production of bLF previously assessed by (EFSA NDA Panel, 2012a,b).

The Panel considers that the production process is sufficiently described and does not raise safety concerns.

[4] EFSA NDA Panel (EFSA Panel on Dietetic Products, Nutrition and Allergies), Turck D, Bresson J-L, Burlingame B, Dean T, Fairweather-Tait S, Heinonen M, Hirsch-Ernst KI, Mangelsdorf I, McArdle H, Naska A, Neuhäuser-Berthold M, Nowicka G, Pentieva K, Sanz Y, Siani A, Sjödin A, Stern M, Tomé D, Vinceti M, Willatts P, Engel K-H, Marchelli R, Pöting A, Poulsen M, Salminen S, Schlatter J, Arcella D, Gelbmann W, de Sesmaisons-Lecarré A, Verhagen H and van Loveren H, 2016. Guidance on the preparation and presentation of an application for authorisation of a novel food in the context of Regulation (EU) 2015/2283. EFSA Journal 2016;14(11):4594, 24 pp. https://doi.org/10.2903/j.efsa.2016.4594

3.4. Compositional data

The applicant provided batch-to-batch analyses of five different production lots (Table 1). According to the analyses, the NF has a protein content higher than 90%. bLF (25–75%) and bLP (10–40%) are the two main constituents of the NF. Following a question from one MS on other contained proteins, data were provided on the 10 most abundant proteins: secretory component < 5%, complement C3 < 2%, β-lactoglobulin < 2%, αS1-Casein < 2%, lactophorin < 2%, pancreatic ribonuclease (RNase-4) < 2%, tetranectin (CLEC3B) < 1% and β-2-glycoprotein 1 (apolipoprotein H) < 1%. Two proteins found in lower quantities in the NF are transforming growth factor (TGF-β2; 0.012–0.018%) and insulin-like growth factor (IGF-1; 0.0015%).

The content of moisture, lactose and fat was below 5.5, 3.0, and 4.5%, respectively.

Table 1: Batch-to-batch analyses of the NF

Parameter	Manufacturing Lot No.				
	111106	**111217**	**130830**	**131114**	**140724**
Appearance	Yellowish grey powder	Yellowish grey powder	Yellowish grey powder	Yellowish grey powder	Yellowish grey powder
pH (5% solution w/v)	6.2	6.4	6.3	6.1	6.7
Total Protein (%)	91	93	94.3	96	94.5
Lactoferrin (%)	32	51	50	63	70
Lactoperoxidase (%)	32	24	25	19	17
TGF-β2 (mg/100 g)	14.6	13.6	16.2	17.4	14
Moisture (%)	4.6	4.1	4.8	3.7	4.2
Lactose (%)	< 0.09	< 0.09	< 0.09	< 0.09	< 0.09
Fat (%)	2.5	2.8	2	0.5	2
Ash (%)	1.7	1.3	1	0.5	0.5
Iron (mg/100 g)	18.8	19.2	17.4	16.7	16.9
Heavy metals					
Lead (mg/kg)	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02
Cadmium (mg/kg)	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005
Mercury (mg/kg)	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005
Arsenic (mg/kg)	0.002	0.002	0.002	0.002	< 0.05
Microbiological parameters					
Aerobic mesophilic count (CFU/g)	50	50	330	20	10
Enterobacteriaceae (CFU/g)	0	0	0	0	0
Yeasts (CFU/g)	0	0	0	0	0
Moulds (CFU/g)	0	0	0	0	0
Escherichia coli (in 1 g)	Negative	Negative	Negative	Negative	Negative
Coagulase positive *Staphylococci* (in 1 g)	Negative	Negative	Negative	Negative	Negative
Salmonella (in 25 g)	Negative	Negative	Negative	Negative	Negative
Listeria (in 25 g)	Negative	Negative	Negative	Negative	Negative
Cronobacter spp. (in 25 g)	Negative	Negative	Negative	Negative	Negative

The Panel considers that the information provided on the composition of the NF is sufficient and does not raise safety concerns.

3.4.1. Stability

In the dossier, the applicant provided a summary of stability studies of the NF in a double ethylene bag packed in a cardboard box under normal storage conditions (20°C and relative humidity 40–50%). The studies showed that no significant microbial growth in the NF and the total protein and TGF-β2 contents did not show pronounced changes over time. However, the moisture content increased by

6% after 12–18 months in one of the stability studies and after 27 months in one batch of the other study. The applicant, therefore, suggested limiting the use by date of the product to 12 months. In response to a comment from a MS, a stability study with dietary foods for special medical purposes was provided by the applicant. The product was supplemented with the NF to a content of TGF-β2 of between 77 and 118 μg/100 g after formulation. The samples were stored under ambient conditions up to 18 months. After storage for 12 months, the TGF-β2 content had decreased by 10% of the initial TGF-β2 content.

Responding to comments from two MS regarding the enzymatic activity and the potential effect of peroxidases to form free radicals in the NF, the panel agreed with the applicant's response that such reaction does not occur in the NF since both thiocyanate ion and hydrogen peroxide are required to exert its peroxidase activity (FAO, 2002).

The Panel considers that the data provided are sufficient with respect to the stability of the NF.

3.5. Specifications

The specifications as proposed by the applicant are presented in Table 2. The analyses were performed using validated methods.

