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September 12, 2016

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GRN 000669

Dr. Antonia Mattia
Director, Division of Biotechnology and GRAS Notice Review
Office of Food Additive Safety (HFS-255)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
4300 River Road
College Park, MD 20740

RE: Submission of new GRAS Notification for Bovine Milk-derived Lactoferrin

Dear Dr. Mattia:

In accordance with 21 CFR § 170.36 (Notice of a claim for exemption based on a GRAS determination) published in the Federal Register (62 FR 18939-18964), the attached new GRAS Notice for the use of Bovine Milk-derived Lactoferrin is submitted on behalf of the notifier, Synlait Milk Ltd.

We request that all correspondence be directed to the agent for Synlait at the address below:

Drummond Food Science Advisory Ltd
1137 Drain Road, Killinchy
RD 2, Leeston 7682 New Zealand
Lynley_dfsa@me.com

We are enclosing three (3) paper copies and one (1) CD-Rom, which we have checked and found to be virus-free.

Sincerely,

(b) (6)

Edward A. Steele
Chairman & CEO

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FOOD ADDITIVE SAFETY

EAS Consulting Group, LLC
1700 Diagonal Road, Suite 750, Alexandria, Virginia 22314
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Generally Recognized As Safe (GRAS) Notice: The Use of Bovine Milk-derived Lactoferrin In Term Milk-based Infant Formulas and Toddler Formulas

Prepared for:

Synlait Milk Ltd,
1028 Heslerton Road,
RD 13, Rakaia 7783
New Zealand

Prepared by:

Drummond Food Science Advisory Ltd,
1137 Drain Road, Killinchy,
RD 2, Leeston 7682 New Zealand

September 2016

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Generally Recognized As Safe (GRAS) Notice: The Use of Bovine Milk-derived Lactoferrin In Term Milk-based Infant Formulas and Toddler Formulas

Prepared for:

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RD 2, Leeston 7682 New Zealand

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PART 1 SIGNED STATEMENTS AND CERTIFICATION

1.1 INTRODUCTION

Pursuant to the criteria detailed in 21 CFR§170 Subpart E – Generally Recognized as Safe (GRAS) Notice [81 FR 55047 (August 17, 2016)], Synlait Milk Ltd. (Synlait) hereby notifies the Food and Drug Administration that the use of bovine milk-derived lactoferrin (bLf) in milk-based term infant (birth to 12 months) and toddler (13 to 36 months) formulas under the intended conditions of use is exempt from the requirement of premarket approval requirements of the Federal Food, Drug, and Cosmetic Act because Synlait has determined that such uses are Generally Recognized As Safe (GRAS) through scientific procedures in accordance with 21 CFR§107.30 (a) and (b).

Synlait Milk Ltd. (Synlait) is submitting this GRAS notice for use of its bovine milk lactoferrin (bLf) in exempt (defined in 21 CFR§107.3) milk-based term infant formula and toddler formula as described in this document. This Notice is based on scientific procedures as described in the following sections, under the conditions of the intended use of bLf in infant formula and toddler formulas.

A comprehensive search of the scientific literature for use, safety and toxicity information on bLf in infant formula, toddler formulas, and other foods was conducted through (November 2015 to July 2016) and made available to the GRAS Panel. The GRAS Panel, independently and critically, evaluated materials submitted by Synlait and other information deemed appropriate or necessary. Synlait accepts responsibility for the GRAS notice that has been made for bLf as described herein. Following an independent, critical evaluation, the GRAS Panel conferred and unanimously agreed to the conclusion described herein. Synlait is also of the opinion that other qualified and competent scientists, reviewing the same publicly available toxicological and safety information, would reach the same conclusion.

Synlait hereby certify, that to the best of our knowledge, this GRAS Notice is a complete, representative, and balanced submission that includes both unfavorable information, together with favorable information, known to Synlait and pertinent to the evaluation of the safety and GRAS status of bLf and it intended uses. Synlait is of the view that the notified substance, bLf, is not subject to the premarket approval requirements of the

Federal Food, Drug, and Cosmetics Act (FD&C Act) based on the conclusion that the notified substance, bLf, is GRAS under the conditions of its intended use.

Signed,

(b) (6)

Date: 12th September 2016

Michael Stein
General Manager Quality & Regulatory
Synlait Milk Ltd
1028 Heslerton Road, RD 13,
Rakaia 7783
NEW ZEALAND

1.2 NAME AND ADDRESS OF THE NOTIFIER

1.2.1 Business Address

Synlait Milk Ltd
1028 Heslerton Road,
RD 13,
Rakaia 7783
NEW ZEALAND
Telephone: +64 3 373 3000
Facsimile: +64 3 373 3001

1.2.2 Mailing Address

Synlait Milk Ltd
1028 Heslerton Road,
RD 13,
Rakaia 7783
NEW ZEALAND
Email: techservices@synlait.com

Manufacturer:

Synlait Milk Ltd
1028 Heslerton Road,
RD 13,
Rakaia 7783
NEW ZEALAND

Person Responsible for GRAS Notice:

Michael Stein, General Manager Quality & Regulatory – **Signatory**
Iain Halcrow, Product & Technical Manager – **Post Submission Inquiries**
Synlait Milk Ltd
1028 Heslerton Road, RD 13,
Rakaia 7783
NEW ZEALAND

1.3 NAME OF GRAS SUBSTANCE

The substance that is the subject of this Generally Recognized As Safe (GRAS) Notice is bovine (*Bos taurus*) milk-derived lactoferrin (bLf). Bovine lactoferrin is also referred to as lactotransferrin.

1.4 INTENDED USE

Synlait intends to add bLf to term bovine milk-based term infant formulas (for infants from birth to 12 months), excluding exempt formulas as defined in 21 CFR §107.3, and to toddler formulas (for older infants and young children (13 to 36 months)) at levels of up to 100 mg /100 g of formula solids. This level is consistent to what has already been accepted by FDA in a previous GRAS Notice (GRN 465).

1.5 BASIS FOR GRAS NOTICE

This notice that the intended use of bovine milk-derived lactoferrin (bLF) manufactured by Synlait Milk Ltd. is GRAS, is based on scientific procedures as described under 21 CFR § 170.30 (b). A comprehensive search of the scientific literature was undertaken between November 2015 and July 2016, in support of this

notice. Based on the results of this search, Synlait has concluded that there exists sufficient qualitative and quantitative scientific evidence, including human and animal data, to determine the safety of the use of bLf in infant formula and toddler formula. This is further supported by the fact that FDA has received and reviewed a GRAS Notice related to the use of bLf in infant formula in which FDA issued a "No Question" letter. Information related to the FDA notice is discussed in this document in support of Synlait's bLf, as the composition of the bLf and intended end use are virtually identical.

The protein, bLf is naturally present in bovine (*Bos taurus*) (or cow's) milk which has a long history of human consumption, including consumption by infants from cow's milk-based formula. bLf is one of the whey proteins in cow's milk, accounting for 0.3% (0.1 g/L of milk) of the total milk protein, or 1.4% of the total whey protein in milk. The highest background exposure of infants to bLf from cow's milk-based infant formula is for infants aged 0-5 months, whose sole source of nutrition is formula, is estimated to be 75 – 137 mg/day (Table 3-1).

Milk-based infant formula and formula for toddlers fortified with bLf are approved for use, and sold in the USA, the European Union, China, Japan, Korea and Taiwan. Bovine lactoferrin has a long and safe history of use in infant formula, with the first bLf fortified formulas becoming available in Japan in 1986 (GRN 465, 2014, p. 4 (pdf))[1]. Synlait has manufactured bLf fortified formulas for sale in China since 2011, and using its own internally manufactured source of bLf since late 2014.

The bLf manufactured by Synlait conforms to accepted food grade specifications and utilizes only approved food grade raw materials and processing aids. The product has a minimum protein content of 95.0 % of which more than 95% is lactoferrin, with maximum levels of 4.5% moisture, 1.0% fat and 1.3% ash. Synlait production facilities are approved under all mandatory dairy manufacturing and export standards in New Zealand, and are FDA registered.

Studies on the metabolic fate of bLf, using animal models appropriate for infants, and where available evidence from infants demonstrate how lactoferrin is absorbed, distributed, metabolized and ultimately excreted. Lactoferrin is only partially hydrolyzed

[1] Page references for other GRAS Notices (GRN) are the pdf page number of the GRN documents available on the FDA GRAS Inventory website at http://www.accessdata.fda.gov/scripts/fdcc/?set=GRASNotices

in the stomach; intact and large fragments may then be partially absorbed from the intestinal lumen via lactoferrin receptors, into the systemic circulation. Lactoferrin may be excreted into the bile and then re-absorbed into the bloodstream for further distribution. The metabolic fate of lactoferrin is supported by studies in infants, where lactoferrin from dietary sources has been detected in both the urine and feces.

Safety studies of bLf included an acute toxicity studies, including 4-week and 13-week subchronic oral toxicity studies. The estimated the no observed adverse effect level (NOAEL) to be at least 2,000 mg/kg/day. Chronic oral toxicity studies (40- and 65-week) supported safe consumption of lactoferrin, and a mutagenicity (Ames) test indicated bLf was not genotoxic.

The addition of bLf to formulas according to the intended uses results in mean estimated daily intakes (EDI's) from the added bLf of 101.3 mg/day for infants 0 – 6 months, 92.2 mg/ day for infants 7 – 12 months and 60.1 mg/day for toddlers (13 – 36 months). The 90^{th} percentiles for the age groups are 148.6 mg/ day, 135.6 mg/day and 132.0mg /day, respectively. Accounting for the background levels in infant formula, the addition of bLf to formulas will increase the daily exposure for term infants by approximately 160% and for toddlers approximately 2-fold.

The tolerance and safety of bLf for infants and children is the subject of a significant number (36) of published intervention studies. The studies consistently report the addition of bLf to formula or supplementation is well tolerated, or that no adverse treatment-related effects are observed. In term infants (0 – 6 months), the mean and 90^{th} percentile EDI's subject of this notification are 101 mg/day and 149 mg/day respectively, and for older infants (7-12 months) 92 mg/day and 136 mg/day respectively. Studies in healthy toddlers report safe exposures of 100 mg/day to 1000 mg/day (Ochoa et al., 2013), with further evidence of safe use at higher levels from studies using bLf (3 g/day) in immunocompromised children (Zuccotti, Salvini, Riva, & Agostoni, 2006).

Based upon scientific procedures, bLf, manufactured by various processes and manufacturers, has previously been determined to be GRAS for specific uses, including term infant formula at levels up to 100 mg/100 g of formula solids and for infants up to 12 months of age (GRN 465). Since the notification of GRN 465, additional studies supporting the safety of bLf in infant formula and potential mechanisms of action in animal models have become available. Those studies further support the safe use of bLf and are included in this notification.

bLf is also GRAS for wider use in cow's milk-based products (100 mg/100 g), powdered milk (400 mg/100 g), ice cream and sherberts (200 mg/100 g) and chewing gum (30 mg/g) (GRN 464).

The estimated daily intake (EDI) of bLf for the intended uses has been determined to be safe, and has also been determined to be GRAS by demonstrating that the safety of the levels of intake under the intended uses is based on publicly available and accepted information. This document was provided to a panel of experts, qualified by scientific training and experience to evaluate the safety of bLf, who collectively and individually reviewed the material and have unanimously agreed to the conclusion cited herein. The *Curriculum vitae* of each of the GRAS Panel members are provided in Appendix 6 of Part 7 of this Notice.

The signed statement of the GRAS Panel follows in Section 1.6

1.6 GRAS PANEL STATEMENT

1.6.1 Introduction

Drummond Food Science Advisory Ltd. (DFSA), New Zealand, on behalf of Synlait Milk Ltd. (Synlait), convened a panel of experts (GRAS Panel), to conduct a critical and comprehensive evaluation of the available pertinent data and information on the use of bovine lactoferrin (bLf) manufactured by Synlait, in term milk-based infant and toddler formulas. The GRAS Panel was specifically requested to determine whether the use of the Synlait manufactured bLf at a level of up to 100mg bLf /100g of formula solids is Generally Recognized as Safe (GRAS) based on known scientific information. The Expert Panel consisted of the below-signed qualified experts: Dr. Craig Jensen, Pediatric Gastroenterologist (Texas Childrens Hospital & Baylor College of Medicine, USA); Distinguished Professor Emeritus Bo Lönnerdal (University of California, Davis, USA); Distinguished Professor Paul Moughan (Riddet Institute, New Zealand); Associate Professor Theresa Ochoa (Cayetano Heredia University, Peru & University of Texas School of Public Health, USA); Professor Bing Wang (Charles Sturt University, Australia).

The GRAS Panel, independently and collectively, critically evaluated the available information presented in documents prepared by DFSA, New Zealand in a dossier: "Generally Recognized As Safe (GRAS): Determination for Bovine Milk-derived Lactoferrin Use In term Milk-based Infant Formulas and Toddler Formulas, and made

several recommendations for revision of the first draft, primarily concerned with expanding the information presented on some key issues. The revised document was read and approved by the panel.

Following its independent critical evaluation of the available information, the GRAS Panel convened by teleconference on 26 July 2016 with representatives of DFSA and Synlait. The GRAS Panel unanimously concluded and without any reservation, that the intended use of Synlait bLf, meeting appropriate food-grade specifications, and manufactured using current Good Manufacturing Practice (cGMP) is GRAS. The GRAS Panel has prepared a summary statement of its conclusions, which is provided below.

1.6.2 Signed Statement

GRAS (Generally Recognized as Safe) Panel Statement Concerning the Generally Recognized as Safe GRAS Status of the Proposed Use of Bovine Lactoferrin (BLf) in Term Milk-based Infant and Toddler Formulas

We, the members of the GRAS (Generally Recognized as Safe) Panel, qualified by scientific training and relevant experience to evaluate the safety of food and food ingredients, have performed a comprehensive and critical review of available information and data on the safety and GRAS status of the use of bovine milk-derived lactoferrin (bLf), manufactured by Synlait, in term milk-based infant and toddler formulas (100mg bLf /100g formula solids). This critical review has been based upon scientific procedures as described under 21 CFR §170.30(b). The data and information perused are summarized in the GRAS determination document ("Generally Recognized As Safe (GRAS) Notice: Bovine Milk-derived Lactoferrin Use In term Milk-based Infant Formulas and Formulas for Toddler Formulas"), prepared by Drummond Food Science Advisory Ltd., on behalf of Synlait Milk Ltd (Synlait).

Based upon our review of the information and data available, we have determined using scientific procedures that the amount of bLf consumed (the intake) for the intended uses specified, has been shown to be safe and GRAS.

The intended use of bLf, manufactured by Synlait, in term milk-based infant and toddler formulas has been determined through the application of scientific procedures to be safe as described under 21 CFR § 170.30 (b) based on the following information:

1. Bovine lactoferrin shares a high degree of homology with human lactoferrin and has similar physiological effects. Lactoferrin is one of the predominant whey proteins in human milk and is considered to have important physiological properties. The mean daily intake of human lactoferrin for breast fed infants is approximately 1100 mg/day.

2. There is a long history for the safe ingestion of bovine milk and bovine lactoferrin by adult and infant humans. The human population has consumed bovine milk for centuries. Bovine milk protein consists of 79.2% casein proteins (27 mg/ml of milk) and 20.8% whey proteins (7.1 mg/ml of milk).

Lactoferrin is one of the whey proteins and accounts for 0.3% (0.1 mg/ml of milk) of the total milk protein, or 1.4% of the whey protein. Infant formula has been made from bovine milk since the 19th century, with the introduction of whey predominant milk-based formulas in the early 1960's. There have been no known significant health problems attributable to the background bLf in milk or to the addition of bLf to infant and toddler formulas.

3. The digestion and metabolic fate of lactoferrin has been evaluated from studies of both human lactoferrin and bLf. Lactoferrin from both sources is handled similarly by the body. Lactoferrin is relatively poorly digested, with a substantial proportion of intact lactoferrin and its peptides persisting throughout the gastrointestinal tract, ultimately being excreted in the feces. Lactoferrin and its peptides may also be partially absorbed from the gastrointestinal tract via specialized lactoferrin receptors and enter the blood. Lactoferrin is removed from the systemic circulation for distribution into organs and a proportion of the absorbed lactoferrin is ultimately excreted in the urine.

