

**From:** [Morissette, Rachel](#)  
**To:** [Cathryn Sacra](#)  
**Subject:** EAS/Glanbia meeting with FDA 11/29/18 - response  
**Date:** Friday, May 10, 2019 11:47:00 AM  
**Attachments:** [image013.png](#)

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Dear Cathryn,

This is in response to your email of April 10, 2019. Shayla is currently on detail and asked me to handle your request moving forward. We reviewed Glanbia's response to the November 29, 2018 meeting memorandum that you sent and have the following comments below. In addition, we strongly recommend that Glanbia FOIA the responsive records for GRN 000716 for the intended use of osteopontin in infant formula, another bioactive substance for which FDA has similar questions regarding its safety. If you decide to submit a FOIA request, please specifically request the amendments and correspondence for GRN 000716, including the scientific memo, policy memo, and memorandum of meeting between CFSAN and the Center for Biologics Evaluation and Research (CBER).

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FDA notes that Glanbia has not satisfactorily explained why and how a GRAS conclusion can be made for the use of high levels of bovine lactoferrin (bLF) based on a standard toxicological testing paradigm. We note our concerns as follows:

- Toxic chemicals exert their toxicity through interactions with their targets in the cells/tissues causing cellular/tissue damage. Such tissue damage creates a pathological state that can be detected through standard toxicological endpoint analysis. bLF is a bioactive protein and not a toxic chemical. Safety issues related to immunomodulation do not create a detectable pathological state and cannot be reliably addressed by simply investigating the standard toxicological endpoints.
- Furthermore, whereas homeostatic compensation may potentially buffer effects in adults and reduce potential for long-term adverse consequences, this is less likely in the rapidly developing and more sensitive infant population. This is true both for immunological effects and for the additional issue [\[1\]](#) of iron storage and metabolism subsequently identified by FDA.
- Glanbia appears to misconstrue FDA's questions about systemic effects, which do not relate to systemic presence of bLF and associated food allergy concerns but rather systemic immunomodulatory effects mediated either through local interactions with the gut or through systemic entry via the relatively porous infant gut wall.
- Glanbia presents anticipated benefits as evidence of safety, but beneficial effects cannot compensate for potential risks in the same individual or in other individuals in the population under FDA's food ingredient safety assessment paradigm. Evidence of benefits

in a conventional food ingredient context can raise questions about concurrent risks arising from the same modes of action, increasing the importance of clarifying all consequences that may arise rather than simply arriving at a conclusion of net benefit.

- Glanbia does not engage with the question of differential exposure in their reference to previous GRAS notices. In arriving at a conclusion of no questions for these notices, FDA relied in much greater part on widespread and longstanding exposure to roughly comparable quantities of bLF in bovine milk-based infant formulas. However, as Glanbia seeks to move up the dose-response curve for bLF and away from historical exposure patterns to bLF in infant populations, FDA will have more questions about the mechanistic and physiological basis for concluding that there is reasonable certainty that all effects will be beneficial or neutral in all individuals. We are not aware of published studies that assess dose-dependent effects of bLF on aspects of systemic immune function in infants. This information by itself would not be sufficient to establish safety, but its absence significantly hinders evaluation of the safety of this intended use.

Because the GRAS conclusion is Glanbia's, it is up to them to identify or develop a safety assessment strategy that incorporates the bioactivity and anticipated effects of bLF at this exposure level in this population, using relevant biomarkers, analysis of modes of action and their consequences, well-defined comparators, and other tools. If accepted by experts in the field, this strategy, potentially a new paradigm for safety assessment, could provide a basis for GRAS status for the intended bLF use.

The safety data presented by Glanbia does not reflect the physiological properties of bLF, the anticipated effects in this population, or the relationship between exposure and effects. Based on the current review of the published literature, FDA would expect to respond with a "no basis" letter to any GRAS notice whose intended use involves bLF levels higher than use levels proposed in previous GRNs, given the questions FDA has identified and the information Glanbia has provided in response.

[1] FDA had previously raised questions about iron homeostasis based on available scientific literature. Excess iron can have a variety of negative effects on development. The possibility that higher levels of bLF in infant formula may alter iron homeostasis and storage and may require changes in iron content was not adequately addressed by Glanbia in its response.

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Until new data and information becomes available to address our safety concerns, we will continue to question the basis for a GRAS conclusion for bLf at these use levels and do not feel that further meetings to discuss Glanbia's current GRAS conclusion would be productive. If new data and information becomes available, please feel free to contact us again to discuss.

Best regards,

**Rachel Morissette, Ph.D.**

*Regulatory Review Scientist*

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**From:** [Cathryn Sacra](#)  
**To:** [Morisette, Rachel](#)  
**Subject:** RE: EAS/Glanbia meeting with FDA 11/29/18 - response  
**Date:** Thursday, June 13, 2019 11:38:07 AM  
**Attachments:** [image001.png](#)  
[image013.png](#)

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Thank you, Rachel. We really appreciate it.

Best regards,  
Cathryn

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**From:** Morisette, Rachel <[Rachel.Morisette@fda.hhs.gov](mailto:Rachel.Morisette@fda.hhs.gov)>  
**Sent:** Thursday, June 13, 2019 11:28 AM  
**To:** Cathryn Sacra <[csacra@easconsultinggroup.com](mailto:csacra@easconsultinggroup.com)>  
**Subject:** RE: EAS/Glanbia meeting with FDA 11/29/18 - response

Hi Cathryn,

I apologize for the delay. The FOIA office has changed our procedures and that has led to an unexpected delay in your request. However, we are going to prioritize your request today and see that you receive the documents hopefully by COB Friday. Again, I'm sorry for the delay.

Best regards,

*Rachel*

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**Rachel Morisette, Ph.D.**

*Regulatory Review Scientist*

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**From:** Cathryn Sacra <[csacra@easconsultinggroup.com](mailto:csacra@easconsultinggroup.com)>  
**Sent:** Monday, June 10, 2019 3:02 PM  
**To:** Morissette, Rachel <[Rachel.Morissette@fda.hhs.gov](mailto:Rachel.Morissette@fda.hhs.gov)>  
**Subject:** RE: EAS/Glanbia meeting with FDA 11/29/18 - response

Dear Rachel,

I hope that you are well. I wanted to follow up on the recommendation that we put in an FOI request for all the amendments and correspondence for GRN 000716, including the scientific memo, policy memo, and memorandum of meeting between CFSAN and the Center for Biologics Evaluation and Research (CBER). We submitted that request on May 10<sup>th</sup>, but have not received the information to date. I know that your office would do the redactions, if any, so wanted to see if there were any updates.