Table 2: Specifications of whey protein isolate

Parameter	Limit	Method
Appearance	Yellowish grey powder	Visual Inspection
Foreign matter (Scorched particles)	Absent	Visual inspection in 25 g (ADMI chart, solubilised with 0.15 M NaCl solution)
pH (5% solution w/v)	5.5–7.6	5% (w/v) solution, pH meter
Total Protein (%)	≥ 90	Kjeldahl method (IDF20/ISO 8968) [N x 6.38]
Lactoferrin (%)	25–75	HPLC (modified GB1903.17.2016)
Lactoperoxidase (%)	10–40	HPLC (modified GB1903.17.2016)
TGF-β2 (mg/100 g)	12–18	ELISA (Quantikine human TGF-β2, R&D Systems)
Moisture (%)	≤ 6.0	ISO 5550
Lactose (%)	≤ 3.0	Enzymatic method (Lactose/D-Galactose kit, Boehringer Mannheim/R-Biopharm)
Fat (%)	≤ 4.5	AFNOR Chimie II 3B 1986
Ash (%)	≤ 3.5	ISO 5545
Iron (mg/100 g)	≤ 25	AAS
Heavy metals		
Lead (mg/kg)	< 0.1	ICP-MS
Cadmium (mg/kg)	< 0.2	ICP-MS
Mercury (mg/kg)	< 0.6	ICP-MS
Arsenic (mg/kg)	< 0.1	ICP-MS
Microbiological specifications		
Aerobic mesophilic count (CFU/g)	≤ 10,000	ISO 4833
Enterobacteriaceae (CFU/g)	≤ 10	ISO 21528-1
Yeasts (CFU/g)	≤ 50	ISO 6611 | IDF 94:2004
Moulds (CFU/g)	≤ 50	ISO 6611 | IDF 94:2004
Escherichia coli (in 1 g)	Negative	ISO 7251
Coagulase positive *Staphylococci* (in 1 g)	Negative	ISO 6888-3
Salmonella (in 25 g)	Negative	VIDAS Easy Salmonella method (equivalent to ISO6579)
Listeria (in 25 g)	Negative	VIDAS LIS method (equivalent to ISO 11290-1/A1:2004)
Cronobacter spp. (in 25 g)	Negative	ISO/TS 22964:2006

The results from batch-to-batch analyses provided in Section 3.4. were within the specification limits and contaminants were below the limits set for both the microbiological and chemical contaminants.

The Panel considers that the information provided on the specifications of the NF is sufficient and does not raise safety concerns.

3.6. History of use of the NF and/or of its source

3.6.1. History of use of the source

Bovine whey, whey protein concentrates and demineralised whey powder are consumed in the EU. Whey protein concentrates produced with a range of techniques including ion exchange resins were approved as a food ingredient in the USA in 1981 (US FDA, 1981). The per capita consumption of whey was estimated at 1.6 lbs (dry matter)/year for 2013, equivalent to approximately 2 grams (dry matter)/person per day in the USA (USDA-ERS, 2016).

3.6.2. History of use of the NF

Some whey basic protein isolates have been granted GRAS status and are permitted to be marketed in the United States (US FDA, 2000, 2006). Also, several bLF products have received GRAS status (US FDA, 2001a,b, 2003, 2014a,b, 2017). According to the applicant, bLF is permitted for use in Japan, China, Korea and Taiwan for both infant formulae and conventional foods, and in infant and follow-on formulae since 1986 in Japan.

In 2012, EFSA published two opinions on the safety of bLF (EFSA, 2012a,b). In these opinions, the safety of bLF was assessed for addition to several food categories (i.e. infant and follow-on formulae, dietetic food for special medical purposes, sports nutrition, non-alcoholic beverages, cakes and pastries, products derived from cheese, milk-based products, cold snacks and sweets). The proposed maximum use of reconstituted (ready-to-drink) infant formulae and follow-on formulae was of 100 mg bLF/100 mL. The Panel considered that the estimated maximum daily intake of 3.4 g bLF for adults, and for toddlers, the 95th percentile intake of 203 mg/kg bw per day were safe.

EFSA has not previously evaluated the safety of bLP. Bovine lactoperoxidase is accepted in dietary supplements (1–2 mg/serving) in the USA (NIH, 2016), and in Japan as food additive (MHLW, 2014) and food supplements. In addition, bLP-containing systems have been assessed by JECFA (1990) and accepted in New Zealand (FSANZ, 2002) as processing aids.

Table 3 provides information on the concentrations of lactoferrin, lactoperoxidase and TGF-β2 found in human and bovine milk and infant formulae.

Table 3: Contents of bLF, bLP and TGF-β2 in the human and cow's milk and infant formulae

Item	lactoferrin (mg/100 mL)	lactoperoxidase (mg/100 mL)	TGF-β2 (μg/100 mL)
Human milk	100–320[(a)]	0.077[(b)]	0.04–1.9 (mean ≈ 0.53)[(c)] 0.05–1.3 (geometric mean = 0.27)[(d)] 0.2–1.5 (mean = 0.6)
Cow's milk	10–15[(e)]	2.4–3.4[(f)]	6.6[(g)] 1.3–7.1[(h)]
Infant formulae	12.7–19.1[(i)]	3.0–4.5[(i)]	0.28–1[(j)]

(a): Pamblanco et al. (1986); Prentice et al. (1987); Hirai et al. (1990); Hennart et al. (1991); Rudloff and Kunz (1997).
(b): Ferenc Levay and Viljoen (1995); Korhonen and Pihlanto (2003).
(c): Srivastava et al. (1996).
(d): Jouni et al. (2009).
(e): Shin et al. (2001).
(f): Indyk et al. (2006).
(g): Elfstrand et al. (2002).
(h): Gauthier et al. (2006).
(i): Calculated value based on an infant formula with protein content of 14 g per 100 mL, whey to casein ratio of 60:40 as well as the content of the constituents found in cow's milk.
(j): Jouni et al. (2009).

3.7. Proposed uses and use levels and anticipated intake

3.7.1. Target population

The applicant intends to target infants and toddlers (infant and follow-on formulae), the general adult population (meal replacement beverages) and the general population (foods for special medical purposes and food supplements).

3.7.2. Proposed uses and use levels

The applicant intends to market the NF as an ingredient for the food categories presented in Table 4. The rationale for the use level in infant and follow-on formulae given by the applicant is to achieve intake levels of TGF-β2 comparable to those of breast-fed infants.

Table 4: Proposed conditions of use of the NF

Proposed Food use	Suggested Serving Size	Max. Use Level	
Infant formula	N/a	30 mg/100 g[(a)] 3.9 mg/100 mL[(b)]	
Follow-on formula	N/a	30 mg/100 g[(a)] 4.2 mg/100 mL[(b)]	
Meal replacement beverages	250 g	100 mg/meal replacement	
		For adults	For children (1–3 years)
FSMP		610 mg per day	58 mg per day
Food supplements	–	610 mg per serving (and per day)	58 mg per serving (and per day)

(a): Powder.
(b): Reconstituted.