4. No published evidence exists that bLf is a clinically relevant allergen.

5. The estimated daily intakes (EDIs) for bLf based on the intended use have been concluded to be safe and GRAS based on absorption, distribution, metabolism, and excretion (ADME) studies, animal toxicology studies, studies investigating physiologic effects, and clinical studies in infants and children. These published studies support the safety of intake of bLf for the intended use.

6. A significant body of evidence from published intervention studies supports the safety of bLf for infants. In the 26 clinical trials identified in infants (from preterm and term at birth – 12 months) and in children (>12 months) and involving approximately 4000 subjects, no adverse events related to the administration of bLf have been reported. The identified studies, completed in both healthy and vulnerable infants and young children, consistently report that bLf is well tolerated. The level of bLf administered in these studies (up to 2,300 mg/ day in term infants and up to 3,000 mg/day in children) adequately addresses the maximum predicted EDI's of bLf of this notification, and supports the safe use of bLf for the intended uses.

7. The estimated mean intake level of bLf from standard term milk-based infant 0 – 6 months, older infants 7- 12 months, and toddler (13-36 months) formulas is 168 mg/day, 143 mg/day and 60 mg/day respectively. The intended use of bLf at up to 100 mg/100g of formula solids results in additional mean EDIs of 101 mg/day (90^{th} percentile 149 mg/day) for infants 0 – 6 months, 92 mg/day (90^{th} percentile 136 mg/day) for older infants 7 – 12 months, and 60 mg/day (90^{th} percentile 132 mg/day) for toddlers (13 – 36 months). The totals of up to approximately 269 mg/day for term infants (0-6 months), 235 mg/day for older infants (7 – 12 months) and 120 mg/day for toddlers are deemed to be safe.

8. In the United States, bLf has previously been determined safe and GRAS for use in term infant formula (GRN 465); 100 mg /110g (formula powder), 13 mg/100ml of ready-to-feed formula, and 26 mg/100ml of liquid formula concentrate. Bovine lactoferrin has also been determined safe and GRAS for use as an ingredient in a range of foods: cow's milk-based food products, including yogurt (100 milligrams (mg) per 100 grams (g)) powdered milk (400 mg/100 g) and ice cream and sherbets (200 mg/100 g); chewing gum (30 mg/g) (GRN 464); sports and functional foods (100 mg/serve) (GRN 77). Bovine lactoferrin has also been determined safe and GRAS for uses as an antimicrobial spray on beef carcasses (GRN 77) and as an antimicrobial agent for use on uncooked beef (GRN 67).

9. Infant and toddler formulas containing added bLf are approved for use and are available to consumers in the USA (100mg/100g (GRN 465)), European Union (100mg/ 100ml for infants and 200mg/100g young children), Japan (no limit), China (100mg / 100g), Taiwan (as practically needed), and also Korea, Indonesia and Pakistan. Bovine lactoferrin has been added to infant formula in Japan since 1986. Synlait currently manufactures infant and toddler formulas containing added bLf for sale in China.

10. Bovine lactoferrin manufactured by Synlait under current Good Manufacturing Practice (cGMP) complies with established food grade specifications and utilizes only food grade raw materials and processing aids. Synlait bLf has a minimum protein content of 95%, of which bLf is ≥95%, a maximum water content of 4.5%, and a maximum ash content of 1.3%. The iron saturation of bLf is ≤ 20%, and the total iron content is ≤ 200 mg/kg. Synlait manufactured

bLf typically has low levels of the endotoxin LPS, known to inhibit the bioactivity of bLf.

CONCLUSION OF THE GRAS PANEL

Based on a critical evaluation of the publicly available data and observations, which are summarized herein, we, the members of the GRAS Panel, have individually and collectively, and without conflict of interest, concluded that bovine lactoferrin (bLf), manufactured consistent with current Good Manufacturing Practice, meeting the food grade specifications cited herein, and when added to term infant formula and toddler formula at added use levels of up to 100 mg/100 g of formula solids, as described in this monograph and resulting in the 90th percentile for term infants of total estimated intake of up to 290 mg of bovine lactoferrin/day and for toddler formula of approximately 240 mg/day, is safe.

It is also our opinion that other qualified and competent scientists reviewing the same publicly available toxicological and safety information would reach the same conclusion. Therefore, we have also concluded based on scientific procedures that bovine lactoferrin, when used as described, is GRAS.

Signatures

Dr. Craig Jensen, Pediatric Gastroenterologist, Texas Childrens Hospital and Associate Professor, Section of Gastroenterology, Hepatology and Nutrition, Department of Pediatrics, Baylor College of Medicine, USA

(b) (6)

Date (b) (6) 9/7/2016

Distinguished Professor Emeritus Bo Lönnerdal, University of California, Davis, USA

(b) (6)

Date 9/7/2016

Distinguished Professor Paul Moughan, Riddet Institute, Massey University, New Zealand

(b) (6)

Date: 7 September 2016

Associate Professor Theresa Ochoa, Universidad Peruana Cayetano Heredia, Peru & University of Texas Health Science Center at Houston, USA

(b) (6)

Date September 6, 2016

Professor Bing Wang, Charles Sturt University, Australia

(b) (6)

Date: 6 September 2016

1.7 AVAILABILITY OF INFORMATION

The data and information that are the basis for Synlait's conclusion of the GRAS status of bLf under the intended conditions of use are available for the FDA's review, both during or after the evaluation of this Notice. Upon request, a complete copy of the data and information will be provided to the FDA either in an electronic format that is accessible for FDA evaluation, or on paper. Upon request, the data and information are available for the FDA to review and copy during customary business hours at either of the following addresses:

Lynley Drummond
Drummond Food Science Advisory Ltd,
1137 Drain Road, Killinchy,
RD 2, Leeston 7682 New Zealand
lynley_dfsa@me.com
Telephone: + 64 3 324 7284

Or,

Synlait Milk Ltd
1028 Heslerton Road,
RD 13,
Rakaia 7783
NEW ZEALAND
info@synlait.co.nz

Synlait has identified Confidential and not generally available data and information contained in Part 7 (Appendices 1, 2, 3, 4, 5 and 6) which it considers are exempt from disclosure under the Freedom of Information Act, 5 U.S.C. §552. Synlait has not identified any trade secrets included as a part of this Notice and authorizes for all information within this Notice to be provided to the Food Safety and Inspection Service (FSIS) of the U.S. Department of Agriculture, as required.

PART 2 IDENTITY OF BOVINE LACTOFERRIN

2.1 INTRODUCTION

The substance that is the subject of this Generally Recognized as Safe (GRAS) Notice is bovine milk-derived lactoferrin (bLf), for use in term milk-based infant formulas and toddler formulas. Bovine lactoferrin, manufactured by various processes and manufacturers, has previously been determined to be GRAS for specific uses (Table 2-1). It has previously been determined to be GRAS for use in term cow's milk-based infant formulas at levels of 100 mg/100 g (GRN 465, 2014) and in other cow's milk-based beverages which may include toddler milk formula at levels of 100 mg/100 g (GRN 464, 2014)

Currently, there are two pending GRAS Notices for a bovine-derived milk whey protein isolate containing 20-75% of the total protein as lactoferrin (Fractionated whey protein isolate containing cow's milk derived lactoferrin, lactoperoxidase, and transforming growth factor β2) (GRN 611, 2015; GRN 612, 2015). GRN 612 is specifically for use of the basic whey protein isolate, in term infant formulas and toddler formulas. GRN 612 provides support for the safe use of bLf in formulas for term infants and toddlers within the context of the more complex whey protein isolate. The predicted exposure of infants and toddlers to bLf, from the intended use of the whey protein isolate of GRN 612 is 1.2 to 4.1 mg/kg BW / day, over and above background levels (GRN 612, 2015, p. 30 (pdf))

The presence of lactoferrin, described as a red protein, was first reported in bovine milk in 1939, and it was partially purified in 1960 (Groves, 1960). However, it was not until 1960 that the isolation, and identification, was successful from both bovine milk (Groves, 1960), and human milk (Johanson, 1960). Lactoferrin is a member of the transferrin family of iron binding proteins, which is characterized by the capacity to reversibly bind iron with high affinity (Mead & Tweedie, 1990). Lactoferrins are glycoproteins which are expressed in most mammalian biological fluids (e.g., milk, tears, saliva) and are a major component of the mammalian immune defense system (Baker & Baker, 2012; Legrand et al., 2008; Ward & Conneely, 2004). Typically, lactoferrins have a molecular weight of about 80 kDa with 670-690 amino acid residues, with an interspecies sequence identity of about 70% (Baker et al., 2002), the major differences being in the N-terminal amino acid sequences (Baker & Baker, 2012).

Table 2-1 Previous Lactoferrin GRAS Notifications				
GRN No.	**Substance**	**Date of closure**	**Applicant**	**Purpose / Use**
465	Cow's milk-derived lactoferrin	Feb 18, 2014	Morinaga Milk Industry Co. Ltd	As an ingredient in cow's milk-based term infant formulas at a levels of 100 milligrams (mg) per 100 grams (g) powdered formulas, 26 mg per 100 milliliters (ml) liquid concentrates, and 13 mg /100ml ready-to-feed formula
464	Cow's milk-derived lactoferrin	Feb 18, 2014	Morinaga Milk Industry Co. Ltd	As an ingredient in cow's milk-based food products, including yogurt (100 milligrams (mg) per 100 grams (g)), powdered milk (400 mg/100 g), and ice cream and sherbets (200 mg/100 g), and in chewing gum (30 mg/g)
130	Bovine milk-derived lactoferrin	Aug 21, 2003	aLF Ventures	Antimicrobial spray for meat carcasses
77	Milk-derived lactoferrin	Aug 14, 2001	DMV International	Sports & functional foods at 100mg / serve
67	Milk-derived lactoferrin	Oct 23, 2001	Farmland National Packaging Co.	Use as antimicrobial in meat packing

The structure of lactoferrin is similar to that of transferrin and ovotransferrin, and is characterized by the presence of two homologous lobes (N and C), each capable of binding an iron molecule together with a synergistically bound carbonate ion (Baker & Baker, 2012; Lien et al., 2004). The conformational state of lactoferrin is highly dependent on its metal ion status. In the metal bound state (holo-lactoferrin) it has a closed highly stable and relatively rigid form, in contrast to the metal-free state (apo-lactoferrin) where the lobes are open (Anderson, Baker, Norris, Rumball, & Baker, 1990; Moore, Anderson, Groom, Haridas, & Baker, 1997). Apo-lactoferrin typically has less than 5% iron saturation, in contrast to the iron saturated from (hololactoferrin) with an

iron saturation approximating 100%, and the natural form of bLf (15-20% iron saturation) (Bokkhim, Bansal, Grondahl, & Bhandari, 2013).

Human lactoferrin (hLf) and bLf exhibit a high degree of similarity both structurally and functionally, whilst differing in some properties as summarized in Table 2-2 (adapted from Latorre, Berlutti, Valenti, Gessani, & Puddu, 2012).

Table 2-2. Similarities and differences between human and bovine lactoferrin

Lactoferrin properties	Human versus bovine	References
Nucleic acid sequence homology	77%*	Mead and Tweedie (1990); Metz-Boutigue et al. (1984); Pierce et al. (1991)
Amino acid sequence homology	69%*	Pierce et al. (1991)
Secondary structure	100%*	Crichton (1990)
Disulfide bonding	100%*	Crichton (1990)
Lobe orientation	Different in relative orientation	Baker et al. (1994)
Glycosylation sites	Lower – hLf has 3 sites compared to 5 for bLf	Haridas, Anderson, and Baker (1995); (Moore et al., 1997)
N-acetyllactosamine glycans	Different – glycan present are species specific	Spik, Coddeville, and Montreuil (1988)
Iron content	Iron content of hLf is approximately 4-fold lower than bLF. Native bLf is typically 15-20% iron	Wang, Chan, and Kloer (1984) (Bokkhim et al., 2013)
Thermoresistance	Higher	Sanchez et al. (1994); Stanciuc et al. (2013); Paulsson, Svensson, Kishore, and Naidu (1993)
Proteolysis resistance	hLf more resistant to proteolysis than bLf possibly due to conformation	Brines and Brock (1983); van Veen, Geerts, van Berkel, and Nuijens (2004)
Dendritic cell capture through C-type lectin receptors (DC-SIGN binding)	Lower – bLf is a more efficient inhibitor of transmission than hLf	Groot et al. (2005)
Nuclear factor (NF)-κB activation	Difference in glycans results in different levels of activation	Ando et al. (2010)
Lipopolysaccharide (LPS) binding	100%*	Elass-Rochard et al. (1995)
Poring binding	100%*	Kishore et al. (1991)

* Percent similarity

Although differences in some structural and biochemical properties exist between hLf and bLf, there is a relatively high level of homology, and importantly their biological activities, as assesses *in vitro* or in animal models is comparable (Steijns & van Hooijdonk, 2000).

2.2 PHYSICAL AND CHEMICAL COMPOSITION OF BLF

The Chemical Abstracts Service (CAS) Registry Number for bovine lactoferrin is CAS Reg. No.146897-68-9. Bovine lactoferrin is a 689 amino acid glycoprotein, with 5 potential glycosylation sites (Mead & Tweedie, 1990; Pierce et al., 1991; van Veen et al., 2004), the mature bLf protein associated with a 19 amino acid signal peptide (Lonnerdal & Suzuki, 2013). It contains N-glycosidically-linked glycans possessing N-acetylneuraminic acid, galactose, mannose, fucose, N-acetylglucosamine, and N-acetylgalactosamine (Coddeville et al., 1992). More recently, van Leeuwen, Schoemaker, Timmer, Kamerling, and Dijkhuizen (2012) identified and quantified 42 different N-glycan structures in bLf. The generally accepted physical and chemical properties of bLf are outlined in Table 2-3. Currently there is no monograph for bovine lactoferrin in the Food Chemicals Codex (FCC); however, it is noted that the FCC has listed lactoferrin in the List of Priority New Food Ingredients Monographs (Updated 27-Apr-2015) (Appendix 4, pg. A2: 2) (www.usp.org/food-ingredients/development-process/priority-new-food-ingredient-monographs). A schematic of the 3-dimensional structure of bLf is shown in Figure 2-1.

The heat stability of bLf is a function of iron binding status and pH. Holo-lactoferrin is more resistant to heat induced changes than apo-lactoferrin, with the ability of both forms to bind a range of bacterial species not affected by pasteurization (72°C for 15 seconds) conditions (Paulsson et al., 1993). Bokkhim, Tran, Bansal, Grondahl, and Bhandari (2014) established that mono- and di-saturated bLf display similar thermal stability and tertiary structure, and that the increased thermal stability can be attributed to the binding of the first iron ion of bLf. Apo-lactoferrin is heat stable at pH 4, resisting heating at 90°C for 5 minutes without any significant loss of iron-binding capacity, antigenic activity, or antibacterial activity (Abe et al., 1991). Sanchez et al. (1994) showed first-order reaction kinetics for denaturation of bLf between 72°C and 85°C , and concluded that the standard pasteurization regimes used in the dairy industry had practically no effect on lactoferrin structure (Steijns & van Hooijdonk, 2000). More recently, the rapid heating conditions typical of pasteurization were shown to minimize conformation changes even in slightly alkaline conditions (pH 7.5) (Schwarcz, Carnelocce, Silva,

Oliveira, & Goncalves, 2008). This work addresses concerns regarding the potential for heat-induced conformational changes (Stanciuc et al., 2013), and subsequent loss of bioactivity, that may occur during pasteurization and spray-drying of lactoferrin. Spray-dried bLf is known to retain its activity (Lönnerdal, Jiang, & Du, 2011).