Best regards,  
Cathryn

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**From:** Morissette, Rachel <[Rachel.Morissette@fda.hhs.gov](mailto:Rachel.Morissette@fda.hhs.gov)>  
**Sent:** Friday, May 10, 2019 11:48 AM  
**To:** Cathryn Sacra <[csacra@easconsultinggroup.com](mailto:csacra@easconsultinggroup.com)>  
**Subject:** EAS/Glanbia meeting with FDA 11/29/18 - response

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Best regards,

**Rachel Morissette, Ph.D.**

*Regulatory Review Scientist*

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DATE: Wednesday, 10 April 2019

SUBJECT: RESPONSE MEMORANDUM OF MEETING (COR2018-6011)

Dear Dr. West-Barnette,

This is in response to your email message dated February 26, 2019 in which you provided a memorandum of the meeting that was held at FDA involving FDA representatives, Glanbia and EAS representatives on November 29, 2018. You also provided an attachment entitled "Illustrative References" which listed twelve references that your toxicologist considered to be important in ascertaining the safety of bovine lactoferrin in general and is of particular concern in infants who consume infant formulas. We note that three of the references are authored/co-authored by Dr. Bo Lonnerdal, who is one of the expert panel members on Glanbia's GRAS Notice. Dr. Lonnerdal was also an expert panel member of GRN 669 as well.

We have also added an additional individual to the Expert Panel, Dr. Marian Kruzel, an internationally known immunologist. You may recall that Dr. Kruzel attended and participated in our November 29, 2018 meeting. At that meeting, Dr. Kruzel gave a brief synopsis of the state of knowledge regarding lactoferrin. In fact, all sections of his narrative are included in this letter.

Your discussion of these issues is of great concern to us as we believe that bovine lactoferrin (bLf) is safe for use in infant formula (IF). In fact, we note that FDA has already approved six GRAS Notices for bLf; two of these GRNs (GRNs 465 and 669) are specific for the use of bLf in IF. As Glanbia has established that its bLf is equivalent to the bLf in the other GRNs, it stands to reason that these concerns are also applicable to the other GRNs as well.

We have placed our responses to the questions/issues that you raised directly below each question. We also note that the twelve references you provided were not tied to a specific question/concern that you raised. Where possible, we have cited the reference(s) that you provided and we believe pertains to that question/issue.

Also, at our meeting, you invited Glanbia to provide a copy of our responses for review prior to incorporating them into the document. We thank you for your review of our responses that we hope will satisfy your concerns.

Additionally, we thank you for the opportunity to address the Agency's five questions/concerns regarding whether there continues to be consensus among qualified experts that the kind of studies and endpoints presented by Glanbia, in light of currently available information on the functionality of lactoferrin, are still accepted as appropriate and sufficient to establish the safety of the intended use level of bLf in infant formula. We appreciate FDA's input and statement of questions to help support the GRAS status of bLf at levels up to 1000 mg/L

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***1) What is Glanbia’s basis for concluding that there is consensus, among scientists qualified by training and experience to assess the properties and activities of bLf in the context of the infant immune system, that no adverse effects will result from the use of bLf in the general infant population at the intended use level in infant formula?***

**Response:**

With respect to the use of bLf in infant formula, we note that FDA has already received, reviewed, and issued “good day” letters for two GRAS Notices (GRNs 465 and 669) for the use of bLf in IF. The GRAS Notice that was prepared by Glanbia contains essentially the same information and data that was used to support these IF GRAS Notices. The Expert Panel members chosen by Glanbia to review the GRAS determination are well recognized as experts in their respective fields of science and in Glanbia’s opinion, are well qualified to offer an opinion on the GRAS use of bLf in IF. The Expert Panel consists of world renowned experts, and most have published extensively in the field of lactoferrin, including an immunologist, a pediatric gastroenterologist, a professor of nutrition and internal medicine, a Dean with expertise in food science and human nutrition, a food regulatory advisor, and a toxicologist. In fact, two of the members also were involved in the preparation and review of GRN 669. Additionally, several recognized international regulatory bodies have also reviewed the use of bLf in IF and have determined that its use is safe in IF. In fact, they have reviewed the bLf produced by the same firms as were/are reviewed by FDA using essentially the same database. In addition, the use level of >600 mg/L was discussed and documented in GRN 669 even though the Notifier elected to use a lower level in one of their IFs. International regulatory bodies recognize the maximum, use level of 1000 mg/L. We are aware of at least one infant formula (IF) on the US market at this time that contains 600 mg/L and is currently on the market.

The intended use of Glanbia’s bLf in this GRAS Notice is for term infants using infant formula. These infants are presumed to be healthy at birth and are typically under the routine care of health professionals. The level of bLf in the IF is chosen to approximate the level that is found in mother’s breast milk which is considered to be the gold standard.

There is a large volume of scientific literature on lactoferrin, with consistent confirmations, for its safe use in IF. In addition, a number of papers also support the safe use at levels up to 1000 mg/L. While we note that active research is ongoing with regard to the use of bLf in IF, the prevailing consensus is that, based on the totality of the evidence currently available, the use of bLf in IF is safe when used as intended.

The reference papers that you have provided support this conclusion. For example, in the reference by Buccigrossi, V., de Marco, G., Bruzzese, E., Ombrato, L., Bracale, I., Polito, G., and Guarino, A. (2007). Lactoferrin induces concentration-dependent functional modulation of intestinal proliferation and differentiation. *Pediatr Res* 61, 410-414, the authors offered the following statement (page 414):

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“Finally, our data also have practical implications. They indicate that bovine LF exerts effects on human intestinal cells that are similar to those induced by the human isoform. The comparative experiments showed that bovine isoform is even more potent than human LF in inducing cell growth and lactase expression. LF has been proposed for a number of therapeutic purposes in human disorders, including intestinal inflammation, cancer prevention, and rotavirus infection (39– 41). Our findings add to this concept and suggest that bovine LF could be used as a functional component of infant formula to promote intestinal epithelial growth and differentiation. This effect is highly desirable, particularly in premature newborn infants or in intestinal diseases associated with epithelial atrophy.”

We concur with the assessment that bLf exerts effects on human intestinal cells that are similar to those induced by the human isoform, and also note that this paper brings up the need for more research.

In the reference paper by Fernandez-Menendez, S., Fernandez-Sanchez, M.L., Gonzalez-Iglesias, H., Fernandez-Colomer, B., Lopez-Sastre, J., and Sanz-Medel, A. (2017). Iron bioavailability from supplemented formula milk: effect of lactoferrin addition. *Eur J Nutr* 56, 2611-2620, the authors state as follows:

#### “Results

The observed results with supplemented rats were compared with those found in rats receiving maternal feeding. Interestingly, differences were found between groups in iron for transport and storage compartments, but not in the functional one, depending upon the dose of iron administered and the chemical species.

#### Conclusion

Considering the results obtained, supplementation with iron salts in excess of LF appears to be the best way of iron supplementation of formula milk.” (Page 2611)

The results of this study are further confounded as the authors further conclude:

“Finally, it should be noted to conclude that feeding rats with non-supplemented iron milk formula caused “latent iron deficiency”, that is, iron body stores became mildly depleted while the serum iron level dropped (even if, as stressed before, no change in the iron RBCs concentration was detected).