Table 5 provides the calculated maximum use levels for bLF, bLP and TGF-β2 by considering their specification upper limits and the proposed maximum use levels of the NF for the intended food categories.

Table 5: Calculated maximum use levels of bLF, bLP and TGF-β2 for the proposed food categories

Proposed Food use	Suggested Serving Size	Calculated Maximum Use Level		
		bLF	**bLP**	**TGF-β2**
		mg/100 mL	mg/100 mL	μg/100 mL
Infant Formula[(a)]	n/a	2.9	1.6	0.7
Follow-on formula[(a)]	n/a	3.2	1.7	0.76
		mg/per serving	mg/per serving	μg/serving
Meal replacement Beverages	250 g (100 mg of the NF)	75	40	18
Foods for special medical purposes	610 mg (per serving and per day)	457.5	244	110
Food supplements	610 mg (per serving and per day)	457.5	244	110

(a): Reconstituted.

The Panel notes that the use levels for bLF in two NF applications assessed and considered safe by the Panel were up to 100 mg/100 g in reconstituted (ready-to-drink) infant formulae, which is about 30 times higher than the bLF concentration (i.e. 2.9 mg/100 mL, Table 5), potentially resulting from the addition of the proposed use level of the NF. In 2012, the Panel considered bLF as safe in infant formula at the proposed use level in infant formula at 1 g/L (EFSA NDA Panel, 2012a).

The applicant reports that analytical data on the occurrence of bLP in infant formula could not be retrieved, but provided an estimate (i.e. 3.0–4.5 mg/100 mL, Table 3). Considering the proposed use level of the NF for reconstituted infant formula (i.e. 3.9 mg/100 mL) and the specification upper limit

for bLP in the NF (40%), the addition of the NF to infant formulae could result to an increase of about 35% (4.5 mg/100 mL + 1.6 mg/100 mL).

The addition of the NF could result in a maximum increase of 0.7 μg/100 mL of bovine TGF-β2 present in bovine milk-based infant formulae at reported concentrations ranging from 0.28 to 1 μg/100 mL according to Jouni et al. (2009). This increase could result in a total concentration of TGF-β2 in infant formula at the higher range reported for the TGF-β2 in human breast milk (up to 1.9 μg/100 mL; Table 3).

3.7.3. Anticipated intake of the NF

Infant and follow-on formulae

Proposed use levels of the NF through infant and follow-on formulae could lead to a consumption of the NF of 20 and 23 mg/kg bw per day in infants and toddlers, respectively (Table 6).

Table 6: Mean and 95th percentile intakes of the NF from infant and follow-on formulae, estimated on the basis of consumption data from Germany (Kersting et al., 1998), the United Kingdom (DNSIYC, 2013) and the EFSA Comprehensive Food Consumption Database (EFSA, 2011)

Database	Population group	Mean intake (mg/kg bw/day)	95th percentile (mg/kg bw/day)
EFSA Comprehensive Food Consumption Database	Infants	3.86–14.71	19.99[(a)]
	Toddlers	0.68–11.84	23.05[(b)]
DONALD study (Kersting et al., 1998)	0–4 months	8.3	–
DNSIYC (2013)	4–6 month	2.78	4.57
	7–12 months	1.95	3.35
	13–17 months	1.25	2.42
	18–23 months	1.00	1.56
	2–3 years	1.06	2.20

(a): Bulgaria Nutrichild.
(b): Finland DIPP.

Meal replacement beverages

The applicant provided intake estimates from meal replacement beverages in the UK National Diet and Nutrition Survey (UKDA, 2014). The number of consumers of meal replacement beverages was low, so only the all-user data are presented (Table 7). Under the proposed conditions of use, the intake of meal replacement beverages is highest in children between the age of 4 and 10 where they can account for up to 3.9 mg/kg bw per day. In the adult population, they can account for between 1 and 1.7 mg/kg bw per day. It is worth noting that the statistical reliability of the results is low due to the low number of consumers. In a scenario with the consumption of 3 meals per day, the intake of the NF in the adult population would be 300 mg/day (for a 70 kg adult) or 4.29 mg/kg bw per day. For children (4–10 years of age), a daily consumption of 300 mg of the NF would correspond to 13 mg/kg bw per day when considering a mean default body weight of 23 kg (EFSA Scientific Committee, 2012).

Table 7: Estimated mean intakes of the NF from meal replacement beverages estimated on the basis of consumption data from the United Kingdom (UKDA, 2014)

Population group	% Users	No of Consumers	Mean consumption	
			(mg/d)	(mg/kg bw per d)
Children 4–10 years	0.6	6	80.1	3.90
Female Teenagers 11–18 years	0.2	1	260.0	3.46
Male Teenagers 11–18 years	1.8	9	98.7	1.64
Female Adults 19–64 years	0.8	14	138.0	1.66
Male Adults 19–64 years	2.7	24	112.0	1.32
Adults ≥ 65 years	1.5	6	84.7	1.03

Foods for special medical purposes

For the adult population, the maximum intended intake level (i.e 610 mg/day) proposed as food for special medical purposes would lead to an intake of 8.7 mg/kg bw per day, when considering a body weight of 70 kg.

For the younger population (specified by the applicant as children ≤ 3 years of age), the maximum intended intake levels (i.e. 58 mg per day) proposed as food for special medical purposes would lead to an intake of 4.8 mg/kg bw per day, when considering the mean body weight of 12 kg suggested by the EFSA Scientific Committee (2012) to be used in risk assessment for this age group.

Food supplements

The intended target population and use levels correspond to those as indicated for FSMPs.

Combined intake of the NF

The proposed uses and use levels could lead to intakes of the NF up to 24.8 mg/kg bw per day for infants (20 + 4.8 mg/kg bw from infant formula and FSMP or food supplements) and 27.8 mg/kg bw per day for toddlers (23 + 4.8 mg/kg bw from follow-on formula and FSMP or food supplements).