Table 2-3. Physical and Chemical Properties of Bovine Lactoferrin

Property		Value	Reference
Molecular Mass (Da)			
	Sedimentation co-efficient (aqueous)	77,100 ± 1,500	Castellino, Fish, and Mann (1970)
	SDS-PAGE	76,400 ± 2,400	Castellino et al. (1970)
	Iron Titration	78,000	Aisen and Leibman (1972)
Isoelectric Point (pH)			
	Chromatofocusing	8.2-8.9	Shimazaki et al. (1993)
	Isoelectric focusing	9.5-10.0	Yoshida and Xiuyun (1991)
Absorption Spectra			Aisen and Leibman (1972)
	Apo-form at 280 nm	12.7	
	Holo-form at 470 nm	0.400	
Protease sensitivity		Relatively low	Brines and Brock (1983)
Iron-binding			Aisen and Leibman (1972)
	Equilibrium dialysis (K1 x 10^{-4})	3.73	
Thermal Denaturation			Paulsson et al. (1993)
	Apo-Lf denaturation (T_{max}: °C)	71 ± 0.3 and 90 ± 0.3	
	Apo-Lf enthalpy (ΔH_{cal}: J/g)	12 ± 0.4 and 2 ± 0.5	
	Holo-Lf denaturation (T_{max}: °C)	65 ± 0.3 and 93 ± 0.3	
	Holo-Lf enthalpy (ΔH_{cal}: J/g)	2 ± 1 and 37 ± 1	

For the purposes of bovine lactoferrin use as a food ingredient, the European Commission has defined bovine lactoferrin as:

"*Bovine lactoferrin (bLF) is a protein that occurs naturally in cow's milk. It is an iron-binding glycoprotein of approximately 77kDa and consists of a single polypeptide chain of 689 amino acids.*

bLF is isolated from skimmed milk or cheese whey via ion exchange and subsequent ultra-filtration steps. Finally it is dried by freeze drying or spraying and the large particles are sieved out" (European Commission, 2012a, 2012b, 2015).

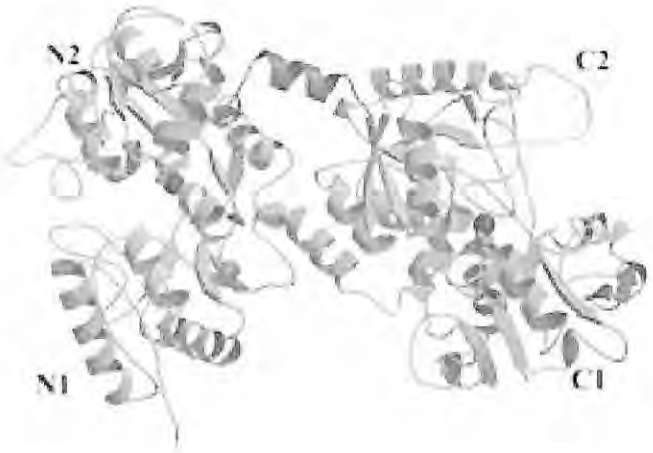

Figure 2-1. Schematic structure of bLf

(Baker, Baker, & Kidd, 2002)

2.3 BIOACTIVITY OF LACTOFERRIN

In infants and young children, lactoferrin is known to exert immunoregulatory, antibacterial, and antiviral activity, and is involved in iron uptake (Lönnerdal, 2016), together with the potential to improve early neurodevelopment and cognition (Wang, 2016). The range of biological activities that lactoferrin consumed in the diet may provide is related to its ability to resist proteolysis. The activity of lactoferrin can be divided into local effects in the gut lumen, and systemic effects mediated by lactoferrin receptors (LFR) present in the apical membrane of the small intestine (Lönnerdal et al., 2011). Lactoferrin (including bovine lactoferrin) is able to bind to intestinal LFR's and be internalized by a clathrin-mediated endocytic mechanism (Liao, Jiang, & Lönnerdal, 2012; Lönnerdal, 2014; Suzuki, Lopez, & Lönnerdal, 2005). Once internalized lactoferrin will localize to the nucleus, and is able to act as a transcription factor (Liao et al., 2012), and influence the expression of cytokines and growth factors (Lönnerdal, 2014). This internalization may also enable lactoferrin to influence iron uptake (Lönnerdal et al., 2011). Non-LFR mediated activity of lactoferrin is related to its structure, and in particular its high affinity for iron (Brock, 2012). Lactoferrin actively sequesters iron, resulting in a bacteriostatic effect by withholding iron form iron-requiring bacteria by forming localized iron-deficient regions (Vogel, 2012). The cationic N-terminal region (Figure 2-1) may act directly on bacteria, also resulting in antibacterial effects (Brock, 2012; Elass-Rochard et al., 1995; Vogel, 2012)

The N-terminal and C-terminal lobes of lactoferrin (Figure 2-1) of lactoferrin are unevenly glycosylated, the C-lobe typically containing more N-linked glycosylation sites

(Albar, Almehdar, Uversky, & Redwan, 2014). The glycosylation state of lactoferrin can modify the structural conformation of the protein, its susceptibility to proteolysis and consequently its biological activity (Le Parc et al., 2014; van Veen et al., 2004). The glycoprofile of lactoferrin shows a degree of inter-species homology (Table 2-2), however known inter-species variation occurs and may be responsible for potential differences in bioactivity of lactoferrin between species (Le Parc et al., 2014). In addition the glycosylation of lactoferrin changes during the course of lactation and is tightly regulated by gene expression (Barboza et al., 2012). Some of the immunomodulatory properties of lactoferrin are related to its glycoprofile (Almond et al., 2013), and importantly the glycosylation may respond to the changing bacterial population in the neonatal gut, importantly providing protection from pathogenic bacteria (Barboza et al., 2012).

The bioactivity of lactoferrin can be affected by several factors. Lactoferrins high affinity to the negatively charged lipopolysaccharide (LPS) (bacterial endotoxin) means the presence of LPS can block the ability of lactoferrin to bind to both LFR receptors, and thus prevent cell-mediated bioactivity (Lönnerdal, 2014) or reduce its bactericidal effectiveness (Elass-Rochard et al., 1995). The presence of large amounts of LPS in early commercial preparations of bLf may have contributed to the limited and mixed findings of early bLf research in infants (Lönnerdal, 2011). The degree of iron saturation also affects lactoferrins bacteriostatic ability (Lönnerdal et al., 2011), and potential effects on intestinal cells (Oguchi, Walker, & Sanderson, 1995).

Peptides from lactoferrin (lactoferricin, lactoferrampin) are known to exhibit strong antibacterial activity both *in vitro* and *in vivo* (Vogel, 2012), and although there is little evidence to support their the formation of these peptides during normal digestion (Brock, 2012; Lönnerdal, 2014)

2.4 MANUFACTURING PROCESS FOR BLF

2.4.1 General Description of the Manufacturing Process for bLf

The Synlait manufacturing process for bLf is outlined in Figure 2.2. The raw milk is separated, the skim milk stream providing the feedstock used for the chromatographic separation of the bLf. The skim milk (unpasteurized) is cooled to below 8°C to prevent microbial growth and then passed through a clarifier to remove any particulate matter,

prior to being pre-filtered through a 1 μm filter to remove any finer insoluble material, reduce the microbial load, remove fat and fat-soluble compounds.

The skim milk filtrate is then passed over an ion exchange column containing Sepharose Big Beads (GE Healthcare). The filtrate bound to the column is thoroughly rinsed with demineralized water, and then washed with a dilute sodium chloride (NaCl) solution to remove potential contaminants that are weakly bound to the column. The bound bLf is then eluted with a more concentrated NaCl solution (approximately 10% w/v) and desalted using ultrafiltration.

The pH of the bLf ultrafiltrate solution is adjusted to, and maintained at below, pH 6.5; the solution is then pasteurized at 73.5°C for 18 seconds. These conditions exceed the pasteurization requirements for both NZ as defined in DCP3 (NZFSA, 2010) and the heat treatment conditions described for "Grade A' Pasteurized Milk (USFDA Department of Health and Human Services, 2015). The pasteurization ensures the final bLf product is produced in compliance with 21 CFR§1240.61 Mandatory pasteurization for all milk and milk products in final package form intended for direct human consumption.

The pasteurized bLf solution is further concentrated with a microfiltration unit (1.2μm) prior to evaporation and spray drying. Spray drying provides a controlled powder particle size, and a less hygroscopic finished material compared to freeze-dried bLf. Spray-drying is the drying technology used in the manufacture of bLf that is the subject of a previous GRAS Notice (GRN 67, 2001, p. 13 (pdf)). The typical particle size distribution of bLf from this process is provided in Appendix 3 (pg. A3: 9). The dried bLf powder is hygienically packed into food grade packaging and sealed to protect it from light, air and moisture. Each batch is tested to ensure it meets the specifications (Table 2-5) set. The bLf content is determined using a high performance liquid chromatography (HPLC) method, details of which are presented in Appendix 3 (pg. A3: 2).

Critical (quality) control points (CCP) are monitored routinely as a part of the third party audited RMP Hazard Analysis Critical Control Point (HACCP) process. Details of the raw materials and processing aids used in the manufacture of bLf are presented in Table 2-4.

The manufacturing process used by Synlait Milk Ltd is similar to that used by Morinaga Milk Industry Co. Ltd (GRN 464, 2014, p. 27 (pdf); GRN 465, 2014, p. 26 (pdf)), the key difference being the technology used to dry the concentrated bLf solution. Synlait utilizes spray-drying technology, whereas Morinaga use freeze-drying followed by pulverization to produce the powder. Spray-drying technology is also used for the

manufacture of bLf in Europe by Friesland Campina (formerly DMV International) (EFSA Panel on Dietetic Products Nutrition and Allergies (NDA), 2012).

2.4.2 Materials and Processing Aids

The primary raw material used in the production of bLf is raw milk. All milk is of New Zealand origin. It is sourced from registered and accredited suppliers of fresh cow's milk, collected from the farms by Synlait Milk Ltd and processed under an approved "Risk Management Programme" (RMP) certified and approved by the New Zealand (NZ) Government agency, the Ministry for Primary Industries (MPI). A copy of the current RMP certificate is included in Appendix 2 (pg. A2: 2). An RMP is a program designed to identify, control, manage, eliminate or minimize hazards and other risks during the processing of animal materials and products. Raw milk supply in NZ is governed by the Animal Products Act (1999), and the Animal Products (Raw Milk Product Specifications) Notice 2009. The Synlait Raw Skim Milk Specification is presented in Appendix 1 (pg. A1: 16), and is fully compliant with the requirements of the Animal Products (Raw Milk Product Specifications) Notice 2009.

Synlait Milk Ltd is a registered Dairy Processor approved by MPI with the designated Unique Location Identifier – manufacturing 540. It is approved for the manufacture of a range of dairy products. Synlait Milk Ltd is a USFDA registered facility (USDFA Registration No. 15930127872), a copy of the current certificate is presented in Appendix 2 (pg. A2: 4). Synlait has an operational and registered HACCP plan (Appendix 2, pg. A2: 3).

The ion exchange column material, Sepharose Big Beads, is approved for use as a food contact substance (USFDA, 2004). Sepharose Big Beads are approved for food use as an ion exchange resin, and also for repeated use in extracting individual proteins or substances present in low concentrations from aqueous food materials such as milk, whey, fruit juice, beer and wine. The approved process conditions include pH 3-14 and temperatures of 5-60°C, which covers the operating range for the extraction of bLf from skim milk. The manufacturer's specifications and safety data sheet for Sepharose Big Beads are presented in Appendix 1 (pg. A1: 18). Following separation of the bLf by ion exchange, the bLf-containing solution is concentrated by ultrafiltration. Details of the FDA and USDA compliant semi-permeable polyethersulfone (PES) ultrafiltration membrane are provided in Appendix 1 (pg. A1: 7). The materials and membranes used for separation, concentration and packaging of the Synlait bLf are safe and suitable and used in accordance with regulations for food contact materials as stated in 21

CFR§177.1520 1.1 (olefin polymers), 21 CFR§177.2800 (textile and textile fibers), 21 CFR§178.3400 (emulsifiers and / or surface-active agents) (Appendix 1, pg. A1: 6), 21 CFR§177.2550 (reverse-osmosis membranes), and 21 CFR§177.2910 (ultra-filtration membranes).

The processing aids used in the production of bLf, are all designated food grade (Table 2-4). The potable water undergoes reverse osmosis treatment prior to use in this process. Given the minor differences in process design, these are essentially the same as those used in the manufacture of lactoferrin by Morinaga (GRN 464, 2014, p. 27 (pdf); GRN 465, 2014, p. 26 (pdf)). A table comparing the materials and processing aids between those used by Morinaga (as cited in GRN 465 (2014, p. 27 (pdf)) is given in Appendix 1 (pg. A1: 17). Specifications for the salt (sodium chloride) and Synlait in-house water supply are presented in Appendix 1 (pg. A1:9). Synlait draws its water from local subterranean aquifers, treats with chlorine to a residual of <5ppm, and filters prior to use in milk or product contact processing requirements. The testing schedule to ensure that the water quality meets the NZ Drinking Water Standards (Ministry of Health, 2008) is outlined in the water quality specification (Appendix 3, pg. A3: 33).

Figure 2-2. Synlait Production Process of Milk-derived Lactoferrin

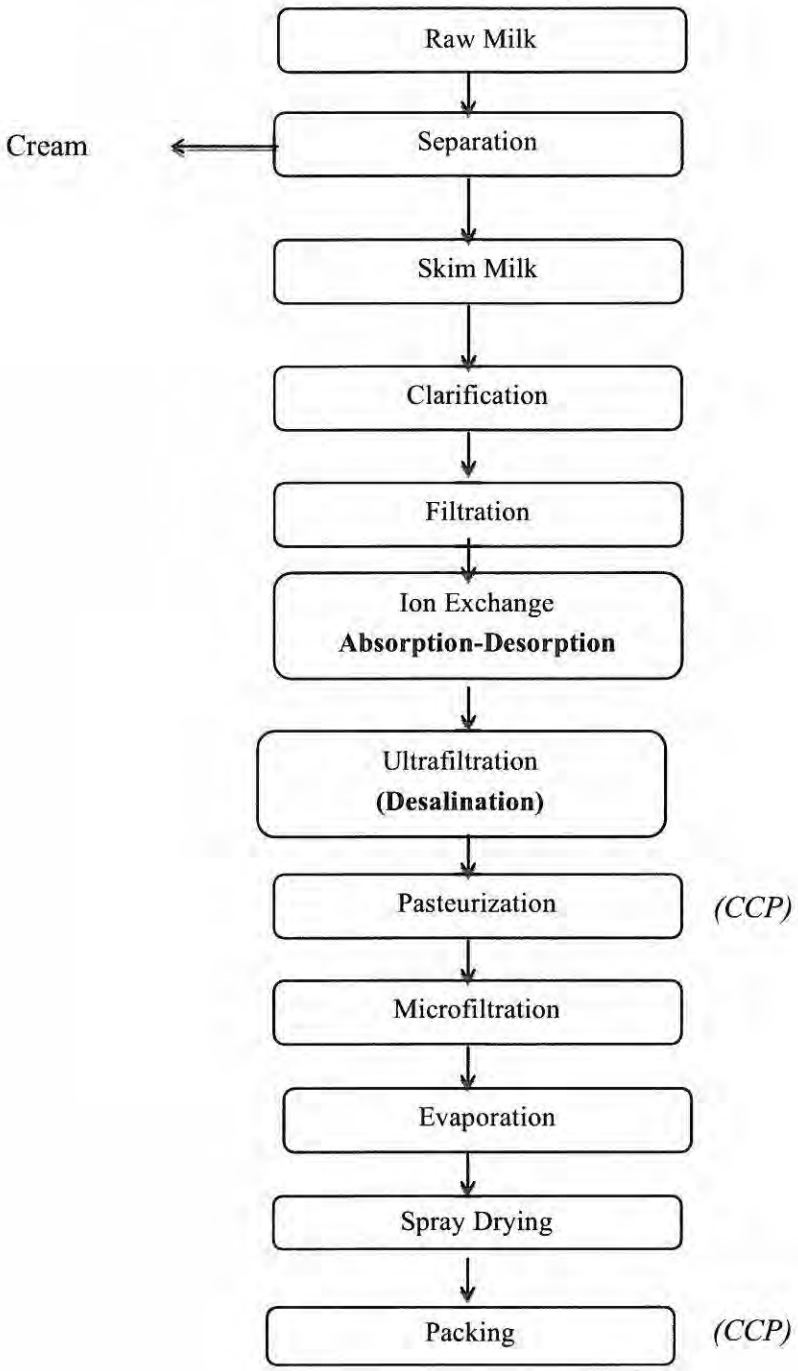

Table 2-4. Raw Materials and Processing Aids Used in the Production of Synlait Milk-derived Bovine Lactoferrin

Material	CAS Number	Purity (%)	Function	Source	Regulatory Approvals
Raw Materials					
Raw milk	N/A	100%	Raw material	Synlait Raw Milk Supply	Conforms to NZ Animal Products Act, and Animal Products (Raw Milk Product Specifications) Notice 2009
Skim milk (unpasteurized)	N/A	100%	Raw material	In process milk	N/A
Processing Aids					
Demineralized water	N/A	100%	Diluent for salt solution	On site RO water	Conforms to NZ Drinking Water Standards
Sodium Chloride	7647-14-5	min. 99.67%	Salt squeeze for ion exchange resin	Dominion Salt, NZ	Meets FCC requirements
Sepharose Big Beads			Ion exchange resin	GE Healthcare	Food Contact Substance Notification 000433
Ultrafiltration Membrane	N/A	N/A.	Protein concentration and demineralization	Koch Membranes	USDA 3-A Standards and 21 CFR
Microfiltration Membrane	N/A	N/A	Microbial load, bLf concentration and particulate reduction	Tami	USDA 3-A Standards

Abbreviations:

CAS Chemical Abstract Service
FCC Food Chemical Codex
N/A Not applicable
NZ New Zealand
RO Reverse Osmosis

2.5 FINISHED PRODUCT SPECIFICATIONS

Specifications (Table 2-5), batch data for 5 batches manufactured between February 2015 and February 2016 (Table 2-7) and stability data (Table 2-10), are presented below for the Synlait manufactured bLf. To demonstrate conformance with the food-grade specifications, Synlait analyzed several batches of bLf. Analytical results from five lots (Table 2-7) suggest that Synlait's bLf is consistently manufactured to meet the standard specifications. The specification parameters comprise physical appearance, purity, total bLf levels, moisture, etc., as well as limits for potential chemical and microbiological impurities, and contaminants. A comparison of Synlait's bLf specifications to those of bLf that was the subject of GRAS notified substances reviewed by the FDA without any questions [including GRN 465 (Morinaga, 2014) and GRN 464 (Morinaga, 2014)] demonstrate that the bLf that is the subject of this GRAS Notice is substantially equivalent to the bLf that was the subject of those GRNs.