It is important to remark that the weight distribution of the animals among groups is different, despite that they were distributed into groups randomly. Differences in weight/development of the pups could result in differences in the demands and/or bioavailability of iron (that is, values observed in all the tissues and fluids analysed could not represent an accurate figure of the real bioavailability of iron from the supplement). In order to perform a statistical analysis in detail, further studies with larger number of individuals would be needed.” (page 2619)

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Based on the results in this study, we conclude that this issue is not settled and additional research is needed.

In the reference paper by Manzoni, P., Dall'Agnola, A., Tome, D., Kaufman, D.A., Tavella, E., Pieretto, M., Messina, A., De Luca, D., Bellaiche, M., Mosca, A., *et al.* (2018). Role of Lactoferrin in Neonates and Infants: An Update. *Am J Perinatol* 35, 561-565, they state as follows:

“Lactoferrin is one of the most represented and important bioactive proteins in human and mammal milk. In humans, lactoferrin is responsible for several actions targeting anti-infective, immunological, and gastrointestinal domains in neonates, infants, and young children. Evidence-based data vouch for the ability of supplemented lactoferrin to prevent sepsis and necrotizing enterocolitis in preterm infants and to reduce the burden of morbidity related to gastrointestinal and respiratory pathogens in young children. However, several issues remain pending regarding answers and clarification related to quality control, correct intakes, optimal schedules and schemes of supplementations, interactions with probiotics, and different types of milk and formulas. This review summarizes the current evidence regarding lactoferrin and discusses the areas in need of further guidance prior to the adoption of strategies that include a routine use of lactoferrin in neonates and young children.” (Page 561)

The authors’ conclusion is stated below:

“Conclusion

LF is a defense protein with diverse physiological functions. It has potent antimicrobial action against bacteria, fungi, viruses, and even some antibiotic-resistant strains. It has bacteriostatic, bactericidal, and anti-adhesion effects. LF is gaining evidence for its role in neonatal and infant medicine, although several pending issues remain regarding quality of the commercial products, safety, schedules, and safety, warranting further studies.” (Page 564)

This paper indicates that there are open questions regarding lactoferrin that should be answered. We concur. Nevertheless, the use of lactoferrin as currently used is accepted as being beneficial.

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In the review reference paper by Legrand, D. (2016). Overview of Lactoferrin as a Natural Immune Modulator. *J Pediatr 173 Suppl*, S10-15, the author's stated conclusion is below:

“Conclusions

Lactoferrin is a unique molecule within the molecular arsenal of immunity, acting on the immune system both as a weapon and as a moderator. The many molecular and cellular targets of lactoferrin, and the complex and multi-parametric networks governing immune responses relative to the threat, make understanding the mechanisms of action of lactoferrin a challenge. The observation that dietary lactoferrin may mimic the protective and immune-modulating properties of endogenous lactoferrin strongly supports the hypothesis that lactoferrin may directly influence immune cells in the gut, very likely through cell receptors, resulting in systemic responses. Elucidating the exact way lactoferrin controls the Gut-Associated Lymphoid Tissue, the major lymphoid tissue in vertebrates, will provide the key to uncovering most mechanisms by which endogenous lactoferrin controls immunity. Such knowledge will also bring invaluable information to the fine-tuning of infant milk formula development.”

We concur with the author's conclusions in that additional research regarding open questions about lactoferrin is needed.

To summarize, it is widely accepted that there are open questions regarding lactoferrin including mechanisms of action, production, utility, etc. There are some published reports that need to be replicated to confirm the results. Some of the open questions are primarily theoretical and need to be discussed and vetted more thoroughly in open forums. It is the totality of the existing evidence which should be the determining factor in assessing the safety of lactoferrin at present.

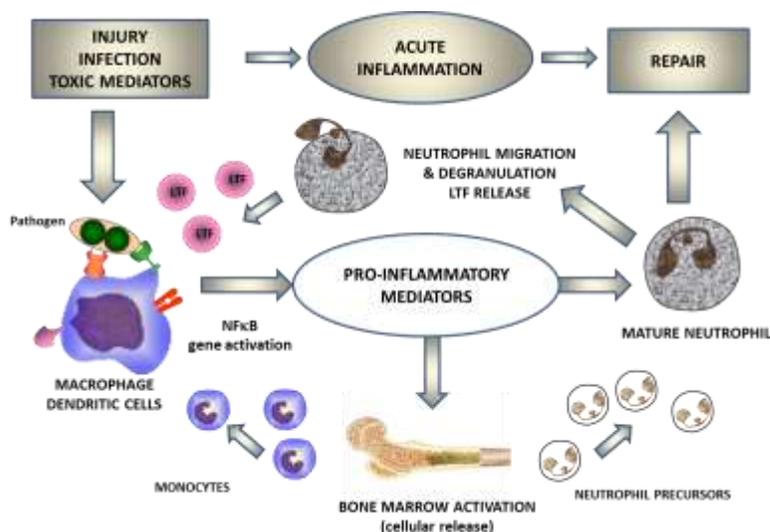
With this in mind, we offer a brief summary on lactoferrin structure and function below.

### **Lactoferrin**

Lactoferrin (LF) exhibits complex functionality with respect to immune function. It is a multifunctional iron-binding glycoprotein found in mammalian milk, tears, saliva, sweat, cerebrospinal fluid and neutrophils. It is secreted to all mucosal sites of mammals and is part of the innate immune response. By virtue of chelating iron, LF not only inhibits microbial growth but also reduces oxidative stress. It has been demonstrated that LF is able to reduce allergic response, and also protect against insult-induced mitochondrial dysfunction. While suppressing microbial growth, LF also directly exerts its first-line defense activity with significant impact on the development of adaptive immune responses by promoting the maturation of T-cell precursors into competent helper cells and by the differentiation of immature B cells into efficient antigen presenting cells. In addition, LF augments the delayed type hypersensitivity (DTH) response to antigens, leading to a strong induction of cell-mediated immunity (CMI). This summarizes what we know about functionality of LF.

The wealth of reliable literature regarding the immune regulatory functions of LF is mostly derived from *in vivo* and *in vitro* observations when species-specific endogenous LF is tested in various experimental protocols. Based on these observations the functionality of LF has been well established in various physiological conditions. Endogenous LF (neutrophil-derived) undeniably plays an important role in mediation of complex cellular responses during the development of inflammation in relation to microbial infection, trauma, or other environmental insult (see Fig. 1).