Adults may have an intake of up to 13 mg/kg bw per day, 8.7 mg/kg bw per day coming from food supplement use in addition to 4.3 mg/kg bw per day from meal replacements (when considering a default body weight of 70 kg).

3.8. Absorption, distribution, metabolism and excretion (ADME)

The applicant has not provided any studies regarding the metabolism of the NF. However, a limited number of studies on ADME of lactoferrin and lactoperoxidase were provided.

In humans, lactoferrin seems to be stable towards proteolytic activity and resistant to digestion in the stomach (Troost et al., 2001). In a human study, where adults (n = 6) were given bLF-radiolabelled iodine-123, about 31% of the initial activity was seen in the plasma within about 60 min (Khan et al., 2000). Plasma levels rapidly decreased and about 5% of the initial dose was observed after 6 h. In breast-fed infants of 1 week of age, 2–6% of the lactoferrin from the breast milk was found in the faeces, which was decreased to less than 2% in 4-month-old infants (Davidson and Lönnerdal, 1987). It has been proposed that absorbed intact lactoferrin is either taken up by receptor-mediated endocytosis into phagocytic cells or by endocytic uptake by liver cells (Ferenc Levay and Viljoen, 1995).

Only very limited information about the metabolic fate of bLP is available. It was reported that lactoperoxidase was inactivated in the presence of pepsin (pH 2.5) but not inactivated by the gastric juice of an infant (pH 5) (Kussendrager and van Hooijdonk, 2000).

With regard to their suitability to assess human safety, rats were considered as a suitable species since adult rats have been shown to absorb native bLF (Takeuchi et al., 2004). 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 enzyme-linked immunosorbent assay (ELISA). The Panel considers that

the provided studies show absorption of bLF in the intestine of rats, and that rats therefore are suitable to study bLF toxicity.

3.9. Nutritional information

The proteins found in the NF are comparable to those found in the native cow's milk as little chemical alteration occurs in processing of the product. Many of these proteins have human equivalents of varying sequence homology. The homology between human and bLF, bLP and TGF-β2 is approximately 70% (Wal, 2004), 83% (Armor proteins, 2014) and 99% (according to BLAST analysis performed in 'NCBI, database Non-redundant protein sequences (nr)' by EFSA), respectively. The applicant proposes to add the NF as an ingredient in infant and follow-on formula, to achieve levels of TGF-β2 comparable to those resulting from consumption of human milk.

Lactoferrin is an iron-binding protein and lactoperoxidase is a haem-bound protein. Considering the specification limit for iron in the NF (≤ 25 mg/100 g; Table 2) as well as the intake estimates of the NF (13 mg/kg bw per day in adults and 24.8 mg/kg bw per day in infants) provided in Section 3.7.3, the intake levels could lead to an intake of iron of 3.3 μg/kg bw per day in adults, and 6.2 μg/kg bw per day in infants. These values represent less than 5% of the dietary reference value for iron established by the Panel, i.e. for infants 8 mg/day, children (aged 1–6 years) 5 mg/day, men 6 mg/day, premenopausal women 7 mg/day (EFSA NDA Panel, 2015).

The Panel considers that consumption of the NF is not nutritionally disadvantageous.

3.10. Toxicological information

The applicant provided two *in vitro* genotoxicity studies, one subchronic 90-day study and one 6-week developmental toxicity study conducted with the NF. Toxicological studies of two commercial whey protein isolates were also provided: 'Milk Basic Protein' (MBP) (54% bLF, 41% bLP) (Kruger et al., 2007) and 'Lactermin' (bLF and bLP together > 50%, smaller amounts of IGF-I, IGF-II, PDGF, FGF, TGF-β and betacelluli) (Dyer et al., 2008) to substantiate the safety of the NF.

3.10.1. Genotoxicity

Two bacterial reverse mutation tests were performed in

Salmonella typhimurium strains TA98, TA100, TA102, TA1535 and TA1537 using the preincubation method and the plate incorporation method, in compliance with OECD TG 471(Forster et al., 2014). Six concentrations of the NF were used for each bacterial strain: from 156.3 to 5,000 μg/plate using three plates per concentration with and without the presence of a metabolic activation system (S9 mix). The NF did not induce an increase in the number of revertants, either with or without S9 mix, in any of the five strains.

The NF was also assessed in an *in vitro* mammalian cell micronucleus test with L5178Y TK+/– mouse lymphoma cells, in compliance with OECD 487 (Forster et al., 2014). Based on the results of the cytotoxicity measurements and the precipitation of the substance at high concentrations, doses for the main study were selected. The test was performed in two independent main experiments in the presence and absence of a metabolic activation system (S9 mix) using seven concentrations from 39.06 to 2,500 μg/mL. In the short-term exposure, cells were incubated for 3 h with and without metabolic activation and three treatments, i.e. 156.3, 312.5 and 625 μg/mL (experiments 1 and 2, with S9 mix and experiment 1, without S9 mix) were evaluated. In the long-term exposure, cells were exposed to the NF for 24 h in the absence of metabolic activation, and three treatments, i.e. 312.5, 625 and 1,250 μg/mL, were evaluated. Without metabolic activation, no significant increase in the frequency of micronucleated cells was noted after either 3- or 24-h treatment. In the short-term exposure with metabolic activation, the frequency of micronucleated cells was increased by 3.5-fold at 625 μg/mL, but this was not statistically significant and remained within historical control range. No increase was seen in the second experiment using the same study conditions. The Panel concludes that the test item did not induce chromosome aberrations or damage to the cell division apparatus.

Taking into account the nature, source and production process of the NF, the Panel considers that there are no concerns regarding genotoxicity. This was supported by the tests performed.

3.10.2. Subchronic toxicity

The applicant provided a subchronic (13 weeks) toxicity study performed with the NF in compliance with OECD TG 408 (Forster et al., 2014). Groups of 6-week-old Sprague–Dawley rats (16/sex per

group in control and high-dose groups and 10/sex per group in mid- and low-dose groups) were administered the NF suspended in 0.9% NaCl by oral gavage at a dosage volume of 10 ml/kg. This corresponded to doses of 0, 600 (low), 1,200 (mid) or 2,000 (high) mg of the NF/kg bw per day. At the end of the treatment period, 10 animals in each group were sacrificed, except for the first six animals per sex of groups 1 and 4, which were kept for a 4-week treatment-free recovery period.