The presence of endotoxins (LPS) in commercial bLf has been identified as a potential inhibition factor for bioactivity (as discussed in 2.3). Synlait regularly monitors the endotoxin levels in the bLf finished product, and works towards continuous improvement in endotoxin levels (Table 2-9). Typical results for endotoxin levels are presented in **Error! Reference source not found.** for batches manufactured between May 2014 and June 2015. Endotoxin measurement is completed by an independent test facility, Callaghan Innovation, a New Zealand Government Research Institute that includes accredited test analytical facilities (www.callaghaninnovation.govt.nz/). Endotoxin levels are measured using the FDA approved (www.fda.gov/ICECI/Inspections/InspectionGuides/InspectionTechnicalGuides/ucm072918.htm) *Limulus* Amebocyte Lysate (LAL) method (U.S. Pharmacopeial Convention, 2016). The average endotoxin level from Table 2-9, is 4.6 EU /mg BLf (average CV <5%). This average is halved to 2.3 EU/mg if the 2 results from the single batch of 1410001141 are omitted. Using the overall average value of 4.6 EU/mg, this equates to a potential contribution of endotoxin in infant formula of 4.6 EU per gram of infant formula powder. In an survey of 75 infant formula from 7 countries (31 formula brands), Townsend, Caubilla Barron, Loc-Carrillo, and Forsythe (2007) found the endotoxin levels in formula ranged from 40 to 5.5 x 10^4 EU per gram of formula powder using the LAL assay. The lower values of that range are consistent with the endotoxin levels in reconstituted infant formula (3.29 to 5.01 EU/mL) recorded by Lönnerdal et al. (2011). Ando et al. (2010) reported the endotoxin level of commercially available human lactoferrin as ranging between 15-26 EU/mg of protein.

In a patented process to produce "endotoxin free" bovine lactoferrin for pharmaceutical-type applications, Thomson, Ward, and Wrobel (2013) described "endotoxin free" bLf as "lactoferrin compositions comprising less than about 20 EU/mg of protein, more preferable less than about 10 EU/mg, and even more preferably less than about 1 EU/mg". Furthermore they identified that bLf derived from sweet whey typically contained endotoxin levels of at least about 250EU /mg (with reports of up to 1250 EU/mg), compared to bLf derived from milk typically being at least about 20 EU/mg (Thomson et al., 2013). In a study evaluating the effects of lactoferrin on intestinal cells, Nguyen, Li, Sangild, Bering, and Chatterton (2014) stated they used a "low" endotoxin bLf (1.6 EU/mg protein determined using the LAL assay) sourced from Morinaga. Therefore, based on this information the levels of endotoxin typically present in Synlait bLf can be considered low.

In conclusion, Synlait's bLf is based on its chemical, physical and physiochemical properties equivalent to the bLf manufactured by Morinaga, presented as GRAS Notices GRN 464 and GRN 465 (GRN 464, 2014, pp. 29-30 (pdf); GRN 465, 2014, pp. 28-29 (pdf)). For comparative purposes, Table 2-8 presents the batch data for Synlait bLf as compared to the Morinaga test results for 6 lots presented as a part of the GRAS Notices GRN 464 (p. 31-32 (pdf)) and GRN 465 (p. 30-31 (pdf)), and batch test data for Friesland Campina bLf extracted from the EFSA Panel on Dietetic Products Nutrition and Allergies (NDA) (2012, pp. 9 - 10). In conclusion, these data further support the substantial equivalence of Synlait's bLf to that subject of GRN 464 and GRN 465. Both the Synlait bLf and the Friesland Campina bLf are manufactured using spray-drying technology. Notably, the European Union does not differentiate bLf on the basis of drying technology, stating both freeze-drying and spray-drying are permitted technologies for the manufacture of bLf (European Commission, 2015):

'Bovine lactoferrin (bLF) is a protein that occurs naturally in cow's milk. It is an iron-binding glycoprotein of approximately 77 kDa and consists of a single polypeptide chain of 689 amino acids. bLF is isolated from skimmed milk or cheese whey via ion exchange and subsequent ultra-filtration steps. Finally it is dried by freeze drying or spraying and the large particles are sieved out.'

The complete EC specification is presented in Appendix 4 (pg. A4: 4), and includes the food categories for which use of bLf is approved and the associated maximum permitted usage levels of uses of bLf within the European Union. As a novel food in the EU, bLf was in the first instance approved specifically for Morinaga and Friesland Campina

(European Commission, 2012a, 2012b, 2015). The EU regulatory system permits the use of "substantial equivalence" for foods and food ingredients already approved. In 2013 the Food Safety Authority of Ireland (FSAI) determined the Glanbia bLf product "Bioferrin®) was substantially equivalent to the Morinaga bLf authorized in 2012 (Appendix 4, pg. A4: 62) (https://www.fsai.ie/uploadedFiles/Science_and_Health/Novel_Foods/Notifications/2013%20Lactoferrin.pdf). In 2016 FSAI also concluded bLf (Vitalarmor® LACTOFERRIN) manufactured by Armor Protéines of France) was substantially equivalent to bLf authorized to Morinaga Milk Industry Co. Ltd. and Friesland Campina through Implementing Decisions 2012/725/EU and 2012/727/EU, respectively, and will be used at the levels set out in the decisions of the EU Commission (Appendix 4, pg. A4: 66) (https://www.**fsai**.ie/uploadedFiles/Science.../2016%20Armor%20**Lactoferrin**.pdf). A comparison of the specifications for bLf produced by Synlait, Morinaga, Friesland Campina, together with the specifications listed by the EC and the proposed specification by China (Table 2-6), show that all of the bLf produced by similar, but slightly different, methods are essentially equivalent. Thus, information generated with one bLf is applicable to the bLf produced by another, similar, method.

Figure 2-3: Synlait's Manufacturing Process for the Addition of bLf to Term Infant and Toddler Formulas

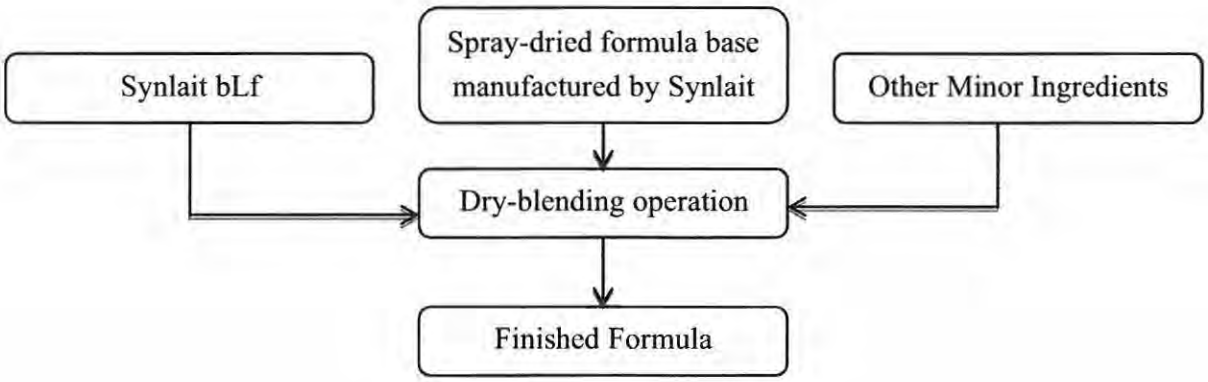

Table 2-5. Manufacturing Specifications for Synlait Milk-derived Bovine Lactoferrin		
Parameter	**Specification**	**Method**
Appearance	Pink to tan colored, free-flowing powder	Visual inspection
Foreign Matter	Absent / 25g	AS2300.4.5:1994
pH (2% solution)	5.2 – 7.2	BS770:1986, ISO 7238 / IDF 104:2004, IDF 115A:1989, APHA (17th Edition) Ch. 15
Total Protein	≥95.0 %m/m	ISO 8968-1 / IDF 20-1:2001, AOAC 991.2
Lactoferrin (Purity)	≥95.0 % of protein	HPLC method (In House Method: TCH-05-0009)
Ash	≤1.3 %m/m	BS 1741:1988 (modified), BS 1743:1968 (modified)
Moisture	≤4.5 %m/m	IDF 26A: 1993
Iron Content	≤200 mg/kg	Acid Digest, ICP OES
Iron Saturation	≤20%	In house method (TCH-05-0011)
Heavy metals	<10 mg/kg	Acid Digest, ICP MS
Lead (Pb)	<0.15 mg/kg	Wet oxidation ICP MS
Cadmium (Cd)	<0.1 mg/kg	Wet oxidation ICP MS
Mercury (Hg)	<0.1 mg/kg	Wet oxidation ICP MS
Arsenic (As)	<0.02 mg/kg	Wet oxidation ICP MS (Detectable Limit)
Solubility		
Transmittance (2% solution, 600nm)	80-100%transmittance Transparent	In house method (2% solution, 20°C) TCH-05-0010
Microbiological Tests		
Aerobic Plate Count	<1000cfu/g	ISO 4833
Coliforms	Not detected/g	ISO 11866-1/IDF 170-1
E. coli	Not detected/g	ISO 11866 – 1:2005 (E)/IDF 170-1 :200

Table 2-5. Manufacturing Specifications for Synlait Milk-derived Bovine Lactoferrin

Parameter	Specification	Method
Coagulase positive *Staphylococcus aureus*	Not detected/g	ISO 6888-3:2003
Yeasts and Molds	<10 cfu/g	ISO 6611/IDF 94:2004
Salmonella	Not detected /250g	ISO 6579
Enterobacteriaceae	Not detected/g	ISO 21528-1:2004
Chronobacter sakazakii	Not detected /300g	ISO/TS 22964 / IDF/RM 210:2006 (see Appendix 3, pg. A3: 20)
Aluminum	<4.8 mg/kg	Wet oxidation ICP-MS
Nitrates	≤50 mg/kg	NZJDST 15, 83-90, 1980, ISO 14673-2, IDF 189-2, AOAC 968.07 (mod)
Nitrites	≤2 mg/kg	NZJDST 15, 83-90, 1980, ISO 14673-2, IDF 189-2, AOAC 968.07 (modified)
Melamine	<0.1 mg/kg	LC-MS/MS (Detectable limit)
Aflatoxin M1	<0.5 µg/kg	AOAC 971.22 (1998) (modified)

Abbreviations:

AOAC Association of Official Analytical Chemists
APHA American Public Health Association
BS British Standards
ICP MS Inductively Coupled Plasma Mass Spectrometry
ICP OES Inductively Coupled optical Emission Spectrometry
HPLC High Performance
Liquid Chromatography
IDF International Dairy Federation
ISO International Organization for Standardization
TCH Technical Manual

Table 2-6. Comparison of Regulatory and Company Specifications for bLf					
Parameter	**European Union** (European Commission, 2012a, 2012b)	**Peoples Republic of China (*Draft*)**	**Synlait Milk Ltd.**	**Morinaga** (GRN 465, 2014, pp. 28-29 (pdf))	**Friesland Campina** (EFSA Panel on Dietetic Products Nutrition and Allergies (NDA), 2012, p. 8)
Physical and Chemical Parameters					
Description	Virtually odorless, light pinkish powder	Pale pink to reddish brown powder	Pink to tan powder	Pink, odorless powder	
Protein of which bovine lactoferrin of which other proteins	> 93.0% > 95.0% < 5.0%	≥ 93.0% ≥ 90.0%	≥ 95.0% ≥ 95.0%	≥ 94.5% ≥ 96.0%	≥ 93.0% ≥ 95.0%
Moisture (loss on drying)	<4.5%	≤4.5%	≤ 4.5%	<4.2%	≤ 4.5%
Ash	< 1.5%	≤1.5%	≤ 1.3%	≤ 1.3%	≤ 1.0%
Arsenic	<2 mg/kg	≤ 1 mg/kg	≤ 0.02 mg/kg	<1 mg/kg	<1 mg/kg (total heavy metals)
Iron Iron Saturation	< 350 mg/kg	≤ 350 mg/kg	≤ 200 mg/kg ≤ 20%	< 350 mg/kg < 25%	100 – 160 mg/kg
pH (2% solution, 20°C)	5.2 to 7.2	5.2 to 7.2	5.2 to 7.2	5.2 to 7.2	5.5 – 6.5
Solubility (2% solution, 20°C)	Complete	Complete, transparent	Complete, transparent	≥80% transmission, Complete	80 – 100% transmission, Complete

Table 2-6. Comparison of Regulatory and Company Specifications for bLf

Parameter	**European Union** (European Commission, 2012a, 2012b)	**Peoples Republic of China (*Draft*)**	**Synlait Milk Ltd.**	**Morinaga** (GRN 465, 2014, pp. 28-29 (pdf))	**Friesland Campina** (EFSA Panel on Dietetic Products Nutrition and Allergies (NDA), 2012, p. 8)
Microbiological					
Total / Standard Plate Count		≤ 1000 cfu/g	≤ 1000 cfu/g		< 1000 cfu/g
Yeasts & Molds		≤ 10 cfu/g	≤ 10 cfu/g	≤ 10 cfu/g	≤ 10 cfu/g
Coliforms		< 3.0 MPN/g	ND / g	Negative / 0.1 g	
Salmonella		ND / 25 g	ND / 250 g	Negative / 25 g	Negative in 10 g
Staphylococcus aureus (coagulase positive)		ND / 25 g	ND / g	Negative / g	Negative in 1 g

cfu = colony forming units
MPN = mean probable number
ND = Not Detected

Table 2-7. Batch Data of Synlait Milk-derived Bovine Lactoferrin

Specification Parameter	**Limit**	**Batch Nos**				
		LFN1510001209	LFN1510001130	LFN1510000679	LFN1510000948	LFN1610000308
Appearance	Pink to tan, free-flowing	Typical	Typical	Typical	Typical	Typical
Foreign Matter (in 25g)	Absent /	Absent	Absent	Absent	Absent	Absent
pH (2% solution)	5.2 – 7.2	6.10	5.80	5.90	5.92	5.79
Total Protein (%m/m)*	≥94.0	97.1	96.9	96.6	97.0	96.9
Lactoferrin (% protein)	≥90.0	95.3	97.4	96.8	96.5	96.3
Ash (%m/m)	≤1.3	<0.1	0.2	0.2	0.1	0.4
Moisture (%m/m)	≤4.5	3.6	3.5	4.2	4.1	4.1
Iron Content (mg/kg)	≤200	110	110	110	110	110
Iron Saturation (%)	≤20	11.0	11.0	11.0	11.0	11.0
Minerals						
Sodium (mg/100g)		63	64	84	51	30
Potassium (mg/100g)		<0.91	<0.91	<0.91	<0.91	<0.91
Magnesium (mg/100g)		<0.84	<0.14	<0.14	<0.14	<0.14
Phosphorus (mg/100g)		2.6	2.5	4.5	2.7	0.84
Calcium (mg/100g)		1.2	0.75	1.7	1.5	2.8
Chloride (%m/m)		0.845	0.838	0.824	0.788	1.0
Copper (µg/100g)		<11	22	20	<11	14
Zinc (mg/100g)		0.42	0.39	0.72	0.53	0.57
Manganese (µg/100g)		<0.14	<7	8.1	<7	<7
Heavy metals						