**Figure 1 LF mediates cellular responses to accommodate physiological homeostasis.** According to Kruzel et al. (Kruzel ML, Zimecki M, Actor JK. Lactoferrin in a context of Inflammation-Induced Pathology. Front Immunol. 2017 Nov 6;8:1438)



Briefly, injury defined by infection, or trauma leads to activation of the NF- $\kappa$ B signal transduction pathway within monocyte/macrophages and/or dendritic cells. This in turn stimulates the production of inflammatory mediators, which subsequently stimulates the production of fresh neutrophils and monocytes from bone marrow and activates circulating neutrophils to release various secondary mediators, including LF. By interacting with specific receptors on monocytes/macrophages and other immune and non-immune cells, LF attenuates inflammation and contributes to tissue repair and limits spread of infectious agents (Fig. 1).

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However, biological effects induced by oral administration of LF are different from the action of endogenous LF. Primarily, LF given orally helps to establish and maintain homeostasis of gastro-intestinal (GI) microbiota via safe and effective delivery of iron. It acts locally on intestinal epithelial cells before it is digested to small peptides and single amino acids. Both human and bovine LFs are recognized in a similar way by the mucosal-associated lymphoid tissue (MALT) in human gastrointestinal tract. Within the MALT, unique populations of dendritic cells interact with dietary antigens, and determine the fate of the resulting adaptive response, i.e., immunity versus tolerance. However, a portion of protease-resistant LF persists throughout the gastrointestinal tract providing local biological effects before it is excreted with feces (Dallas, Underwood, Zivkovic, & German, 2012). Subsequent to activation of immune cells in GI, lactoferrin induces various immune functions that are transduced systemically. Welch et al (Welsh KJ, Hwang SA, Boyd S, Kruzel ML, Hunter RL, Actor JK. Influence of oral lactoferrin on Mycobacterium tuberculosis induced immunopathology. Tuberculosis. 2011;91 Suppl 1:S105-13) reported that oral lactoferrin (supplied in drinking water) significantly reduced lung inflammation in mice infected with Mycobacterium tuberculosis.

Similarly, Mohamed, et al., 2019 (Mohamed, W.A.; Salama, R.M.; Schaalán, M.F. 2019. A pilot study on the effect of lactoferrin on Alzheimer's disease pathological sequelae: Impact of the p-Akt/PTEN pathway. Biomed Pharmacother. 111, 714-723), demonstrated that LF, given orally, decreased serum IL-6 and increased serum IL-10 in patients who suffered from Alzheimer Disease, which led to a reduction of cognitive decline in these patients.

In general, however, systemic effects of oral LF are not due to LF crossing the gut wall, but are a result of receptor-induced signal transduction. Therefore, both human lactoferrin (hLf) and bLf when administered orally provide similar biological effects on GI microbiota and activation of the mucosal epithelial cells including cell proliferation, differentiation, and expression of various signaling molecules.

According to Lonnerdal et al. (Bo Lönnerdal; Rulan Jiang; Xiaou Du; 2011. Bovine Lactoferrin Can Be Taken Up by the Human Intestinal Lactoferrin Receptor and Exert Bioactivities. Journal of Pediatric Gastroenterology and Nutrition. 53(6):606–614), bLf administered orally is taken up by the human lactoferrin receptor and exerts similar bioactivities as human LF on human colon epithelial cells such as induction of proliferation, differentiation, and TGFβ expression.

Finally, human milk-derived LF shares similar protein sequence, structure, and bioactivity with its bovine counterpart. Both human and bovine LFs have the same globular structure with two iron-binding sites on each lobe and an active N-terminal. Both are cationic proteins with isoelectric point (pI) around 8.5 and almost identical molecular weight as total amino acid composition is different only by two (2) amino acids (691 - human versus 689 - bovine). As the structure determines the function both human and bovine LFs are very similar in their functionality and should be considered bioequivalent.

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**2) *If the conclusion is based on the view that there are no relevant exposure-related qualitative or quantitative differences in bLF effects in infants between Glanbia's current intended use level and use levels previously considered by FDA in GRAS notices, what is the basis for this view?***

**Response:**

Glanbia's basis for determining that bLf is safe at levels of up to 1000 mg/L is based on the existing scientific data currently available. As Glanbia will be marketing its bLf to different IF manufacturers, it is possible that the manufacturer will choose a level that suits their purpose. In fact, there are IFs currently on the market that disclose various levels of bLf.

The mean daily intake of human lactoferrin for breast fed infants is approximately 1100 mg/day. It is the desire of some IF manufacturers to add bLf at levels that closely parallel the level in human breast milk.

We note that there are reports in the literature which discuss qualitative and quantitative differences on the effects of bLf in IF. In fact, several of the references that you provided discuss this in detail including beneficial effects and putative negative effects. Among these references were;

Hare, D.J., Cardoso, B.R., Szymlek-Gay, E.A., and Biggs, B.A. (2018). Neurological effects of iron supplementation in infancy: finding the balance between health and harm in iron-replete infants. *Lancet Child Adolesc Health* 2, 144-156;

Legrand, D. (2016). Overview of Lactoferrin as a Natural Immune Modulator. *J Pediatr* 173 *Suppl*, S10-15;

Lonnerdal, B. (2010). Bioactive proteins in human milk: mechanisms of action. *J Pediatr* 156, S26-30;

Lonnerdal, B. (2017). Development of iron homeostasis in infants and young children. *Am J Clin Nutr* 106, 1575S-1580S.

These references point to the fact that there are confounding issues related to feeding IF, and for that matter, human breast milk.

With regard to:

Manzoni, P., Dall'Agnola, A., Tome, D., Kaufman, D.A., Tavella, E., Pieretto, M., Messina, A., De Luca, D., Bellaiche, M., Mosca, A., *et al.* (2018). Role of Lactoferrin in Neonates and Infants: An Update. *Am J Perinatol* 35, 561-565;

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Nguyen, D.N., Li, Y., Sangild, P.T., Bering, S.B., and Chatterton, D.E. (2014). Effects of bovine lactoferrin on the immature porcine intestine. *Br J Nutr* *111*, 321-331;

Villavicencio, A., Rueda, M.S., Turin, C.G., and Ochoa, T.J. (2017). Factors affecting lactoferrin concentration in human milk: how much do we know? *Biochem Cell Biol* *95*, 12-21;

Wessling-Resnick, M. (2017). Excess iron: considerations related to development and early growth. *Am J Clin Nutr* *106*, 1600S-1605S.

These reports all point to the fact that more research is needed to determine the effects of all bio-active compounds in milk to determine how they interact with other bio-active molecules, etc. At present, the determination as to how much of any individual compound may be necessary for a healthy infant is based on a case-by-case basis.

There is a little unequivocal evidence that dietary LF can cross the gut wall intact and enter the hepatic portal system in physiologically relevant concentrations. In contrary, it was demonstrated that LF is acting directly on the GI mucosa by inducing epithelial cell proliferation, differentiation and expression of various signaling molecules. In this milieu, both human and bovine LFs are bioequivalent in a way that both are acting locally on brush border cells without crossing the gut wall barrier.