No statistically significant differences were seen in food consumption, body weight, clinical and ophthalmological examinations between control and test groups. One male in the high-dose group suffered from abscess in the lateral region of the thorax and was sacrificed prematurely at Day 33. This finding is most likely due to an injury caused by the gavage. Mean cell haemoglobin concentration was significantly lower in the high-dosed males (32.90 vs. 33.30 in control), but this was not accompanied by findings in related parameters in this group. A significantly higher mean reticulocyte percentage and lower mean thrombin time were seen in mid-dose males. In females, a lower mean cell haemoglobin was seen in the low-dose group. None of the above-mentioned haematologically effects are considered treatment related. In blood biochemistry, no significant differences were seen between high-dosed animals and controls. A few significant findings were seen in the lower dose groups, but the changes are judged as incidental. A decreased urine volume, increased specific gravity and decreased pH were observed in females administered with 2,000 mg/kg bw per day. The differences noted were small, were only observed in one sex and were considered not to be adverse. No differences in organ weights were attributed to treatment with the NF. In addition, no macroscopic or microscopic findings were seen. The Panel considers that the highest dose tested (i.e. 2,000 mg/kg bw per day) is the no observed adverse effect level (NOAEL) of this study.

The applicant also provided subchronic (13 weeks) toxicity studies of MBP (Kruger et al., 2007) and Lactermin (Dyer et al., 2008). In both studies, the study authors came to the conclusion that these protein isolates did not give an indication of toxicity. In Kruger et al. (2007), a significant lower body weight was seen in female rats given 2,000 MBP/kg bw per day (highest dose) between day 14 and day 63. At 90 days, this effect was no longer seen. No effect on body weight was seen in male rats.

3.10.3. Chronic toxicity and carcinogenicity

No studies were provided assessing chronic toxicity or carcinogenicity.

3.10.4. Reproductive and developmental toxicity

A 6-week developmental toxicity study was performed with the NF in compliance with the USFDA Guidance for Industry, Nonclinical Safety Evaluation of Paediatric Drug Products (Forster et al., 2014). The objective of the study was to evaluate the potential effects of the NF on the development of juvenile rats, following daily oral (gavage) administration from post-natal day (PND) 7 to PND 49 inclusive. Pregnant female Sprague–Dawley rats were received in the test facility on gestation day (GD) 14. Following parturition, the litters were culled on PND 4 to standardised litters of 2M + 2F or 3M + 3F. The test group of 16 rats/sex was given 600 mg/kg bw per day of the NF. The control group (16 rats/sex) received the vehicle (NaCl 0.9%) only. At the end of the treatment period, 10 animals in each group were sacrificed. The remaining six animals were kept for a 4-week treatment-free recovery period. Despite the fact that there is exposure to both the NF and maternal milk during the early post-natal period, the Panel notes that there is no information on the relative intake of protein from either source. In addition, only one dose of the NF and one control group were tested. The Panel notes that no conclusion can be drawn from this study.

The applicant also provided a developmental toxicity study on a related whey basic protein isolate (MBP) which was performed on healthy male and female Crj:CD (SD) IGS rats (in compliance with the 'Guidelines for Designation of Food Additives and for Revision of Standards for Use of Food Additives", Notification No. 29 of the Environmental Health Bureau, Ministry of Health and Welfare, Japan, March 22, 1996, Kruger et al., 2007). The study utilised one treatment group and one control group; 20 pregnant females were included per group. MBP was administered daily by gavage at a dose of 2,000 mg/kg bw per day on Days 7–17 of gestation.

No adverse clinical effects were observed in the dams, and no differences between treated and control animals were seen for body weight, body weight gain, food consumption, number of corpora lutea, number of implantation sites, number of live and dead foetuses, number of resorbed embryos, viability indices of foetuses, sex ratio, placental weight and body weight of foetuses. In live foetuses, there were no external, visceral or skeletal anomalies or variations. These results show no adverse effects of MBP on reproduction or development in rats.

3.10.5. Human data

The applicant did not provide human studies performed with the NF.

The applicant provided eight human studies with products containing main components of the NF. Six of these studies, however, investigated dose levels for bLF and bLP which were considerably below the proposed maximum intake levels of this NF. One open-label study tested a product without bLF in psoriasis patients (Armor Protéines SAS., 2013; unpublished) and one randomised, double-blind, placebo-controlled trial with a product containing bLF, bLP and TGF-β2 in amounts complying with the specifications of the NF, studied the effects on markers of atherosclerosis in hypercholesterolemic subjects (Schmitt and Mireaux, 2008; unpublished). The studied dose was, however, below maximum proposed use levels for this NF and safety relevant endpoints included only the level of serum enzymes aspartate transaminase (AST) and alanine transaminase levels (ALT). No effects were noted.

Although the provided human studies do not raise concern with regard to the two main constituents, bLF and bLP, the Panel notes the significant limitations of these studies (i.e. not performed with the NF, dose levels tested, limited number of endpoints) in relation to the safety of the NF.

3.11. Allergenicity

The Panel notes that the source of the NF is a recognised allergen and that, therefore, the NF is potentially allergenic.

4. Discussion

The NF is a mixture of basic whey proteins obtained by ion exchange chromatography from skimmed cow's milk. The main constituents are bLF, bLP and TGF β2, which share an amino acid sequence homology of approximately 70, 83 and 99%, respectively, with their analogues present in human milk. The highest estimated intake of the NF based on the proposed uses and use levels would be 24.8 mg/kg bw per day in infants and 27.8 in toddlers.

The information provided on composition, specifications, production process and stability of the NF does not raise safety concerns. Taking into account the composition of the NF and the intended use levels, the Panel considers that the consumption of the NF is not nutritionally disadvantageous.

The Panel considers that there is no concern with respect to genotoxicity of the NF. The NOAEL of a subchronic rat study was 2,000 mg/kg bw per day (the highest dose tested).