Table 2-7. Batch Data of Synlait Milk-derived Bovine Lactoferrin

Specification Parameter	**Limit**	**Batch Nos**				
		LFN1510001209	LFN1510001130	LFN1510000679	LFN1510000948	LFN1610000308
Lead (Pb) (mg/kg)	<0.02	<0.01	<0.01	<0.01	<0.01	<0.01
Cadmium (Cd) (mg/kg)	<0.1	<0.002		<0.002	<0.002	<0.002
Mercury (Hg) (mg/kg)	<0.1	<0.01	<0.01	<0.01	<0.01	<0.01
Arsenic (As) (mg/kg)	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02
Solubility (2% solution, 600 nm)	Transparent	Transparent	Transparent	Transparent	Transparent	Transparent
Transmittance (2% solution, 600 nm) (%)	80-100	96.0	95.8	94.7	97.9	95.2
Microbiological Tests						
Aerobic Plate Count (cfu/g)	<1000	<10	<10	<10	<10	<10
Coliforms (in 1g)	Not detected	Not detected	Not detected	Not detected	Not detected	Not detected
Coagulase positive *Staphylococcus aureus* (in 1g)	Not detected	Not detected	Not detected	Not detected	Not detected	Not detected
Yeasts and Molds (cfu/g)	<10	<1	<1	<10	<10	<10
Salmonella (in 250 g)	Absent	Absent	Absent	Absent	Absent	Absent
Chronobacter sakazakii (in 300g)	Not detected	Not detected	Not detected	Not detected	Not detected	Not detected
Aflatoxin M1	<0.5	<0.025	<0.025	<0.025	<0.025	<0.025

*%m/m = % mass/mass

Table 2-8. Batch Data of Synlait, Morinaga and Friesland Campina Milk-derived Bovine Lactoferrin

Specification Parameter	Synlait bLf Batches					Morinaga bLf Batch Data (GRN 465, 2014, pp. 30-31 (pdf))						Friesland Campina Batch Data (EFSA Panel on Dietetic Products Nutrition and Allergies (NDA), 2012, pp. 9 - 10)					
	LFN1510001209	LFN1510001130	LFN1510000679	LFN1510000948	LFN1610000308	131110	151110	171110	101110	161210	211210	10371335	10388040	10390371	10391587	10393723	10409699
Appearance	Typical	Typical	Typical	Typical	Typical	Conform	Conform	Conform	Conform	Conform	Conform						
Foreign Matter (in 25g)	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent						
pH (2% solution)	6.10	5.80	5.90	5.92	5.79	5.53	5.58	5.75	5.50	5.59	5.20	5.8	5.8	5.8	5.8	5.7	5.8
Total Protein (% m/m)	97.1	96.9	96.6	97.0	96.9							97.4	97.7	97.7	97.8	96.6	97.3
Total Protein (% dry weight)						98.8	99.4	99.3	99.2	98.8	98.7						
Lactoferrin (% protein)	95.3	97.4	96.8	96.5	96.3	97.3	96.8	96.8	97.0	97.7	97.2	>95	>95	>95	>95	>95	>95
Ash (% m/m)	<0.1	0.2	0.2	0.1	0.4							0.20	0.21	0.32	0.25	0.30	0.12
Ash (% dry weight)						0.13	0.17	0.11	0.07	0.09	0.05						
Moisture (% m/m)	3.6	3.5	4.2	4.1	4.1	0.41	0.05	0.35	0.54	0.70	0.43	3.04	3.39	3.25	3.29	3.53	2.95
Iron Content (mg/kg)	110	110	110	110	110	211	217	197	82.0	95.9	95.1						
Iron Saturation (%)	11.0	11.0	11.0	11.0	11.0	15.1	15.5	14.1	5.9	6.9	6.8						
Minerals																	
Sodium (mg/100g)	63	64	84	51	30	38.0	39.9	42.0	33.5	47.1	48.2						
Potassium (mg/100g)	<0.91	<0.91	<0.91	<0.91	<0.91	1.00	5.36	5.30	1.09	1.11	2.70						
Magnesium (mg/100g)	<0.84	<0.14	<0.14	<0.14	<0.14	0.49	0.51	0.55	0.56	0.55	0.53						
Phosphorus (mg/100g)	2.6	2.5	4.5	2.7	0.84	3.25	3.87	3.99	2.95	3.14	3.57						
Calcium (mg/100g)	1.2	0.75	1.7	1.5	2.8	8.14	8.87	9.15	7.81	7.93	7.69						
Chloride (%m/m)	0.845	0.838	0.824	0.788	1.0	0.766	0.816	0.758	0.795	0.764	0.889						
Copper (µg/100g)	<11	22	20	<11	14	280	90	310	ND	ND	ND						
Zinc (mg/100g)	0.42	0.39	0.72	0.53	0.57	0.15	0.64	0.32	0.32	0.29	0.25						

Table 2-8. Batch Data of Synlait, Morinaga and Friesland Campina Milk-derived Bovine Lactoferrin

Specification Parameter	**Synlait bLf Batches**					**Morinaga bLf Batch Data** (GRN 465, 2014, pp. 30-31 (pdf))						**Friesland Campina Batch Data** (EFSA Panel on Dietetic Products Nutrition and Allergies (NDA), 2012, pp. 9 - 10)					
	LFN151 0001209	LFN151 0001130	LFN151 0000679	LFN151 0000948	LFN161 0000308	1311 10	15111 0	17111 0	10111 0	16121 0	211210	10371 335	10388 040	10390 371	10391 587	10393 723	1040969 9
Manganese (µg/100g)	<0.14	<7	8.1	<7	<7	ND	ND	ND	0.01	0.01	0.01						
Heavy metals																	
Lead (Pb) (mg/kg)	<0.01	<0.01	<0.01	<0.01	<0.01	ND	ND	ND	ND	ND	ND						
Cadmium (Cd) (mg/kg)	<0.002		<0.002	<0.002	<0.002	ND	ND	ND	ND	ND	ND						
Mercury (Hg) (mg/kg)	<0.01	<0.01	<0.01	<0.01	<0.01	ND	ND	ND	ND	ND	ND						
Arsenic (As) (mg/kg)	<0.02	<0.02	<0.02	<0.02	<0.02	ND	ND	ND	ND	ND	ND						
Solubility (2% solution, 600 nm)	Transparent	Transparent	Transparent	Transparent	Transparent	100	100	100	100	100	100						
Transmittance (2% solution, 600 nm) (%)	96.0	95.8	94.7	97.9	95.2	91.9	92.0	90.6	81.4	95.2	85.7	91	88	91	90	90	93
Microbiological Tests																	
Aerobic Plate Count (cfu/g)	<10	<10	<10	<10	<10	0	0	0	0	0	0	<1000	<1000	<1000	<1000	<1000	<1000
Coliforms (in 1g)	ND	ND	ND	ND	ND	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.						
Coagulase positive *Staphylococcus*	ND	ND	ND	ND	ND	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.

Table 2-8. Batch Data of Synlait, Morinaga and Friesland Campina Milk-derived Bovine Lactoferrin

Specification Parameter	**Synlait bLf Batches**					**Morinaga bLf Batch Data** (GRN 465, 2014, pp. 30-31 (pdf))						**Friesland Campina Batch Data** (EFSA Panel on Dietetic Products Nutrition and Allergies (NDA), 2012, pp. 9 - 10)					
	LFN151 0001209	LFN151 0001130	LFN151 0000679	LFN151 0000948	LFN161 0000308	1311 10	15111 0	17111 0	10111 0	16121 0	211210	10371 335	10388 040	10390 371	10391 587	10393 723	1040969 9
aureus (in 1g)																	
Yeasts and Molds (cfu/g)	<1	<1	<10	<10	<10	0	0	0	0	0	0	<10	<10	<10	<10	<10	<10
Salmonella (in 25g)	Absent	Absent	Absent	Absent	Absent	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
Enterobacteriaceae (cfu/g)	ND	ND	ND	ND	ND							<10	<10	<10	<10	<10	<10
Chronobacter sakazakii (in 300g)	ND	ND	ND	ND	ND	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.						
Aflatoxin M1	<0.025	<0.025	<0.025	<0.025	<0.025	ND	NT	NT	ND	NT	NT						

*%m/m = % mass/mass
ND= Not Detected
NT = Not Tested

Table 2-9. Endotoxin Levels in Synlait Milk-derived Bovine Lactoferrin

Sample ID	Endotoxin (EU/mg bLf)[1]	Recovery (%)	CV (%)
1410001046	6	107	3.5
1410001141-T1	17	92	2.2
1410001141-T2	13	86	23
1510000949	3.4	86	1.4
1510001129	5.4	147	2
1510001207-T1	1.15	106	0
1510001207-T2	1.42	113	2.7
1510001314-T1	0.5	135	5.2
1510001314-T2	1.08	109	7.7
1510001314-T3	1.18	155	2.5
1510001314-T4	0.74	121	4.6

[1] Endotoxin measured using the *Limulus* amoebocyte assay (LAL) method (Endosafe LAL Test Kit, SC Bio Limited, NZ).

2.6 bLf STABILITY

The stability of Synlait's bLf was evaluated using an in-house accelerated storage protocol, as detailed below. Samples were packed as for commercial purpose, into multilayer oxygen and water vapor resistant 5 kg packs (packaging specification is in Appendix 1, pg. A1: 27), and stored in temperature controlled incubators at 40°±1°C for 36 weeks. The accelerated storage protocol is based on prediction of the kinetics of degradation described by the Arrhenius equation. This is one of the most common models used for shelf-life prediction (Anderson & Scott, 1991; Robertson, 1993). A historically useful generalization based on the Arrhenius equation is that for many common reactions at room temperature, the reaction rate doubles for every 10°C increase in temperature (Kramer, 2009). According to the accelerated storage protocol 1 week at 40°C is approximately equivalent to 1 month at ambient temperature. Hence, 36 weeks at 40°C represents an equivalent ambient storage period of 36 months (3 years). Analysis of the samples at t=0, t=30 and t=36 weeks are presented in Table 2-10. Samples were analyzed according to methods outlined in the specifications (Table 2-5).

Results of the accelerated shelf-life test (Table 2-10 and Table 2-11) show no significant changes in any of the physical, chemical, physicochemical or microbiological parameters over the 36

weeks at 40°C. Based on the accelerated storage protocol, bLf powder manufactured by Synlait, and commercially packed, is stable and has a shelf life of at least 3 years (36 months) at ambient temperatures. Ambient (<25°C, <65% RH) shelf life testing, in the 5 kg commercial packs, has been evaluated at 12 months and is on-going in order to validate the accelerated storage data in real time. At 12 months there were no notable changes to the product or its' microbiological status.

As noted above, Synlait has been manufacturing term infant and toddler formulas containing bLf for more than five (5) years. The production process for the manufacture of the formulas containing bLf is outlined in Figure 2-3. The bLf is added to the formulas using dry-blending technology to enable accurate addition rates, whilst ensuring homogeneity in the finished product. The use of dry-blending technology minimizes any potential losses through reconstitution and additional thermal processes in the manufacture of the formulas. The stability of bLf in powdered formula for up to 3 years is well established (GRN 465, 2014 p.34 (pdf)).

Table 2-10. Accelerated Stability Testing of Synlait Milk-derived Bovine Lactoferrin – Physicochemical

(Lot (b) (6) ; Date of Manufacture 01 May 2014)

Test Parameter	Units	Specification Minimum	Specification Maximum	Week 0	Week 30 (@ 40°C)	Week 36 (@ 40°C)
Lactoferrin	% protein	95		94.6	95.7	95.5
Protein	%m/m	95		97.5	96.2	96.7
Ash	%m/m		1.3	0.2	0.2	0.3
Moisture	%m/m		4.5	3.8	4.3	3.9
Iron	mg/100g		20	11	11	11
Iron Saturation	%		20	11.3	11	11
Fat	%m/m		<1.0	Not tested	<0.1	0.1
Sediment (/25 g)		A		A		
Foreign Matter (/25g)			Absent	Absent	Absent	Absent
pH (2% solution)		5.2	7.2	6.2		6.37
Solubility Transmittance	%	Transparent 80	100	Transparent 91.8	Transparent 94.0	Transparent 93.3
Appearance		Typical		Typical	Typical	Typical
Heavy Metals	mg/kg		<10	<3	<3	<3

Table 2-10. Accelerated Stability Testing of Synlait Milk-derived Bovine Lactoferrin – Physicochemical

(Lot (b) (6) ; Date of Manufacture 01 May 2014)

Arsenic	mg/kg		<0.02	Not detected	<0.02	<0.02
Aluminum	mg/kg		<4.8	<1	<1.0	<1.0
Cadmium	mg/kg		<0.1	<0.002	<0.002	<0.002
Mercury	mg/kg		<0.1	<0.01	<0.01	<0.01
Lead	mg/kg		<0.02	<0.01	<0.01	<0.01
Aflatoxin M1	µg/kg		<0.05	<0.02	<0.025	<0.025
Melamine	ppm		<0.1	<0.05		Not detected

Table 2-11. Accelerated Stability Testing of Synlait Milk-derived Bovine Lactoferrin - Microbiological

(Lot (b) (6) ; Date of Manufacture 01 May 2014)

Microbiological Parameters					
	Unit	**Specification Maximum**	**Week 0**	**Week 30 (@ 40°C)**	**Week 36 (@ 40°C)**
Aerobic Plate Count	cfu/g	1000	<10	<10	<10
Thermophilic Aerobic Spores	cfu/g		<10	<1	<10
Mesophilic Aerobic Spores	cfu/g		<1	<1	<1
Yeasts and Molds	cfu/g	10	<1	<10	<10
Coliforms	/g	Not detected	Not detected	Not detected	Not detected
Escherichia coli	/g	Not detected	Not detected	Not detected	Not detected
Presumptive *Bacillus cereus*	cfu/g	100	<10	<10	<10
Enterobacteriaceae	cfu/g	Not detected	Not detected	Not detected	<1
Coag. Positive *Staph. aureus*	/g	Not detected	Not detected	Not tested	Not detected
Chronobacter sakazakii	/300g	Not detected	Not detected	Not tested	Not detected
Listeria	/125g	Not detected	Not detected	Not tested	Not detected
Salmonella	/250g	Not detected	Not detected	Not tested	Not tested
Salmonella (n=5)	/25g	Not detected	Not detected	Not tested	Not detected

PART 3 DIETARY EXPOSURE

3.1 INTRODUCTION

In the first months of life, infants are exposed exclusively to the components in human milk, infant formula, or a combination of human milk and infant formula. Human milk is regarded as the preferred source of nutrition for infant feeding. The exclusive use of human milk during the first six months of life, and in combination with solid foods from about six months through the first year of life and beyond, as determined by mother and child, is recommended by the American Academy of Pediatrics (AAP) (American Academy of Pediatrics, 2005).