Biological effects of dietary compounds are relevant to the function of the GI tract as well as the total composition of the diet. It is worth mentioning that the diet is quickly changing over the first year of infant growth, including the introduction of solid foods, and it would be difficult to select the endpoints specific to LF but no other compounds of diet. The Johnston et al. (2015) and the Vaarala, et al. (2015) studies (see below) do discuss some specific endpoints that are discussed below.

The most relevant study for looking at the higher dose, is a double-blind, parallel-designed, gender-stratified prospective study (William H. Johnston, Claude Ashley, Michael Yeiser, Cheryl L. Harri, Suzanne I. Stolz, Jennifer L. Wampler, Anja Wittke and Timothy R. Cooper, 2015. Growth and tolerance of formula with lactoferrin in infants through one year of age: double-blind, randomized, controlled trial *BMC Pediatrics* *15*:173) 480 infants were randomized to receive a marketed routine cow's milk-based infant formula (Control; n= 155) or one of two investigational formulas with bLf at 600 mg/L (n= 165) or 1000 mg/L (n= 160) from 14–365 days of age. Investigational formulas also had a prebiotic blend of polydextrose (PDX) and galactooligosaccharides (GOS) and adjusted arachidonic acid (ARA). The primary outcome was weight growth rate from 14–120 days of age. Anthropometric measurements were taken at 14, 30, 60, 90, 120, 180, 275, and 365 days of age. Parental recall of formula intake, tolerance, and stool characteristics was collected at each time point. Medically-confirmed adverse events were collected throughout the study period. The concentrations of bLf in the test formulas are within the range of LF concentration in human milk.

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There were no group differences in growth rate (g/day) from 14–120 days of age; 353 infants completed the study through 365 days of age (Control: N =110; LF- at 600 mg/L N= 127; LF @ 1000 mg/L N= 116). Few differences in growth, formula intake, and infant fussiness or gassiness were observed through 365 day of age. Group discontinuation rates and the overall group incidence of medically-confirmed adverse events were not significantly different. From 30 through 180 days of age, group differences in stool consistency ( $P < 0.005$ ) were detected with softer stools for infants in the 600 mg/L and 1000 mg/L groups versus Control. Compared to the Control, infants who received investigational formulas with bLf and the prebiotic blend of PDX and GOS experienced a softer stooling pattern similar to that reported in breastfed infants.

Vaarala et al. (Vaarala O, Saukkonen T, Savilahti E, Klemola T, Akerblom HK. 1995. Development of immune response to cow's milk proteins in infants receiving cow's milk or hydrolyzed formula. *J Allergy Clin Immunol.* Dec;96(6 Pt 1):917-23) showed that feeding infants with cow's milk-based formula induced systemic humoral and cellular responses to cow's milk proteins. However, T-cell response later declined, supporting the concept of oral tolerization to the T-cells.

The Johnston study demonstrated that routine infant formulas with the higher levels of bLf were safe, well-tolerated, and associated with normal growth when fed to healthy term infants through 365 days of age. This combined with the Vaarala study that showed oral tolerization with regard to T-cells, in addition to a history of safe use, as described in the GRAS document, help support the safety of bLf at 1000 mg/L.

Indeed, a significant body of evidence from published intervention studies supports the safety of bLf for infants (GRN 669). 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. As discussed above, the mean daily intake of human lactoferrin for breast fed infants is approximately 1100 mg/day. 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 at 1000 mg/L for the intended uses. In addition, they show that both lower and higher concentrations of bLf are safe.

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The EFSA opinion (EFSA Panel on Dietetic Products Nutrition and Allergies (NDA). (2012b). Scientific opinion on bovine lactoferrin. *EFSA Journal*, 10 (5), 2701, [Available at: <http://onlinelibrary.wiley.com/doi/10.2903/j.efsa.2012.2701/epdf>)] includes the following:

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

Therefore, based on the most recent evaluation of the publicly available data and observations, regarding bioequivalence of bovine milk derived LF and its human counterpart we believe that the total estimated intake up to 1000 mg/L of bLF is safe.

**3) *If the conclusion is based on the view that none of the physiological effects generated by the properties and activities of bLF at the intended use level in this population are relevant factors in a safety assessment, what is the basis for this view?***

**Response:**

We believe the references by Lonnerdal speaks to this issue.

Lonnerdal, B. (2010). Bioactive proteins in human milk: mechanisms of action. *J Pediatr* 156, S26-30.

“Although many factors are likely to be responsible for these differences, it is apparent that breast milk provides a multitude of bioactive proteins that are capable of physiological activities in the newborn infant and therefore can affect short- and long-term outcomes.” (page S26).

“...From this study, it cannot be ascertained that lactoferrin was responsible for the observed effect because there was no lactoferrin-only group. There is also a possible synergistic effect of lactoferrin and lysozyme as reported in an in vitro study by Ellison and Giehl.<sup>14</sup> Lactoferrin has also been shown to have antiviral activity against hepatitis C virus, cytomegalovirus, Herpes simplex virus, rotavirus, adenovirus, and human immunodeficiency virus.<sup>15</sup>

Three recent studies support that lactoferrin may prevent infections in children.” (page S26).

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In addition, we uncovered another article by Lonnerdal et al. and Bhatia which also addresses this issue.

Bovine Lactoferrin Can Be Taken Up by the Human Intestinal Lactoferrin Receptor and Exert Bioactivities, Lönnerdal, Bo; Jiang, Rulan; Du, Xiaou, Journal of Pediatric Gastroenterology and Nutrition: **December 2011 - Volume 53 - Issue 6 - p 606–614** [Available at: [https://journals.lww.com/jpgn/Fulltext/2011/12000/Bovine Lactoferrin Can Be Taken Up by the Human.8.aspx#pdf-link](https://journals.lww.com/jpgn/Fulltext/2011/12000/Bovine_Lactoferrin_Can_Be_Taken_Up_by_the_Human.8.aspx#pdf-link)].

“Conclusions: CbLF is biologically active and is likely to exert several of the bioactivities of hLF if added to infant formula.” (page 606).

Bovine Lactoferrin, Human Lactoferrin, and Bioactivity, Bhatia, Jatinder, Journal of Pediatric Gastroenterology and Nutrition: **December 2011 - Volume 53 - Issue 6 - p 589** [Available at: [https://journals.lww.com/jpgn/FullText/2011/12000/Bovine Lactoferrin, Human Lactoferrin, and.4.aspx#print-article-link](https://journals.lww.com/jpgn/FullText/2011/12000/Bovine_Lactoferrin,_Human_Lactoferrin,_and.4.aspx#print-article-link)].

“Bovine LF (bLF) inhibits the growth of a wide variety of bacteria, fungi, viruses, and parasites. Furthermore, a high homology between the human and bovine forms of LF suggests that supplementation of infant formulas with LF may provide similar protection against sepsis as observed with the use of human milk.” (page 589).

Biological effects of dietary compounds are relevant to the function of the gastro-intestinal tract as well as total composition of the diet.