Considering the source and nature of the NF, the Panel considers the margin of exposure (MOE) of 154 to be sufficient for the adult population (on an estimated high intake of 13 mg/kg bw per day).

For infants and toddlers, the MOE would be at least 81 and 72, respectively. Noting the occurrence of bLF, bLP and TGF-β2 present in bovine-based infant formulae, the Panel considers that the increase of their intakes from the NF at the proposed use level for infant formulae would not be of concern. Taking into account the source, the production process, the nature of the NF as well as the history of consumption of the main components of the NF, and that the NOAEL in a subchronic rat study, which was the highest dose tested, the Panel considers that the MOE of 81 and 72 for infants and toddlers are sufficient.

5. Conclusions

The Panel concludes that the NF, whey basic protein isolate, is safe under the proposed uses and use levels.

The Panel could not have reached the conclusion on the safety of the NF under the proposed conditions of use without the data claimed as proprietary by the applicant, i.e. 90-day subchronic toxicity study by Silvano (2012; unpublished) to determine the MOE.

Steps taken by EFSA

1) Letter from the European Commission to the European Food Safety Authority with the request for a scientific opinion on the safety of ARES(2017)6052616, dated 11 December 2017.
2) As the application was re-submitted by the applicant through the e-submission portal in order for it to be treated as an application under Regulation (EU) 2015/2283 as set out in article 35(1) of Regulation (EU) 2015/2283, the mandate submitted under ARES(2017) 6052616 was replaced by the present mandate Ares(2018)994596, dated 21 February 2018.

3) On 22 02 2018, EFSA received a valid application from the European Commission on whey basic protein isolate as NF, which was submitted by Armor Protéines S.A.S., and the scientific evaluation procedure started.
4) During its meeting on 27 June 2018, the NDA Panel, having evaluated the data, adopted a scientific opinion on the safety of whey basic protein isolate as a NF pursuant to Regulation (EU) 2015/2283.

References

Armor Protéines SAS., 2013. Clinical Trials on TGB-β2-rich Milk Protein Fractions. Unpublished report

Armor Protéines SAS., 2014. NCBI Blast:AAI07168:Lactoperoxidase [Homo sapiens], Unpublished report

Armor Protéines SAS., 2017. SDS PAGE Analysis of Vitalarmor® GF 100 Unpublished Report

Davidson LA and Lönnerdal B, 1987. Persistence of human milk proteins in the breast-fed infant. Acta Paediatrica Scandinavica, 76, 733–740.

DNSIYC (Diet and Nutrition Survey of Infants and Young Children), 2013. (SN 7263). Prepared by National Centre for Social Research (NatCen), London, Engl. / Medical Research Council (MRC), Human Nutrition Research, Cambridge, UK / MRC Epidemiology Unit, Cambridge, UK / MRC Epidemiology Unit, Cambridge, UK / Human Nutrition Research Centre (HNRC), Newcastle University for Department of Health / Food Standards Agency (FSA), London, UK and Distributed by UK Data Archive, Colchester, UK; 2013. Available online: https://discover.ukdataservice.ac.uk/doi?sn=7263 [Version: 16 May 2013; open full catalogue record].

Dyer AR, Burdock GA, Carabin IG, Haas MC, Boyce J, Alsaker R and Read LC, 2008. In vitro and in vivo safety studies of a proprietary whey extract. Food and Chemical Toxicology, 46, 1659–1665.

EFSA (European Food Safety Authority), 2011. Use of the EFSA Comprehensive European Food Consumption Database in Exposure Assessment. EFSA Journal 2011;9(3):2097, 34 pp. https://doi.org/10.2903/j.efsa.2011.2097

EFSA NDA Panel (EFSA Panel on Dietetic Products, Nutrition and Allergies), 2012a. Scientific Opinion on bovine lactoferrin. EFSA-Q-2010-01269, adopted on 27 April 2012. EFSA Journal 2012;10(5):2701, 26 pp. https://doi.org/10.2903/j.efsa.2012.2701. Available online: http://www.efsa.europa.eu/de/efsajournal/pub/2701.htm

EFSA NDA Panel (EFSA Panel on Dietetic Products, Nutrition and Allergies), 2012b. Scientific Opinion on bovine lactoferrin. EFSA-Q-2011-00974, adopted on 28 June 2012. EFSA Journal 2012;10(7):2811, 14 pp. https://doi.org/10.2903/j.efsa.2012.2811. Available online: http://www.efsa.europa.eu/de/efsajournal/pub/2811

EFSA NDA Panel (EFSA Panel on Dietetic Products, Nutrition and Allergies), 2015. Scientific Opinion on Dietary Reference Values for iron. EFSA Journal 2015;13(10):4254, 115 pp. https://doi.org/10.2903/j.efsa.2015.4254

EFSA Scientific Committee, 2012. Guidance on selected default values to be used by the EFSA Scientific Committee, Scientific Panels and Units in the absence of actual measured data. EFSA Journal 2012;10(3):2579, 32 pp. https://doi.org/10.2903/j.efsa.2012.2579

Elfstrand L, Lindmark-Månsson H, Paulsson M, Nyberg L and Akesson B, 2002. Immunoglobulins, growth factors and growth hormone in bovine colostrum and the effects of processing. International Dairy Journal, 12, 879–887. https://doi.org/10.1016/S0958-6946(02)00089-4

FAO, 2002. Lactoperoxidase - Frequently Asked Questions. Food and Agriculture Organization of the United Nations (FAO), Rome, Italy. Available online: http://www.fao.org/ag/againfo/themes/documents/lps/dairy/MPV/Lactoperoxidase/faq.htm#faqtop

Ferenc Levay P and Viljoen M, 1995. Lactoferrin: a general review. Haematologica, 80, 252–267.

Fischer R, Debbabi H, Blais A, Dubarry M, Rautureau M, Boyaka PN and Tome D, 2007. Uptake of ingested bovine lactoferrin and its accumulation in adult mouse tissues. International Immunopharmacology, 7, 1387–1393.