3.2 LACTOFERRIN IN HUMAN MILK

Human milk is a dynamic and complex substance consisting of thousands of constituents such as immune factors, hormones, and live cells in addition to macronutrients, vitamins, and minerals (Picciano, 2001). The lactoferrin content of human milk varies as a function of time *postpartum*, and is associated with functional roles in gut maturation and immune function in infants (Lönnerdal, 2003; Lönnerdal, 2011).

A range of concentrations of lactoferrin in human milk has been reported both as a function of the stage of lactation, and geography (Lien et al., 2004; Mehta & Petrova, 2011; Rai et al., 2014; Trend et al., 2016). Lien et al. (2004) found a range of mean concentrations of between 1.37 g/L in Mexico to 2.12 g/L in China, with an overall mean of 1.83 ± 0.67g/L. The decline in lactoferrin concentration over the duration of lactation was typified by the pattern observed in Canada, falling from a little over 2 g/L at birth through to 1.5 g/L at 1 year (Lien et al., 2004). In a systematic review of the longitudinal changes in lactoferrin concentration in human milk, Rai et al. (2014) found that across the 52 studies from around the world, the unweighted mean of mean (+/-SEM) concentrations of lactoferrin in early milk (<28 days lactation) was 4.91 +/- 0.31 g/L (range of means 0.34-17.94 g/L; median 4.03). For mature milk, the mean of means was 2.10 +/- 0.87 g/L (range of means 0.44-4.4 g/L; median 1.91).

More recently, Trend et al. (2016) reported the lactoferrin concentration in breast milk of mothers who had delivered term infants to decline from approximately 5g/L in colostrum (days 2-5 postpartum) to 3 g/L in mature breast milk (days 26-30 postpartum). Furthermore they identified the concentration of lactoferrin in breast milk varied as a function of gestation (extremely preterm, very preterm, preterm and term deliveries), with a trend for increased

concentrations of lactoferrin in the colostrum (days 2-5 post-partum) and transition milk (days 8-12) in the milk of mothers who delivered extremely and very preterm infants (Trend et al., 2016).

For exclusively breast-fed infants the average mean daily intake of lactoferrin in the USA is estimated to range between 933 mg/day for infants 0 to < 1month, and 3 – 6 months respectively, based on the recommended values for human milk intake rates (U.S. EPA, 2011) and the average values of lactoferrin in human breast milk (Table 3-1).

Table 3-1: Estimated of Intake of Lactoferrin in Breast-fed Infants in the USA

Human Milk	Average Concentration in Source Food (mg/L)	Mean Intake (mg/kg-bw/day)	Mean Intake (mg/day)
Infants Birth to <1 month	1,830[a]	275[b]	933[b]
Infants 1 to <3 months		256[b]	1,263[b]
Infants 3 to <6months		201[b]	1,409[b]
Infants 6 to < 12months		152[b]	1,134[b]

[a] Lien et al. (2004)
[b] Based on recommended human milk intakes (U.S. EPA, 2011)

3.3 CURRENT SOURCES OF bLf IN THE DIET OF INFANTS AND TODDLERS

The dietary exposure of infants and toddlers to lactoferrin from non-human milk sources is predominantly from milk-based formulas currently available, and bovine milk.

Cows' "Milk is defined as the lacteal secretion, practically free from colostrum, obtained by the complete milking of one or more healthy cows." (21 CFR§131.110). The lactoferrin concentration of bovine milk is approximately 0.1 mg/ml (Barth & Behnke, 1997; Korhonen & Pihlanto, 2007). The lactoferrin content of mature bovine milk is, like that of human milk dynamic, and varies predominantly as a function of stage of lactation (Cheng et al., 2008; Soyeurt et al., 2007). One 240 ml serving of bovine milk will provide approximately 24 mg of bLf. Bovine milk protein (approximately 34.1 mg/L) contains casein proteins (27.0 g/L, 79.2% of total protein) and whey proteins (7.1 g/L, 20.8% of total protein). The amount of lactoferrin in bovine milk accounts for approximately 1.4% of the total whey proteins (Barth & Behnke, 1997).

The protein content of standard term cow's milk-based infant and toddler formulas means that infants and toddlers are exposed to bLf when consuming those formulas, as well as cow's milk. The protein content, and ratio of whey to casein, of formulas available in the USA varies according to brand and type of formula (Bartram & Montoya, 2014).

The milk protein composition of 3 formula brands (Similac (Abbott_Nutrition, 2015) from Abbott Nutritionals, Enfamil (MeadJohnson_Nutrition, 2014) from Mead Johnson Nutritionals, and Good Start (Gerber.com, 2014) from Gerber/Nestlé) were used to estimate the background exposure to bovine lactoferrin from cow's milk-based formulas that are not currently supplemented with bLf in the USA (Table 3-2). The whey:casein ratio for Similac formula for infants ranged from 20:80 to 60:40, and was 20:80 for formulas for toddlers. The whey:casein ratio for Enfamil products ranged from 20:80 to 80:20 for infant formula, and 20:80 to 60:40 for toddler formula. All Gerber Good Start formulas for infants are based on 100% partially hydrolysed whey protein and formulas for toddlers have a whey:casein ratio of 20:80.

Table 3-2: Estimated Exposure of Infants and Toddlers to Bovine Lactoferrin from Consumption of Currently Marketed Term Cow's Milk-based Formula

Manufacturer			**Abbott Nutrition**	**Mead Johnson Nutritionals**	**Gerber / Nestle**
Product			*Similac*	*Enfamil*	*Good Start*
Protein Content[a,b]	Infant (per 100 g)		1.36 to 1.74 g	1.38 to 2.0 g	1.44 g
	Toddler (per 100 g)		1.98 g	1.71 to 1.78 g	2.0 g
Whey:Casein Ratio[c]	Infant		20:80 to 60:40	20:80 to 80:20	100:0
	Toddler		20:80	20:80 to 60:40	20:80
Estimates of Bovine Lactoferrin Concentrations in Reconstituted, Ready-to-Feed Formulas	Infant	per 100 mL	4.0 to 15.0 mg	4.0 to 17.3 mg	20.8 mg
		per 100 g[d]	3.9 to 14.6 mg	3.9 to 16.8	20.2 mg
	Toddler	per 100 mL	5.7 mg	5.2 to 14.9 mg	5.8 mg
		per 100 g[d]	5.5 mg	5.0 to 14.4 mg	5.6 mg

[a] Protein levels obtained from the USDA National Nutrient Database for Standard Reference (Release 28 May 2016) (USDA, 2016 - http://ndp.nal.usda.gov/) and cross-checked with information available from manufacturer's web sites [Abbott Nutrition, 2015 - (Similac (http://abbottnutrition.com/brands/similac); Mead Johnson Nutrition, 2014 - Enfamil (http://www.enfamil.com); Gerber.com, 2016 - Good Start (https://www.gerber.com/Home)]. All products listed comply with 21 CFR 107 (CFR, 2016b).

[b] Nutritional composition is derived from reconstituted, ready-to-feed formulas or based on manufacturer's reconstitution instructions.

[c] The whey:casein ratio was based on information available from Albert Health Services' (AHS, 2014)"Infant

Formula for Health Term Infants – Compendium" (http://www.albertahealthservices.ca/assets/info/nutrition/if-nfs-ng-healthy-infants-infant-formula-compendium.pdf), and from information made available by the manufacturers (*i.e.*, websites, labels).

[d] Bovine lactoferrin concentrations were determined by multiplying the protein content with the percentage of whey protein, then multiplying by 1.4%, the approximate amount of lactoferrin in whey (Barth and Behnke, 1997). Concentrations per 100 mL were converted to per 100 g by dividing the per 100 mL values by the densities of reconstituted formula, which are approximately 1026 and 1040 g/L for formulas for infants and toddlers, respectively (Synlait Milk Ltd. – unpublished data)

The more extensive range of formula product options now available is reflected in the wider range of potential exposure levels than those previously estimated in GRN 465 (2014, p. 38 (pdf)): average exposure of infants (0 - 4 months) consuming solely milk-based formula 74 to 137 mg bLf per day, older infants (5 – 11 months) consuming formula an average exposure of 65 – 120 mg bLf per day, and toddlers (12 – 35 months) 54 – 100 mg /day.

3.3.1 Existing use of bLf in Formula in the United States

Early 2016, Mead Johnson released a new infant formula "Enspire™" containing added lactoferrin, and a whey to casein ratio of 60:40. The level of bLf in this formula is 0.6 g/L (600 mg/L or 60 mg /100ml), which equates to approximately 440 mg bLf /100g solids based on a reconstitution rate of 13.5% w/v (https://www.meadjohnson.com/pediatrics/us-en/products/about-enfamil-enspire (*accessed 29 June 2016)*). This level exceeds that of the intended use of bLf subject to this notification, and the levels of intended use in GRN 465 (100 mg/100 g powdered formulas, 26 mg/100ml liquid concentrates, and 13 mg/100ml ready-to-feed formulas (GRN 465, 2014, p. 18 (pdf)), but is less than the maximum level (1.0 g/L) shown to support normal growth and tolerance in term infants (Johnston et al., 2015). The clinical study used bLf provided from DMV International bv.) which is the spray-dried bLf manufactured by Friesland Campina. This bLf is essentially the same as that manufactured by Synlait, the bLf being isolated and concentrated by the same manufacturing processes, and spray dried.

3.4 ESTIMATED DIETARY CONSUMPTION OF BOVINE LACTOFERRIN BASED ON INTENDED USE OF bLf IN FORMULA IN THE UNITED STATES[2]

3.4.1 Introduction

Bovine lactoferrin, produced by Synlait Milk Ltd., is intended for use as an ingredient in infant formulas. Estimates for the intake of bovine lactoferrin in the United States (U.S.) were based on the proposed food uses and use levels for bovine lactoferrin in conjunction with food consumption data included in the U.S. National Center for Health Statistics (NCHS) National Health and Nutrition Examination Surveys (NHANES). The NHANES data are collected and released in 2-year cycles with the most recent cycle containing data collected in 2011-2012. To have a more robust sample size, intake data from 3 consecutive NHANES cycles (*i.e.,* 2007 to 2008; 2009 to 2010; 2011 to 2012) were used in the intake assessment; results are presented separately for each cycle and for all 3 cycles combined (CDC, 2010, 2011, 2015; USDA, 2010, 2012, 2014).

For all 3 NHANES cycles, data were collected from individuals and households *via* 24-hour dietary recalls administered on 2 non-consecutive days (Day 1 and Day 2). Calculations for the mean and 90th percentile all-user intakes were performed for the proposed uses of bovine lactoferrin in infant formula (intended for infants 0 to 6 months), follow-on formula (intended for infants 7 to 12 months), and toddler formula (intended for toddlers 13 to 36 months). In addition, the percentage of consumers was determined. Amongst users, the per person and per kilogram body weight intakes were reported for the following population groups:

infants, ages 0 to 6 months;
infants, ages 7 to 12 months; and,
toddlers, ages 13 to 36 months.

The number of users of infant formula in the toddler age group was determined to be very low (*i.e.,* only 48 of 1,334 toddlers included in the 2007 to 2012 NHANES consumed infant or toddler formula on either day of the 2-day survey). It was considered that, as a worst-case scenario, caregivers would replace fluid milk with toddler formula; thus, a second intake assessment was conducted for the toddler age group, in which intakes of fluid milk were assessed

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[2] The Estimated Dietary Consumption analysis was completed by:
Intertek Scientific and Regulatory Consultancy
Food and Nutrition Group
2233 Argentia Road, Suite 201
Mississauga, Ontario
L5N 2X7

at the mean and 90th percentile, as proxies for the intake of toddler formula, and intakes of bovine lactoferrin from its addition to toddler formula were estimated.
It should be noted that the dietary intake estimates presented herein are highly conservative estimates, given that an inherent assumption in the modeling is that all infant and toddler formulas within the formula category will contain bovine lactoferrin at the maximum specified level of use.
More details on the NHANES methodology and the dietary intake estimates are presented below.

3.4.2 Food Consumption Survey Data

3.4.2.1 Survey Description

NHANES data for the years 2007 to 2012 are available for public use. NHANES are conducted as continuous, annual surveys, and are released in 2-year cycles. Each year about 7,000 people from 15 different locations across the U.S. are interviewed, and approximately 5,000 complete the health examination component of the survey. Any combination of consecutive years of data collection is recognized and used as a nationally representative sample of the U.S. population. To have a more robust sample size, intake data from 3 consecutive NHANES cycles (*i.e.,* 2007 to 2008; 2009 to 2010; 2011 to 2012) were used in the intake assessment; results are presented separately for each cycle and for all 3 cycles combined (CDC, 2010, 2011, 2015; USDA, 2010, 2012, 2014).

NHANES 2007 to 2008, 2009 to 2010, and 2011 to 2012 survey data were collected from individuals and households *via* 24-hour dietary recalls administered on 2 non-consecutive days (Day 1 and Day 2) throughout all 4 seasons of the year. Day 1 data were collected in-person, and Day 2 data were collected by telephone in the following 3 to 10 days, on different days of the week, to achieve the desired degree of statistical independence. The data were collected by first selecting Primary Sampling Units (PSUs), which were counties throughout the U.S., of which 15 PSUs are visited per year. Small counties were combined to attain a minimum population size. These PSUs were segmented and households were chosen within each segment. One or more participants within a household were interviewed. For NHANES 2007 to 2008, 12,943 individuals were selected for the sample, 10,149 were interviewed (78.4%), and 9,762 were sampled (75.4%). For NHANES 2009 to 2010, 13,272 individuals were selected for the sample, 10,537 were interviewed (79.4%), and 10,253 were sampled (77.3%). For NHANES 2011 to 2012, 13,431 individuals were selected for the sample, 9,756 were interviewed (72.6%) and 9,338 were sampled (69.5%) (CDC, 2010, 2011, 2015; USDA, 2010, 2012, 2014).
In addition to collecting information on the types and quantities of foods consumed, information on socio-economic status, physiological parameters, and demographic variables also was

collected from individual participants in the NHANES 2007 to 2012, such as sex, age, height and weight, and other variables useful in characterizing consumption. The inclusion of this information allows for further assessment of food intake based on consumption by specific population groups of interest within the total population. The sample design for NHANES 2007 to 2012 includes an oversample of Asian Americans; however, sample weights were incorporated to allow estimates from these subgroups to be combined to obtain national estimates that reflect the relative proportions of these groups in the population as a whole (CDC, 2010, 2011, 2015; USDA, 2010, 2012, 2014).

3.4.2.2 Statistical Methods

For the intake assessment, consumption data from individual dietary records, detailing food items ingested by each survey participant, were collated by computer and used to generate estimates for the intake of bovine lactoferrin from its proposed addition to infant and toddler formulas by infants ages 0 to 6 months, infants ages 7 to 12 months, and toddlers ages 13 to 36 months[3]. Estimates for the daily intake of bovine lactoferrin from infant or toddler formulas represent projected 2-day averages for each infant/toddler from Day 1 and Day 2 of NHANES 2007 to 2008, 2009 to 2010, and 2011 to 2012 data; these average amounts comprised the distribution from which mean and percentile intake estimates were generated. Mean and percentile intake estimates were generated incorporating survey weights in order to provide representative intakes for the entire U.S. population All-user intake refers to the estimated intake (CDC, 2010, 2011, 2015; USDA, 2010, 2012, 2014) of formula (or milk[4]) by those infants or toddlers for whom the consumption of infant or toddler formula (or milk[2]) was reported, hence the "all-user" designation. Individuals were considered 'users' if they consumed 1 or more infant, follow-on, or toddler formulas on either Day 1 or Day 2 of the survey.

Mean and 90th percentile intake estimates based on sample sizes of less than 30 and 80, respectively, may not be considered statistically reliable due to the limited sampling size (LSRO, 1995). Any values based on less than 30 or 80 users at the mean or 90th percentile, respectively, are marked with an asterisk.

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[3] Statistical analysis and data management were conducted using Statistical Analysis Software (SAS), version 9.4, SAS Institute Inc. (Cary, NC, U.S.).

[4] Bovine lactoferrin is not intended to be added to fluid milk; rather, given the low prevalence of formula intake in the toddler age group (*i.e.*, only 3.5% of the toddlers in the 2007 to 2012 NHANES consumed infant or toddler formula), fluid milk intakes in the toddler age group were used as a proxy for formula intakes (a conservative, worst-case scenario).