A primary function of the GI tract is to digest dietary macromolecules and absorb the resultant nutrients into the hepatic portal system. Large proteins are first digested to peptides by gastric and pancreatic proteases, then are taken by peptidases present on the enterocytic brush border and broken down to smaller peptides and individual amino acids. Although the absorption of di- and tri-peptides is possible across the apical membrane in human GI, the mechanism of such absorption is still poorly documented. In general, there is little unequivocal evidence that dietary bioactive peptides can cross the gut wall intact in physiologically relevant concentrations (Miner-Williams WM, Stevens BR, Moughan PJ. 2014. Are intact peptides absorbed from the healthy gut in the adult human? Nutr Res Rev 27:308–29).

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However, in large studies on weaning piglets some of orally administered bLf was observed in the bile suggesting an active transport of LF by endocytosis via the epithelial cells into the bloodstream (Harada, E., Itoh, Y., Sitizeo, K., Takeuchi, T., Araki, Y., & Kitagawa, H., 1999). Characteristic transport of lactoferrin from the intestinal lumen into the bile via the blood in piglets. *Comp Biochem Physiol A Mo/ Integr Physiol*, 124(3), 321-327). In general, however; the bioavailability of dietary peptides is very poor.

Again, human milk-derived LF shares similar protein sequence, structure, and bioactivity with its bovine counterpart. Both human and bovine LFs have the same globular structure with two iron-binding sites on each lobe and an active N-terminal. Both are cationic proteins with pI around 8.5 and almost identical molecular weight as total amino acid composition is different by two (2) amino acids (691 - human versus 689 - bovine). As the structure determines the function both human and bovine LFs are similar in their functionality and should be considered bioequivalent.

Based on a long history of safe and beneficial utility of bovine milk in human nutrition it is conceivable that individual components of human and bovine milk are bioequivalent. Mostly, any effects are localized to the GI tract, with some being transduced systemically.

Although the primary function of the GI tract is to digest food to make the macromolecules easy to absorb, there is also an important immune aspect of GI function in humans. The GI tract, which is the largest immunologic organ in the body, is constantly exposed to an enormous array of exogenous antigens including commensal bacteria and ingested proteins. A single epithelial layer separates this antigenic load from the lymphocytes, antigen presenting cells (APC), stromal cells and other immune cells in the lamina propria that together comprise the mucosal-associated lymphoid tissue (MALT). Within the MALT, unique populations of dendritic cells (DCs) interact with dietary antigens, and determine the fate of the resulting adaptive response, i.e., immunity versus tolerance. According to Vaarala et al. (Vaarala O, Saukkonen T, Savilahti E, Klemola T, Akerblom HK. Development of immune response to cow's milk proteins in infants receiving cow's milk or hydrolyzed formula. *J Allergy Clin Immunol*. 1995 Dec;96(6 Pt 1):917-23), feeding infants with cow's milk-based formula induced systemic humoral and cellular responses to cow's milk proteins. T-cell response later declined, supporting the concept of oral tolerization.

Human tolerance and safety of bLf has been established in a large number of intervention studies in infants (pre-term and VLBW, term) and young children (C. William H. Johnston, Claude Ashley, Michael Yeiser, Cheryl L. Harris, Suzanne I. Stolz, Jennifer LBMC *Pediatr*. 2015; 15: 173. Growth and tolerance of formula with lactoferrin in infants through one year of age: double-blind, randomized, controlled trial). The studies consistently report that the addition of bLf to formula or as a supplement was well tolerated, or that no adverse treatment-related effects were observed. Furthermore, the range of bLf safely consumed and tolerated in these studies is higher than the maximum predicted EDI's of bLf in other reports (mean 1023 mg/day, or 179 mg/kg BW/day, 90th percentile 1484 mg/day or 269 mg/kg/BW/day) in term infants aged 0 - 6 months) in term infants aged 0 - 6 months.

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It is important to emphasize that the mean daily intake of human lactoferrin for breast fed infants is approximately 1100 mg/day, which is higher than the bLf level proposed by Glanbia.

**4) *If the conclusion is based on the view that bLF and hLF are equivalent in their effects on infant physiology, what is the basis for that view?***

**Response:**

FDA has already approved six GRAS Notices for bLf; two of these GRNs (GRNs 465 and 669) are specific for the use of bLf in IF. As Glanbia has established that its bLf is equivalent to the bLf in the other GRNs, it stands to reason that these concerns are also applicable to the other GRNs as well. Nevertheless, we have included additional bioequivalent justification.

A primary function of the GI tract is to digest dietary macromolecules and absorb the resultant nutrients into the hepatic portal system. Large proteins are first digested to peptides by gastric and pancreatic proteases, then are taken by peptidases present on the enterocytic brush border and broken down to smaller peptides and individual amino acids. Human milk-derived LF shares similar protein sequence, structure, and bioactivity with its bovine counterpart. Both human and bovine LFs have the same globular structure with two iron-binding sites on each lobe and an active N-terminal. Both are cationic proteins with a similar isoelectric point and almost identical molecular weight as total amino acid composition is different only by two amino acids (691 - human versus 689 - bovine). As the structure determines the function, both human and bovine LFs are very similar in their functionality and should be considered bioequivalent, especially noting a long history of safe and beneficial utility of bovine milk in human nutrition.

As discussed earlier, both human and bovine LFs are bioequivalent in a way that both are acting locally on brush border cells without crossing the gut wall barrier.

This conclusion is also supported by the conclusion/statements made in the reports below:

Bovine Lactoferrin Can Be Taken Up by the Human Intestinal Lactoferrin Receptor and Exert Bioactivities, Lönnerdal, Bo; Jiang, Rulan; Du, Xiaou, Journal of Pediatric Gastroenterology and Nutrition: **December 2011 - Volume 53 - Issue 6 - p 606–614** [Available at: [https://journals.lww.com/jpgn/Fulltext/2011/12000/Bovine\\_Lactoferrin\\_Can\\_Be\\_Taken\\_Up\\_by\\_the\\_Human.8.aspx#pdf-link](https://journals.lww.com/jpgn/Fulltext/2011/12000/Bovine_Lactoferrin_Can_Be_Taken_Up_by_the_Human.8.aspx#pdf-link)].

“Conclusions: CbLF is biologically active and is likely to exert several of the bioactivities of hLF if added to infant formula.” (page 606).

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Bovine Lactoferrin, Human Lactoferrin, and Bioactivity, Bhatia, Jatinder, *Journal of Pediatric Gastroenterology and Nutrition*: **December 2011 - Volume 53 - Issue 6 - p 589** [Available at: [https://journals.lww.com/jpgn/FullText/2011/12000/Bovine Lactoferrin, Human Lactoferrin, and.4.aspx#print-article-link](https://journals.lww.com/jpgn/FullText/2011/12000/Bovine_Lactoferrin,_Human_Lactoferrin,_and.4.aspx#print-article-link)].