Forster R, Bourtourault M, Chung YJ, Silvano J, Sire G, Spezia F, Puel C, Descotes J and Mikogami T, 2014. Safety evaluation of a whey protein fraction containing a concentrated amount of naturally occurring TGF-β2. Regulatory Toxicology and Pharmacology, 69, 398–407.

FSANZ, 2002. Lactoperoxidase System (Application A404 - Final Assessment Report). Food Standards Australia New Zealand (FSANZ), Canberra, Australia. Available online: http://www.foodstandards.gov.au/code/applications/pages/applicationa404thelactoperoxidasesystem/applicationa404lacto1862.aspx [Accessed: 18 December 2002]

Gauthier SF, Pouliot Y and Maubois J-L, 2006. Growth factors from bovine milk and colostrum: composition, extraction and biological activities. Le Lait, INRA Editions, 86, 99–125.

Hennart PF, Brasseur DJ, Delogne-Desnoeck JB, Dramaix MM and Robyn CE, 1991. Lysozyme, lactoferrin, and secretory immunoglobulin A content in breast milk: influence of duration of lactation, nutrition status, prolactin status, and parity of mother. The American Journal of Clinical Nutrition, 53, 32–39.

Hirai Y, Kawakata N, Satoh K, Ikeda Y, Hisayasu S, Orimo H and Yoshino Y, 1990. Concentrations of lactoferrin and iron in human milk at different stages of lactation. Journal of Nutritional Science and Vitaminology, 36, 531–544.

Indyk HE, Filonzi EL and Gapper LW, 2006. Determination of minor proteins of bovine milk and colostrum by optical biosensor analysis. Journal of AOAC International, 89, 898–902.

JECFA, 1990. Lactoperoxidase/thiocyanate/hydrogen peroxide system. In: Evaluation of Certain Food Additives and Contaminants. 35th Report of the Joint FAO/WHO Expert Committee on Food Additives (JECFA). (WHO Technical Report Series, no 789). World Health Organization (WHO), Geneva, Switz.;, pp. 28-29. Available online: http://whqlibdoc.who.int/trs/WHO_TRS_789.pdf

Jouni ZE, Waworuntu R, Vangari G, Silightom R, Rosales R, Ferguson P, Rai G, Ruiz- Palacio G, Guerrero M, Morrow A and McMahon R, 2009. Concentration of transforming growth factor-beta in an infant formula from five countries and human milk from Mexico and the US, The FASEB Journal, 23:1_supplement, LB494-LB494.

Kersting M, Alexy U, Sichert-Hellert W, Manz F and Schöch G, 1998. Measured consumption of commercial infant food products in German infants: Results from the DONALD study (Dortmund Nutritional and Anthropometrical Longitudinally Designed). Journal of Pediatric Gastroenterology and Nutrition, 27, 547–552.

Khan S, Perkins AC, Frier M, Blackshaw PE and Leufkens P, 2000. Biodistribution of human lactoferrin in the gastrointestinal tract. In: Shimazaki K, Tsuda H, Tomita M, Kuwata T and Perraudin JP (eds.). Lactoferrin: Structure, Function and Applications. Proceedings of the 4th International Conference on Lactoferrin: Structure, Function and Applications, May 18-22, 1999, Sapporo, Japan. (Excerpta Medica International Congress Series, no 1195). Elsevier Science B.V., Amsterdam, The Netherlands. pp. 319–325.

Korhonen H and Pihlanto A, 2003. Bioactive peptides: novel applications for milk proteins. Applied Biotechnology, Food Science and Policy, 1, 133–144.

Kruger CL, Marano KM, Morita Y, Takada Y, Kawakami H, Kobayashi T, Sunaga M, Furukawa M and Kawamura K, 2007. Safety evaluation of a milk basic protein fraction. Food and Chemical Toxicology, 45, 1301–1307.

Kussendrager KD and van Hooijdonk AC, 2000. Lactoperoxidase: physico-chemical properties, occurrence, mechanism of action and applications. The British Journal of Nutrition, 84, S19–S25.

MHLW, 2014. List of Existing Food Additives [Complied and published by the Ministry of Health and Welfare on April 16, 1996]. Japan Food Chemical Research Foundation (JFCRF), Tokyo, Japan. Available online: http://www.ffcr.or.jp/zaidan/FFCRHOME.nsf/pages/list-exst.add [Effective from January 30, 2014, Last update: 06/30/2014]

NIH, 2016. Dietary ingredient: Lactoperoxidase. In: Dietary Supplements Labels Database (DSLD) National Library of Medicine (NLM) / National Institutes of Health (NIH), Office of Dietary Supplements, Bethesda (MD). Available online: https://dsld.nlm.nih.gov/dsld/rptIngredient.jsp?db=adsld&item=LACTOPEROXIDASE

Pamblanco M, Ten A and Comin J, 1986. Proteins in preterm and term milk from mothers delivering appropriate or small-for-gestational age infants. Early Human Development, 14, 267–272.

Prentice A, Ewing G, Roberts SB, Lucas A, MacCarthy A, Jarjou LM and Whitehead RG, 1987. The nutritional role of breast-milk IgA and lactoferrin. Acta Paediatrica Scandinavica, 76, 592–598.

Rudloff S and Kunz C, 1997. Protein and nonprotein nitrogen components in human milk, bovine milk, and infant formula: quantitative and qualitative aspects in infant nutrition. Journal of Pediatric Gastroenterology and Nutrition, 24, 328–344.

Schmitt B and Mireaux M, 2008. Rapport Provisoir de L'Etude Sur L'Effet de la Consommation de Lactochol® Sur Les Parametres de L'Atherosclerose. EC 1106. Confidential. Centre d'Enseignement et de Recherche en Nutrition (CERN), [French with English summary] Unpublished report

Shin K, Hayasawa H and Lönnerdal B, 2001. Purification and quantification of lactoperoxidase in human milk with use of immunoadsorbents with antibodies against recombinant human lactoperoxidase. The American Journal of Clinical Nutrition, 73, 984–989.