3.4.3 Food Usage Data

The individual food-uses and use-levels for bovine lactoferrin employed in the current intake analysis are summarized in Table 3-3. Food codes representative of infant formula and fluid milk were chosen from the NHANES 2007 to 2008, 2009 to 2010, and 2011 to 2012 (CDC, 2010, 2011, 2015; USDA, 2010, 2012, 2014). Food codes were grouped in food-use categories according to Title 21, Section §170.3 of the Code of Federal Regulations (CFR, 2016). All food codes for infant and toddler formula included in the current intake assessment are listed in Part 7: Appendix 7.

Table 3-3: Summary of the Individual Infant Formula and Use-Levels for Bovine Lactoferrin in the U.S.

Food Category	Infant Formula	Bovine Lactoferrin		
		Proposed Use Level (mg/100g formula solids)	Proposed Use Level (mg/100 mL)[a]	Maximum Proposed Use Level (mg/100 g)[b]
Infant and Toddler Formulas	Formulas for Infants 0 to 6 months	100	12.5	12.2
	Formulas for Infants 7 to 12 months	100	13.5	13.1
	Formulas for Toddlers 13 to 36 months	100	15.0	14.4

Abbreviations: NHANES = National Health and Nutrition Examination Survey; U.S. = United States.
[a] The proposed use levels are relative to 100 mL of infant formula that is reconstituted and ready-to-feed.
[b] This represents the maximum bovine lactoferrin concentration in ready-to-feed infant formulas. The maximum proposed use levels were calculated by dividing the proposed use levels by the densities of reconstituted formula, which are approximately 1026, 1033, and 1040 g/L for formulas for infants aged 0 to 6 months, infants aged 7 to 12 months, and toddlers aged 13 to 36 months, respectively (Synlait Milk Ltd – unpublished data).

3.4.4 Food Survey Results

Estimates for the total daily intakes of bovine lactoferrin from infant formula and from fluid milk[5] are summarized in Sections 3.4.4.1.1 and 3.4.4.1.2, respectively.

[5] Bovine lactoferrin is not intended to be added to fluid milk; rather, given the low prevalence of formula intake in the toddler age group (*i.e.*, only 3.5% of the toddlers in the 2007 to 2012 NHANES consumed infant or toddler formula), fluid milk intakes in the toddler age group were used as a proxy for formula intakes (a conservative, worst-case scenario).

3.4.4.1 Estimated Daily Intake of Bovine Lactoferrin from Infant Formulas in the U.S.

3.4.4.1.1 Estimated Consumption Based on Intakes of Infant Formula

Based on data from the 2007 to 2012 NHANES, approximately 77.3, 74.1, and 3.5% of infants aged 0 to 6 months, 7 to 12 months, and 13 to 36 months, respectively, consumed infant or toddler formula on either day of the 2-day survey (Table 3-4) (CDC, 2010, 2011, 2015; USDA, 2010, 2012, 2014). Amongst users, the estimated formula intakes were 831.6, 705.3, and 416.5 g/day (or 135.0, 78.5, and 37.8 g/kg body weight/day, respectively) in infants aged 0 to 6 months, 7 to 12 months, and 13 to 36 months, respectively (Table 3-4). Amongst users, the 90th percentile formula intakes were 1,220.0, 1,037.3, and 915.0 g/day (or 212.4, 114.8, and 100.6 g/kg body weight/day, respectively) in infants aged 0 to 6 months, 7 to 12 months, and 13 to 36 months, respectively (Table 3-4).

The maximum bovine lactoferrin concentration intended to be added to formula is 100 mg per 100 g of formula solids. Per 100 g of reconstituted, ready-to-feed infant formula, this equates to 12.2, 13.1, and 14.4 mg for formulas intended for infants aged 0 to 6 months, infants aged 7 to 12 months, and toddlers aged 13 to 36 months, respectively. In addition to the bovine lactoferrin that is intended to be added to formula, there are background levels of bovine lactoferrin present in milk-based infant formula. From a survey of the United States Department of Agriculture (USDA) Food Composition Database (Standard Release 28) (USDA, 2016), background bovine lactoferrin concentrations in reconstituted, ready-to-feed infant and toddler formulas range from 3.9 to 20.2 mg/100 g and 5.0 to 14.4 mg/100 g, respectively. A summary of the products that were surveyed and the calculations used to estimate background bovine lactoferrin concentrations in reconstituted, ready-to-feed infant and toddler formulas is provided in Table 3-2, with further detail in Part 7 Appendix 7 (Table A7.1, pg. A7:2). **Taking the upper background level as a conservative estimate (*i.e.*, 20.2 mg of bovine lactoferrin per 100 g of reconstituted, ready-to-feed infant formula and 14.4 mg of bovine lactoferrin per 100 g of reconstituted, ready-to-feed toddler formula), the total bovine lactoferrin levels (*i.e.*, from the background level and the amount intended to be added, combined) would equate to 32.4, 33.3, and 28.8 mg of bovine lactoferrin per 100 g of reconstituted, ready-to-feed formula for infants aged 0 to 6 months, 7 to 12 months, and 13 to 36 months, respectively.**

Taking into consideration the formula intake estimates, the background bovine lactoferrin levels in milk-based infant and toddler formulas, and the bovine lactoferrin levels that are intended to be added to the infant and toddler formulas (Part 7 Appendix 7 (Table A7.2, pg. A7:4), the mean bovine lactoferrin intake estimates amongst users in the 2007 to 2012 NHANES are 269.4,

234.9, and 120.0 mg/day (or 43.7, 26.1, and 10.9 mg/kg body weight/day, respectively) in infants aged 0 to 6 months, infants aged 7 to 12 months, and toddlers aged 13 to 36 months, respectively. The 90th percentile bovine lactoferrin intake estimates are 395.3, 345.4, and 263.5 mg/day (or 68.8, 38.2, and 29.0 mg/kg body weight/day, respectively) in infants aged 0 to 6 months, infants aged 7 to 12 months, and toddlers aged 13 to 36 months, respectively. The intakes of bovine lactoferrin at the mean and 90th percentile in mg/day and in mg/kg body weight/day are summarized in Table 3-4 and Table 3-5, respectively.

Table 3-4: Summary of the Estimated Daily Intake of Bovine Lactoferrin (in mg/day) from its Addition to Infant and Toddler Formulas in the U.S. by Population Group[a] (2007-2012 NHANES Data) (CDC, 2010, 2011, 2015; USDA, 2010, 2012, 2014)

NHANES Cycle	Age Group	Number Surveyed	Number (%) of Users	All-user Intakes of Infant or Toddler Formulas (g/day)			All-user Intakes of Bovine Lactoferrin (mg/day)[b]		
				Mean (±SEM)	Range	90th Percentile	Mean (±SEM)	Range	90th Percentile
2007 to 2008	0 to 6 months	198	166 (84.3)	799.1 (31.8)	30.5 to 1,641.6	1,220.0	258.9 (10.3)	9.9 to 531.9	395.3
	7 to 12 months	188	145 (73.3)	679.7 (28.3)	7.8 to 1,594.4	1,021.8	226.3 (9.4)	546.7 to 530.9	340.3
	13 to 36 months	449	13 (2.7)	621.5 (147.6)*	90.4 to 1,082.8	915.0*	179.0 (42.5)*	26.0 to 311.8	263.5*
2009 to 2010	0 to 6 months	200	161 (74.8)	842.5 (26.6)	30.4 to 1,631.8	1,250.5	273.0 (8.6)	9.8 to 528.7	405.2
	7 to 12 months	161	129 (75.4)	705.4 (37.4)	15.3 to 1,704.3	1,098.0	234.9 (12.5)	5.1 to 567.5	365.6
	13 to 36 months	496	17 (2.8)	481.3 (67.5)*	31.0 to 1,302.0	732.0*	138.6 (19.4)*	8.9 to 375.0	210.8*
2011 to 2012	0 to 6 months	186	145 (73.4)	854.5 (44.5)	2.5 to 1,830.0	1,235.3	276.9 (14.4)	0.8 to 592.9	400.2
	7 to 12 months	151	112 (73.8)	736.5 (26.1)	30.5 to 1,945.6	1,003.20	245.3 (8.7)	10.2 to 647.9	334.1
	13 to 36 months	389	18 (5.3)	254.0 (76.9)*	77.5 to 1,403.0	442.3*	73.2 (22.1)*	22.3 to 404.1	127.4*
2007 to 2012	0 to 6 months	584	472 (77.3)	831.6 (20.1)	2.5 to 1,830.0	1,220.0	269.4 (6.5)	0.8 to 592.9	395.3
	7 to 12 months	500	386 (74.1)	705.3 (18.2)	7.8 to 1,945.6	1,037.3	234.9 (6.1)	2.6 to 647.9	345.4
	13 to 36 months	1,334	48 (3.5)	416.5 (83.1)	31.0 to 1,403.0	915.0*	120.0 (23.9)	8.9 to 404.1	263.5*

Abbreviations: NHANES = National Health and Nutrition Examination Survey; SEM = standard error of the mean; U.S. = United States.

[a] Intakes presented are for users only. A user was defined as any infant or toddler for whom the consumption of infant or toddler formula on either day of the 2-day survey was reported.

[b] Bovine lactoferrin intake estimates were derived by taking into account the amount of bovine lactoferrin that is intended to be added to the formula (*i.e.*, 12.2, 13.1, and 14.4 mg per 100 g of reconstituted, ready-to-feed formula for infants aged 0 to 6 months, 7 to 12 months, and 12 to 36 months, respectively), as well as the amount of bovine lactoferrin that is naturally present in milk-based formulas (*i.e.*, up to 20.2 mg per 100 g of reconstituted, ready-to-feed infant formula and up to 14.4 mg per 100 g of reconstituted, ready-to-feed toddler formula).

* Indicates an intake estimate that may not be statistically reliable, as the sample size does not meet the minimum reporting requirements

Table 3-5: Summary of the Estimated Daily Intake of Bovine Lactoferrin (in mg/kg body weight/day) from its Addition to Infant and Toddler Formulas in the U.S. by Population Group[a] (2007-2012 NHANES Data) (CDC, 2010, 2011, 2015; USDA, 2010, 2012, 2014)

NHANES Cycle	Age Group	Number Surveyed	Number (%) of Users	All-user Intakes of Infant Formula (g/kg body weight/day)			All-user Intakes of Bovine Lactoferrin (mg/kg body weight/day)[b]		
				Mean (±SEM)	Range	90th Percentile	Mean (±SEM)	Range	90th Percentile
2007 to 2008	0 to 6 months	198	166 (84.3)	129.1 (5.5)	3.4 to 393.6	203.3	41.8 (1.8)	1.1 to 127.5	65.9
	7 to 12 months	188	145 (73.3)	76.7 (3.4)	0.9 to 164.8	114.8	25.5 (1.1)	0.3 to 54.9	38.2
	13 to 36 months	449	13 (2.7)	62.4 (17.6)*	6.6 to 100.6	100.6*	18.0 (5.1)*	1.9 to 29.0	29.0*
2009 to 2010	0 to 6 months	200	161 (74.8)	138.7 (6.4)	5.4 to 344.4	223.3	44.9 (2.1)	1.7 to 111.6	72.3
	7 to 12 months	161	129 (75.4)	78.1 (4.1)	1.6 to 274.9	125.7	26.0 (1.4)	0.5 to 91.5	41.9
	13 to 36 months	496	17 (2.8)	40.5 (5.8)*	3.1 to 99.4	63.7*	11.7 (1.7)*	0.9 to 28.6	18.3*
2011 to 2012	0 to 6 months	186	145 (73.4)	137.0 (8.6)	0.4 to 327.9	217.6	44.4 (2.8)	0.1 to 106.2	70.5
	7 to 12 months	151	112 (73.8)	81.0 (3.6)	3.4 to 214.0	110.6	27.0 (1.2)	1.1 to 71.3	36.8
	13 to 36 months	389	18 (5.3)	21.4 (7.1)*	4.5 to 133.6	32.3*	6.2 (2.0)*	1.3 to 38.5	9.3*
2007 to 2012	0 to 6 months	584	472 (77.3)	135.0 (4.0)	0.4 to 393.6	212.4	43.7 (1.3)	0.1 to 127.5	68.8
	7 to 12 months	500	386 (74.1)	78.5 (2.1)	0.9 to 274.9	114.8	26.1 (0.7)	0.3 to 91.5	38.2
	13 to 36 months	1,334	48 (3.5)	37.8 (9.0)	3.1 to 133.6	100.6*	10.9 (2.6)	0.9 to 38.5	29.0*

Use of bLf as an ingredient in Infant and Toddler Milk-based Formula in Countries Abbreviations: NHANES = National Health and Nutrition Examination Survey; SEM = standard error of the mean; U.S. = United States.

[a] Intakes presented are for users only. A user was defined as any infant or toddler for whom the consumption of infant or toddler formula on either day of the 2-day survey was reported.

[b] Bovine lactoferrin intake estimates were derived by taking into account the amount of bovine lactoferrin that is intended to be added to the formula (*i.e.*, 12.2, 13.1, and 14.4 mg per 100 g of reconstituted, ready-to-feed formula for infants aged 0 to 6 months, 7 to 12 months, and 12 to 36 months, respectively), as well as the amount of bovine lactoferrin that is naturally present in milk-based formulas (*i.e.*, up to 20.2 mg per 100 g of reconstituted, ready-to-feed infant formula and up to 14.4 mg per 100 g of reconstituted, ready-to-feed toddler formula).

* Indicates an intake estimate that may not be statistically reliable, as the sample size does not meet the minimum reporting requirements.

3.4.4.1.2 Estimated Consumption in the Toddler Group Based on Intakes of Fluid Milk

There was a low prevalence of formula intake in the toddler age group (*i.e.,* only 3.5% of the toddlers in the 2007 to 2012 NHANES consumed infant or toddler formula); thus, as a worst-case, fluid milk intakes were used as a proxy for formula intakes in the toddler group. As summarized in Table 3-6 and Table 3-7, 89.5% of toddlers included in the survey reported the consumption of fluid milk on either day of the 2-day survey in NHANES 2007 to 2012 (CDC, 2010, 2011, 2015; USDA, 2010, 2012, 2014). The intake of fluid milk amongst users of fluid milk was 456.1 g/day (or 37.0 g/kg body weight/day) at the mean, and 831.1 g/day or (71.1 g/kg body weight/day) at the 90th percentile. Interestingly, despite the low number of users of infant or toddler formula in the toddler age group, amongst the users, the intakes of formula at the mean [*i.e.,* 416.5 g/day (37.8 g/kg body weight/day)] are similar to those of fluid milk.

Assuming that, in toddlers, fluid milk will be replaced with a toddler formula with a maximum bovine lactoferrin concentration of 28.8 mg per 100 g of reconstituted, ready-to-feed formula (*i.e.,* 14.4 mg/100 g that are intended to be added **plus** 14.4 mg/100 g background lactoferrin levels in milk-based toddler formula), the bovine lactoferrin intake estimates in toddlers are 131.4 mg/day (or 10.7 mg/kg body weight/day) at the mean and 239.4 mg/day (or 20.5 mg/kg body weight/day) at the 90th percentile.

Table 3-6: Summary of the Estimated Daily Intake of Bovine Lactoferrin (in mg/day) by Toddlers from its Addition to Infant and Toddler Formulas in the U.S., Assuming that Fluid Milk Intakes are Replaced with Toddler Formula[a,b] (2007-2012 NHANES Data) (CDC, 2010, 2011, 2015; USDA, 2010, 2012, 2014)

NHANES Cycle	Age Group	Number Surveyed	Number (%) of Users	All-user Intakes of Fluid Milk (g/day)			All-user Intakes of Bovine Lactoferrin (mg/day)[c]		
				Mean (±SEM)	Range	90th Percentile	Mean (±SEM)	Range	90th Percentile
2007 to 2008	13 to 36 months	449	401 (88.1)	454.3 (18.9)	7.6 to 2,173.1	808.3	130.8 (5.4)	2.2 to 625.9	232.8
2009 to 2010	13 to 36 months	496	454 (89.5)	504.8 (18.5)	2.4 to 1,959.6	922.6	145.4 (5.3)	0.7 to 564.4	265.7
2011 to 2012	13 to 36 months	389	348 (91.0)	398 (16.5)	8.3 to 1,296.3	770.1	114.6 (4.8)	2.4 to 373.3	221.8
2007 to 2012	13 to 36 months	1,334	1,203 (89.5)	456.1 (10.3)	2.5 to 2,173.1	831.1	131.4 (3.0)	0.7 to 625.9	239.4

Abbreviations: NHANES = National Health and Nutrition Examination Survey; SEM = standard error of the mean; U.S. = United States.