“Bovine LF (bLF) inhibits the growth of a wide variety of bacteria, fungi, viruses, and parasites. Furthermore, a high homology between the human and bovine forms of LF suggests that supplementation of infant formulas with LF may provide similar protection against sepsis as observed with the use of human milk.” (page 1).

Buccigrossi, V., de Marco, G., Bruzzese, E., Ombrato, L., Bracale, I., Polito, G., and Guarino, A. (2007). Lactoferrin induces concentration-dependent functional modulation of intestinal proliferation and differentiation. *Pediatr Res* 61, 410-414.

“Finally, our data also have practical implications. They indicate that bovine LF exerts effects on human intestinal cells that are similar to those induced by the human isoform. The comparative experiments showed that bovine isoform is even more potent than human LF in inducing cell growth and lactase expression. LF has been proposed for a number of therapeutic purposes in human disorders, including intestinal inflammation, cancer prevention, and rotavirus infection (39–41). Our findings add to this concept and suggest that bovine LF could be used as a functional component of infant formula to promote intestinal epithelial growth and differentiation. This effect is highly desirable, particularly in premature newborn infants or in intestinal diseases associated with epithelial atrophy.” (page 414).

Milk is a complex and complete source of bioactive molecules that help protect the newborn against infectious diseases and promote development while selectively enriching a protective and beneficial gut microbiota (Pacheco A.R., Barile D., Underwood M.A., Mills D.D. The impact of milk glycol biome on the neonate gut microbiota *Annu Rev Anim Biosci.* 2015 Feb 16; 3: 419–445). Epidemiological data suggest that human milk provides unique health benefits during early infancy that extend to long-lasting benefits. Lactoferrin in particular is known to facilitate proper colonization of infant’s gut, and protect against diarrhea by preventing the attachment of enteropathogens in the gut (Ochoa Theresa, Cleary T.G. Effect of lactoferrin on enteric pathogens. *Biochemie* 2009, 91(1); 30-34). Although it may be difficult to assign specific functions for individual components of milk in development of infant’s immune system it is well established that LF is acting on the GI mucosa by inducing epithelial cell proliferation, differentiation and expression of various signaling molecules. In this context, both human and bovine LFs, as structurally similar proteins, exert their bioequivalent functions by acting locally on brush border cells without crossing the gut wall barrier.

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Therefore, based on the most recent evaluation of the publicly available data and observations, regarding bioequivalence of bovine milk derived LF and its human counterpart we believed that the total estimated intake up to 1000 mg/L of bLf is safe.

- 5) *Subsequent to our meeting, we identified an additional issue. Given that: a) bLF differs with hLF in iron saturation; b) infants' needs for exogenous iron differ developmentally, as well as individually; c) there appears to be debate about iron homeostasis in infants younger than 9 months, what is the basis for concluding that bLF exposure resulting from the intended use would not be a safety concern?*

**Response:**

The following five articles indicate that there are open questions regarding iron supplementation and status in infants as they mature to children and adults, and that more research is needed. We concur. Nevertheless, the use of naturally occurring higher levels of LF in nursing infants and exposures of lactoferrin in IF as currently used is accepted as being beneficial.

Fernandez-Menendez, S., Fernandez-Sanchez, M.L., Gonzalez-Iglesias, H., Fernandez-Colomer, B., Lopez-Sastre, J., and Sanz-Medel, A. (2017). Iron bioavailability from supplemented formula milk: effect of lactoferrin addition. *Eur J Nutr* 56, 2611-2620.

In this study rats (N = 3/group) were randomly assigned to groups and it was determined that there was a difference in weight distribution in each dose group initially. Therefore, the study should be rerun with a larger N for statistical robustness.

The authors concluded in part, that the “Finally, it should be noted to conclude that feeding rats

with non-supplemented iron milk formula caused “latent iron deficiency”, that is, iron body stores became mildly depleted while the serum iron level dropped (even if, as stressed before, no change in the iron RBCs concentration was detected).”

Hare, D.J., Cardoso, B.R., Szymlek-Gay, E.A., and Biggs, B.A. (2018). Neurological effects of iron supplementation in infancy: finding the balance between health and harm in iron-replete infants. *Lancet Child Adolesc Health* 2, 144-156.

The authors state:

“What is clear from the evidence presented here is that little consensus exists on what the precise benefits and potential harms of iron supplementation are; numerous studies have identified a benefit in settings where iron deficiency anaemia is endemic, in terms of restoring haematological markers to those considered acceptable (panel 2), although the effects on neurodevelopment are not as obvious. Iron is crucial for

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neurodevelopment, although direct intervention has become somewhat controversial, and will remain so until further large-scale longitudinal trials are able to categorically confirm or refute long-term benefits.”

This reference points to the fact that there are confounding issues related to iron supplementation in IF.

Lonnerdal, B. (2017). Development of iron homeostasis in infants and young children. *Am J Clin Nutr* 106, 1575S-1580S.

The authors concluded:

“Studies in human infants and experimental animals suggest that iron homeostasis is absent or limited in early infancy, which is largely due to a lack of regulation of the iron transporters

DMT1 and ferroportin. The high and unregulated absorption of iron in the newborn period may confer developmental benefits but raises the possibility of excessive iron accumulation.”

Lonnerdal, B., Georgieff, M.K., and Hernell, O. (2015). Developmental Physiology of Iron Absorption, Homeostasis, and Metabolism in the Healthy Term Infant. *J Pediatr* 167, S8-14.

“Iron is unique as there is no natural route for excreting excess iron. Thus, the possibility of overload certainly exists and is well known in adults. However, iron overload from orally provided iron as such has not been described in term human infants, and only implicated in premature infants with a known, or feared, consequence of increased iron-associated oxidative damage. Indications of excessive iron intakes by infants have been observed recently. As mentioned above, we noticed that supplementation of Swedish healthy, term breast-fed infants with iron drops caused decreased linear growth by 9 months of age (54). Since this adverse effect was not noted in Honduran infants, we hypothesized that the adverse effect was due to the iron-replete status of the Swedish infants. Indeed, when the Honduran cohort was divided into iron-replete and iron non-replete infants an adverse effect on growth was observed in the iron-replete group. A few other studies have also shown negative effects of iron supplements on growth (55, 56). However, in those studies the effect was noted for reduced weight gain rather than linear growth. It should be noted, though, that the nutritional status of the infants in those studies was compromised overall, which is known to decrease linear growth and cause stunting. Thus, when linear growth is compromised it is possible that the adverse effect of excess iron may be manifested differently and instead affects weight gain. However, a recent study on breastfed US infants given iron drops showed both a significant reduction in length gain and a trend towards reduced weight gain as compared to infants given iron-fortified cereals (57). In the studies cited above, iron drops were given.