Silvano J, 2012. 13-week toxicity study by the oral route (gavage) in rats followed by a 4-week treatment-free period: [Test Item TM0601p]: (Laboratory Study No. 38668 TCR). Unpublished report

Sire G, 2012a. Bacterial Reverse Mutation Test: [Test Item TM0601p]: (Laboratory Study No. 38665 MMO). Unpublished report

Sire G, 2012b. In Vitro Micronucleus Test in L5178Y TK +1-Mouse Lymphoma Cells: [Test Item TM0601p]: (Laboratory Study No. 38837 MNV). Unpublished report

Spezia F, 2013. Toxicity study by Oral Route (gavage) in Juvenile Rats [Test Item TM0601p]: (Laboratory Study No. 38669 RST). Unpublished report

Srivastava MD, Srivastava A, Brouhard B, Saneto R, Groh-Wargo S and Kubit J, 1996. Cytokines in human milk. Research Communications in Molecular Pathology and Pharmacology, 93, 263–287.

Takeuchi T, Kitagawa H and Harada E, 2004. Evidence of lactoferrin transportation into blood circulation from intestine via lymphatic pathway in adult rats. Experimental Physiology, 89, 263–270.

Troost FJ, Steijns J, Saris WH and Brummer RJ, 2001. Gastric digestion of bovine lactoferrin in vivo in adults. Journal of Nutrition, 131, 2101–2104.

UKDA, 2014. National Diet and Nutrition Survey Years 1-4, 2008/09-2011/12 [computer file], 6th ed. (SN: 6533). UK Data Archive (UKDA) [distributor], Colchester, Essex;. Available online: https://doi.org/10.5255/ukda-sn-6533-2 [open full catalogue record]

US FDA, 1981. GRAS status of whey, whey products and hydrogen peroxide; Final rule (21 CFR Part 184) [Docket No. 78N-0369]. Federal Register (US), 46, 44434–44442.

US FDA, 2000. GRN 000037 [Whey protein isolate and dairy product solids, American Dairy Products Institute, Chicago (IL)]. In: GRAS Notices. U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety & Applied Nutrition (CFSAN), Office of Premarket Approval, Silver Spring (MD). Available online: http://www.accessdata.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=37

US FDA, 2001a. GRN 000067 [Milk-derived lactoferrin, Farmland National Packaging Company, L.P., Liberal (KS)]. In: GRAS Notices. US Food and Drug Administration (US FDA), Center for Food Safety & Applied Nutrition (CFSAN), Office of Food Additive Safety, Silver Spring (MD);. Available online: http://www.accessdata.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=67 [Filed: Jan. 9, 2001; Response letter: Oct. 23, 2001]

US FDA, 2001b. GRN 000077 [Milk-derived lactoferrin, DMV International, Fraser (NY)]. In: GRAS Notices. US Food and Drug Administration (US FDA), Center for Food Safety & Applied Nutrition (CFSAN), Office of Food Additive Safety, Silver Spring (MD); Available online: http://www.accessdata.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=77

US FDA, 2003. GRN 000130 [Bovine milk-derived lactoferrin, aLF Ventures, LLC, Salt Lake City (UT)]. In: GRAS Notices. US Food and Drug Administration (US FDA), Center for Food Safety & Applied Nutrition (CFSAN), Office of Food Additive Safety, Silver Spring (MD);. Available online: http://www.accessdata.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=130

US FDA, 2006. GRN 000196 [Bovine milk basic protein fraction, Snow Brand Milk Products Co., Ltd., Tokyo, Japan AND IAS Co., Ltd.]. In: GRAS Notices. US Food and Drug Administration (US FDA), Center for Food Safety & Applied Nutrition (CFSAN), Office of Food Additive Safety, Silver Spring (MD); Available online: http://www.accessdata.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=196

US FDA, 2014a. GRN 000464 [Cow's milk-derived lactoferrin, Morinaga Milk Industry Co., Ltd., Tokyo, Japan]. In: GRAS Notices. US Food and Drug Administration (US FDA), Center for Food Safety & Applied Nutrition (CFSAN), Office of Food Additive Safety, Silver Spring (MD);. Available online: http://www.accessdata.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=464

US FDA, 2014b. GRN 000465 [Cow's milk-derived lactoferrin, Morinaga Milk Industry Co., Ltd., Tokyo, Japan]. In: GRAS Notices. US Food and Drug Administration (US FDA), Center for Food Safety & Applied Nutrition (CFSAN), Office of Food Additive Safety, Silver Spring (MD); Available online: http://www.accessdata.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=465

US FDA, 2017. GRN 000669 [Bovine Milk-derived Lactoferrin, Synlait Milk Ltd, Rakaia, New Zealand]. In: GRAS Notices. US Food and Drug Administration (US FDA), Center for Food Safety & Applied Nutrition (CFSAN), Office of Food Additive Safety, Silver Spring (MD); Available online: https://www.fda.gov/downloads/Food/IngredientsPackagingLabeling/GRAS/NoticeInventory/ucm592102.pdf

USDA-ERS, 2016. Dairy products [Whey]: Per capita consumption, United States, 1975-2013. In: Food Availability (Per Capita) Data System. U.S. Department of Agriculture (USDA), Economic Research Service (ERS), Washington (DC); 2016. Available online: https://www.ers.usda.gov/data-products/food-availability-per-capita-data-system/ [Excel spreadsheet; Dataset updated August 3, 2016]

Wal J-M, 2004. Bovine milk allergenicity. Annals of Allergy, Asthma & Immunology, 93, S2–S11.

Abbreviations

ALT	Alanine transaminase
AST	Aspartate transaminase
bLF	Bovine Lactoferrin
bLP	Bovine Lactoperoxidase
ELISA	enzyme-linked immunosorbent assay
FSMP	Food for special medical purposes
GD	gestation day
GMP	Good manufacturing practice
HACCP	Hazard Analysis Critical Control Point
HPLC	High-performance liquid chromatography
MBP	Milk basic protein
MOE	margin of exposure
MS	Member State
NDA	Dietetic Products, Nutrition and Allergies
NOAEL	no observed adverse effect level
NF	Novel food
PND	post-natal day
TGF	Transforming growth factor