[a] Intakes presented are for users only. A user was defined as any toddler for whom the consumption of fluid milk on either day of the 2-day survey was reported.

[b] Bovine lactoferrin is not intended to be added to fluid milk; rather, given the low prevalence of formula intake in the toddler age group (*i.e.*, only 3.5% of the toddlers in the 2007 to 2012 NHANES consumed infant or toddler formula), fluid milk intakes in the toddler age group were used as a proxy for formula intakes (a conservative, worst-case scenario).

[c] Bovine lactoferrin intake estimates were derived by taking into account the amount of bovine lactoferrin that is intended to be added to the toddler formula (*i.e.*, 14.4 mg per 100 g of reconstituted, ready-to-feed toddler formula), as well as the amount of bovine lactoferrin that is naturally present in milk-based toddler formulas (*i.e.*, up to 14.4 mg per 100 g of reconstituted, ready-to-feed toddler formula).

Table 3-7: Summary of the Estimated Daily Intake of Bovine Lactoferrin (in mg/kg body weight/day) by Toddlers from its Addition to Infant and Toddler Formulas in the U.S., Assuming that Fluid Milk Intakes are Replaced with Toddler Formula[a,b] (2007-2012 NHANES Data) (CDC, 2010, 2011, 2015; USDA, 2010, 2012, 2014)

NHANES 2007 to 2008	Age Group	Number Surveyed	Number (%) of Users	All-user Intakes of Fluid Milk (g/kg body weight/day)			All-user Intakes of Bovine Lactoferrin (mg/kg body weight/day)[c]		
				Mean (±SEM)	Range	90th Percentile	Mean (±SEM)	Range	90th Percentile
2007 to 2008	13 to 36 months	449	401 (88.1)	36.8 (1.3)	0.7 to 131.2	66.1	10.6 (0.4)	0.2 to 37.8	19.0
2009 to 2010	13 to 36 months	496	454 (89.5)	41.4 (1.7)	0.3 to 158.0	80.4	11.9 (0.5)	0.1 to 45.5	23.2
2011 to 2012	13 to 36 months	389	348 (91.0)	31.9 (1.2)	0.7 to 139.4	60.0	9.2 (0.3)	0.2 to 40.1	17.3
2007 to 2012	13 to 36 months	1,334	1,203 (89.5)	37.0 (0.8)	0.3 to 158.0	71.1	10.7 (0.2)	0.1 to 45.5	20.5

Abbreviations: NHANES = National Health and Nutrition Examination Survey; SEM = standard error of the mean.

[a] Intakes presented are for users only. A user was defined as any toddler for whom the consumption of fluid milk on either day of the 2-day survey was reported.

[b] Bovine lactoferrin is not intended to be added to fluid milk; rather, given the low prevalence of formula intake in the toddler age group (*i.e.*, only 3.5% of the toddlers in the 2007 to 2012 NHANES consumed infant or toddler formula), fluid milk intakes in the toddler age group were used as a proxy for formula intakes (a conservative, worst-case scenario).

[c] Bovine lactoferrin intake estimates were derived by taking into account the amount of bovine lactoferrin that is intended to be added to the toddler formula (*i.e.*, 14.4 mg per 100 g of reconstituted, ready-to-feed toddler formula), as well as the amount of bovine lactoferrin that is naturally present in milk-based toddler formulas (*i.e.*, up to 14.4 mg per 100 g of reconstituted, ready-to-feed toddler formula

3.4.5 Conclusions of Estimated Dietary Consumption of Bovine Lactoferrin Based on Intended Use of BLf in Formula in the United States

Using data collected in the 2007 to 2012 U.S. NHANES, the intakes of infant formula in infants aged 0 to 6 months, infants aged 7 to 12 months, and toddlers aged 13 to 36 months were used to estimate exposures to bovine lactoferrin. In addition, given the low number of users of formula in the toddler age group, the intakes of fluid milk were used as a proxy, assuming that toddler formulas would be used to replace fluid milk. There were a number of assumptions included in the assessment, which mean that the resulting exposure estimates may be considered the 'worst case' values. For example, it has been assumed in both exposure assessments that all infant and toddler formulas contain bovine lactoferrin at the maximum specified level of use. In reality, the levels added to specific foods will vary. In addition, it is well-established that the length of a dietary survey affects the estimated consumption of individual users. Short-term surveys, such as the typical 2- or 3-day dietary surveys, may overestimate the consumption of food products that are consumed relatively infrequently (Anderson, 1988)

In summary, on an all-user basis, the resulting mean intakes of bovine lactoferrin from its addition to infant and toddler formulas, and also accounting for background levels of bovine lactoferrin in milk-based formulas, by infants and toddlers in the U.S., are 269.4, 234.9, and 120.0 mg/day (or 43.7, 26.1, and 10.9 mg/kg body weight/day, respectively) in infants aged 0 to 6 months, 7 to 12 months, and 13 to 36 months, respectively. The 90th percentile bovine lactoferrin intake estimates were 395.3, 345.4, and 263.5 mg/day (or 68.8, 38.2, and 29.0 mg/kg body weight/day, respectively) in infants aged 0 to 6 months, infants aged 7 to 12 months, and toddlers aged 13 to 36 months, respectively.

The mean intakes of bovine lactoferrin by toddlers aged 13 to 36 months, using fluid milk intakes as a proxy for the intake of toddler formulas, amongst users of fluid milk are 131.4 mg/day (or 10.7 mg/kg body weight/day) at the mean, and 239.4 mg/day (or 20.5 mg/kg body weight/day) at the 90th percentile.

Infants aged 0 to 6 months were identified as the population group having the highest mean and 90th percentile all-user bovine lactoferrin intakes at 269.4 mg/day (or 43.7 mg/kg body weight/day) and 395.3 mg/day (or 68.8 mg/kg body weight/day), respectively.

3.4.6 Use of bLf as an ingredient in Infant and Toddler Milk-based Formula in Countries Other than the USA.

Bovine lactoferrin was first used in infant formula (50 mg bLf /100 g formula powder) manufactured by Morinaga Milk Industry Co. Ltd in Japan in 1986 (GRN 465, 2014, p. 39 (pdf)). Since that time it has been used extensively in infant and toddler milk formulas for both domestic and export markets (GRN 465, 2014, p. 40 (pdf)). In Japan, lactoferrin is listed on the "List of Existing Food Additives" (2014): Additive 341 "Lactoferrin concentrates: a substance composed mainly of lactoferrin obtained from mammals' milk": (http://www.ffcr.or.jp/zaidan/FFCRHOME.nsf/pages/list-exst.add). The specifications for "Lactoferrin Concentration" in the existing food additives list in Japan, as submitted in GRN 465 is provided in Appendix 3 (pg. A3: 11), and a copy of the "List of Existing Food Additives" in Appendix 4 (pg. A4: 68). There is no specific restriction of use for bLf in Japan because it is a natural substance.

In addition to Japan, the use of bLf in infant formula is widely accepted throughout a number of other Asian jurisdictions, and is considered a desirable addition to infant formula by many consumers. In Taiwan, the use of bLf (additive code 08112) has been approved since 2000, with addition rate in infant formula prescribed "as practically needed" (Standards for Specification, Scope, Application and Limitation of Food Additives (Appendix 4, pg. A4: 70). In Korea, bLf is listed as an approved Food Additive for use in infant and follow-up formula (Standards for Manufacturing and Preparation: General Standards for Food Additive use in Foods (infant foods) Appendix 4, pg. A4: 59) with no specific restrictions on level of addition. Considered a natural additive, the Korean Food Additives Code contains a specification and identity testing criteria for lactoferrin (Appendix 3, pg. A3: 13). In 2015, a draft amendment to the Food Regulations in Singapore has proposed the permitted use of bovine lactoferrin in infant formulas at levels up to 100 mg/100 ml of formula (Appendix 4, pg. A4: 60).

Synlait has been manufacturing a range of infant formula products containing added bLf, at a range of levels <100 mg/100 g formula powder, for export to China since 2011; however, it is only since late 2014 that bLf has been manufactured and sourced internally by Synlait. Examples of current products are shown in Appendix 5 (pg. A5: 3). Lactoferrin is permitted for addition to infant formula under GB14880 "National Food Safety Standard for the Use of Nutritional Fortification Substances in Foods" at levels < 1.0 g/kg (Appendix 4, pg. A4: 29). In 2015, Chinese authorities notified the WTO (World Trade Organization) of its intended Standard for lactoferrin (Appendix 4, pg. A4 15), based on earlier preparatory work for the draft standard (Appendix 4 pg. A4: 20).

In 2012, the European Food Safety Authority (EFSA) Panel on Dietetic Products Nutrition and Allergy (NDA Panel) released its Scientific Opinion on bovine lactoferrin (EFSA Panel on Dietetic Products Nutrition and Allergies (NDA), 2012), concluding that bLf as a novel food ingredient was safe under the proposed uses and use levels, including infant formula. Following this opinion, the European Commission (EC) authorized bLf as a novel food ingredient, amending a minor technical oversight, regarding permitted drying technologies for the manufacture of bLf in 2015 (European Commission, 2012a, 2012b, 2015). The authorizations also included a specification for the bLf ingredient (Appendix 4, pg. A4: 3). In the EU, bLf can be added to infant and follow-on formula for infants up to 12 months to a maximum of 100 mg/100 ml (ready to drink (RTD)), and to foods on a daily basis intended for young children (e.g., toddler formula) to a maximum of 200 mg/ 100 g RTD. The average intake levels from the approved use of bLf in Europe are 1.2 g bLf/day for infants aged 0 – 6 months, 1.9 g bLf/day for infants 8 – 10 months and for young children (1 – 3 years) a range of 2.7 to 2.8 g/day. The latter value includes intakes of bLf from other foods available in the EU that are approved for bLf supplementation, and to which this age group and older children may be exposed.

3.5 CONCLUSION

Infants consuming cow's milk-based formula, and toddlers consuming either formula or milk, are currently exposed to bLf. Background exposure to currently marketed formulas results in infants (0 – 6 months of age) that typically consume formula as the sole source of nutrition having the highest exposure to bLf of approximately 168 mg/day. Older infants (7 – 12 months) and toddlers (13- 36 months) are exposed to lesser amounts of bLf, 143 and 60 mg/day, respectively. The intended uses of bLf in infant formula will increase the daily average exposure of infants to bLf by approximately 160 – 200% to 269, 235 and 120 mg/day for infants 0 – 6 months, 7 – 12 months, and 13-36 months, respectively. Notably, exposure of US infants to bLf from the intended uses are approximately 5 to 10 times less than those approved in the EU (estimated at 1.2 g/day for infants <12 months and 2.7 to 2.8 g/day for older infants (EFSA Panel on Dietetic Products Nutrition and Allergies (NDA), 2012, p. 12)) , and approximately 4 - 5 times less than lactoferrin exposure from breast milk (estimated mean 1,185 mg/day for infants <12 months (Table 3-1).

Based on the dietary exposure data, Synlait concludes that the exposure of formula-fed infants and toddlers to bLf under the intended use is significantly less than the exposure to hLf of breast-fed infants, and equivalent to or less than the levels otherwise deemed safe and acceptable in other jurisdictions.

PART 4 SELF-LIMITING LEVELS OF USE

Synlait bLf is a pink to tan colored powder. Under the intended use of bLf in infant and toddler milk-based formulas it does not, and is not intended to impart any significant or detectable color to the formula products. Synlait does not intend bLf to function as a color additive and is of the opinion that it does not meet the definition of a color additive under 21 CFR§70.3 (f) under the intended conditions of use. The potential to detect color differences in formula with added bLf sets a self-limiting level of use, as consumers will not accept formula that has detectable color. The maximum addition rate of bLf in formula that results in detectable color change is unknown.

Under section 201(t)(1) and 21 CFR§ 70.3(f), the term color additive means a material that is a dye, pigment, or other substance made by a process of synthesis or similar artifice, or extracted, isolated, or otherwise derived from a vegetable, animal, mineral, or other source, and that is capable (alone or through reaction with another substance) of imparting color when added or applied to a food; except that such term does not include any material which the Secretary, by regulation, determines is used (or intended to be used) solely for a purpose or purposes other than coloring. Under regulation 21 CFR§ 70.3(f) food ingredients such as cherries, green or red peppers, chocolate, and orange juice which contribute their own natural color when mixed with other foods are not regarded as color additives; but where a food substance such as beet juice is deliberately used as a color, as in pink lemonade, it is a color additive. The use of bLf in formula is not intended to impart any color.

Under 21 CFR§ 70.3(g), a material that otherwise meets the definition of color additive can be exempt from that definition on the basis that it is used or intended to be used solely for a purpose or purposes other than coloring, as long as the material is used in a way that any color imparted is clearly unimportant insofar as the appearance, value, marketability, or consumer acceptability is concerned. Synlait considers bLf is not subject to exemption under 21 CFR§ 70.3(g), as any detectable color imparted by bLf in formula products will be unacceptable to consumers.

The use of bLf as the sole protein source in foods has not been investigated. The intended use of bLf in infant and toddler formulas that are the subject of this GRAS notice, is driven by the intent to more closely align the protein composition of the formulas to the protein composition of human milk, of which lactoferrin is approximately 16% of the total protein of mature human milk (Hirai et al., 1990). The amount of protein in infant formula is strictly regulated: 21 CFR§ 170.100 Nutrients requires a minimum and maximum protein content of 1.8 g/100kcal and 4.5 g/100kcal respectively. Global recommended standards also restrict the level of protein in infant formula; minimum and maximum protein content of 1.8 g/100kcal and 3.0 g/100kcal respectively for cow's milk based formulas (CODEX Alimentarius, 2015; Koletzko et al., 2005).

In addition to the total level of protein in formula there are recommendations and requirements regarding protein quality, that may further limit the total amount of bLf that can be used in formula. Protein quality measures such as the protein efficiency ratio (PER), Protein Digestibility-corrected Amino Acid Score (PDCAAS) and Digestible Indispensable Amino Acid Score (DIAAS) are used to evaluate the quality of proteins against nutritional requirements (FAO Expert Consultation, 2013) particularly for infants and young children, however these measures have not been determined for bLf as a sole protein nutrition source as use as such is not appropriate. Recommendations, and in some regulatory jurisdictions requirements, for minimum amino acid compositions of protein in formula (Table 4-1) (CODEX Alimentarius, 2015; Koletzko et al., 2005; Koletzko et al., 2012), mean there will be limitations to the maximum addition amount of bLf that can be used in infant formula. As a potential sole source of protein nutrition bLf is inappropriate as it has lower that required levels of the amino acids histidine, isoleucine, methionine, phenylalanine, tryptophan and tyrosine (Table 4-1). Therefore, other proteins must be used in addition to bLf to ensure minimum amino acid levels are met. The level of other proteins, and by balance the level of bLf, will be contingent on the amino acid profile of any specific protein chosen.

Therefore, from both the regulatory and nutritional perspectives, both the total maximum protein level permitted and the protein quality as determined by the amino acid profile, is a self-limiting factor for the potential maximum level of use of bLf in formula.

Table 4-1: Amino Acid Value of bLf and Requirements for Infants

Amino Acid	bLf (g/100 g protein)[a]	Recommended Minimum for Infants (g/100g protein)[b]
Cystine	3.8	2.1
Histidine	1.4	2.3
Isoleucine	2.6	5.1
Leucine	10.6	9.4
Lysine	7.8	6.3
Methionine	0.4	1.4
Phenylalanine	4.3	4.5
Threonine	5.2	4.3
Tryptophan	1.7	1.8
Tyrosine	3.5	4.2
Valine	6.6	4.9

[a] Wang et al. (1984)

[b] CODEX Alimentarius (2015); Koletzko et al. (2005); Koletzko et al. (2012)