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Since iron fortification is likely to result in less iron being absorbed, the potential risk of excess iron in this form may be lower. However, Lozoff et al (58) found that whereas infants with an initially low Hb (<106 g/L) benefitted from infant formula containing a higher level of iron (12.7 mg/L) and showed better developmental outcomes at 10 years of age than those given formula with less iron (2.3 mg/L) from 6 to 12 months of age, those with an initial Hb above 128 g/L showed worse scores when given formula with the higher level of iron. This suggests that an excess of fortification iron also may result in adverse effects.”

Further studies are needed to explore the mechanism behind the adverse effect of excess iron.

Wessling-Resnick, M. (2017). Excess iron: considerations related to development and early growth. *Am J Clin Nutr* 106, 1600S-1605S.

The authors stated: “In conclusion, whether high iron affects erythropoiesis and, in particular, stress erythropoiesis is not clear. The influence of the iron sensor TfR2 on erythropoietin sensitivity may be relevant in iron-loading anemia. Ineffective erythropoiesis will evolve an anemic state that has been repeatedly shown to be detrimental to early development. Molecular studies to determine what steps in erythropoiesis are sensitive to a high iron condition could provide insight into potential interventions. Similarly, despite our knowledge that several key micronutrients (e.g., vitamin A, copper, manganese, and zinc) support iron’s function in erythropoiesis, how these nutrients interact remains unknown to our knowledge. It is necessary to consider many factors when formulating recommendations for iron supplementation because

these nutrient-nutrient interactions could possibly contribute to iron-induced toxicity. Research on mixtures of micronutrients can be carried out in both human and animal studies to establish a more comprehensive and holistic view of nutritional needs during pregnancy and early childhood.”

Ochoa et al., 2012 (Ochoa, T. J., Pezo, A., Cruz, K., Chea-Woo, E., & Cleary, T. G., 2012. Clinical studies of lactoferrin in children. *Biochem Cell Biol*, 90(3), 457-467) showed no effect on the effects on iron status in infants fed bLf supplemented formula.

One study by Chierici, et al. (Chierici, R., Sawatzki, G., Tamisari, L., Volpato, S., & Vigi, V. (1992). Supplementation of an adapted formula with bovine lactoferrin. 2. Effects on serum iron, ferritin and zinc levels. *Acta Paediatr*, 81(6-7), 475-479) showed that infants receiving the higher dose of bLf (100mg / 100 ml) had significantly higher serum ferritin levels at days 90 and 150, while ferritin levels of breast-fed infants were significantly higher than in non-supplemented formula-fed infants at day 30 and day 90.

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Davidsson, et al. (Davidsson, L., Kastenmayer, P., Yuen, M., Lönnerdal, B., & Hurrell, R. F. (1994). Influence of Lactoferrin on Iron Absorption from Human Milk in Infants. *Pediatr Res*, 35(1), 117-124) measured iron absorption in infants fed breast milk (with its native content of LF) and the same milk from which LF had been removed. Fractional iron absorption was significantly lower from breast milk than from LF-free breast milk. The authors concluded that the results do not support a direct role for LF in the enhancement of Fe absorption from human milk.

Jenkins and Griffiths (Jenkins P and Griffiths J. 2014. Lactoferrin supplementation for very preterm infants. *Infant 10* (5) 147-150. Accessed at: [http://www.infantjournal.co.uk/pdf/inf\\_059\\_ent.pdf](http://www.infantjournal.co.uk/pdf/inf_059_ent.pdf)) of the ELFIN Trial Investigators Group stated because bovine lactoferrin does not bind strongly to the lactoferrin receptor in the human small intestine, it is not absorbed via the gastrointestinal tract and does not generate hypersensitivity or allergic immunological reactions.

More recently, Lonnerdal (Lönnerdal, B. (2016). Bioactive Proteins in Human Milk: Health, Nutrition, and Implications for Infant Formulas. *J Pediatr*, 173 Suppl, S4-9.) stated that the intestinal mucosa of breastfed infants is more developed than that of formula-fed infants. Increased mucosal development caused by lactoferrin may, therefore, increase the mucosal surface and not only enhance the uptake of iron but also of other nutrients.

The iron status of piglets fed control, antibiotic supplemented or bLf supplemented formula for 30 days was determined. Lactoferrin supplementation significantly increased serum iron values over controls by 22% and 23%, on days 15 and 30, respectively, but did not affect serum total iron-binding capacity at either time point (Shan, T., Wang, Y., Wang, Y., Liu, J., & Xu, Z. (2007). Effect of dietary lactoferrin on the immune functions and serum iron level of weanling piglets. *J Anim Sci*, 85(9), 2140-2146).

There is a large volume of scientific literature on lactoferrin with reliable evidence for the safe use of bLf in IF at various levels. We are also aware that there are publications supporting the safe use of bLf at levels up to 1000 mg/L.

A significant body of evidence from published intervention studies supports the safety of bLf for infants (GRN 669). 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 mean daily intake of human lactoferrin for breast fed infants is approximately 1100 mg/day. 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 at 1000 mg/L for the intended uses.

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The EFSA opinion (EFSA Panel on Dietetic Products Nutrition and Allergies (NDA). (2012b). Scientific opinion on bovine lactoferrin. *EFSA Journal*, 10 (5), 2701. [Available at: <http://onlinelibrary.wiley.com/doi/10.2903/j.efsa.2012.2701/epdf>] includes the following, confirming in an exhaustive review that there were not any untoward effects of bLf at levels of 1000 mg/L:

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

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

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

Biological effects of dietary compounds are relevant to the function of gastro-intestinal tract as well as total composition of diet. It is worth mentioning that the diet is quickly changing over the first year of infant growth, and it may be difficult to select the endpoints specific to LF but no other compounds of diet. Therefore, in two studies relevant to LF functionality the endpoints are rather tox related than immune function. In addition, development of humoral and cellular immune responses to orally administered antigens in humans is poorly understood, although antigen administration has been suggested as a treatment for hypersensitivity disorders and autoimmune diseases.

In a double-blind trial (Vaarala O, Saukkonen T, Savilahti E, Klemola T, Akerblom HK. 1995. Development of immune response to cow's milk proteins in infants receiving cow's milk or hydrolyzed formula. *J Allergy Clin Immunol*. Dec; 96 (6 Pt 1):917-23.), 10 infants received cow's milk-based formula, and 10 infants received a casein hydrolysate formula until the age of 9 months. Blood samples were taken at the ages of 6, 9, and 12 months. Cellular responses were assessed by proliferation assay of peripheral blood mononuclear cells to cow's milk proteins (beta-lactoglobulin, bovine serum albumin, and alpha-casein). Humoral responses to the same proteins were measured by ELISA for IgG antibodies.

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Feeding infants with cow's milk-based formula induced systemic humoral and cellular responses to cow's milk proteins. T-cell response later declined, supporting the concept of oral tolerization. Exposure to cow's milk proteins after the age of 9 months resulted in depressed cellular and humoral responsiveness to these proteins.

In other, double-blind, parallel-designed, gender-stratified prospective study (William H. Johnston, Claude Ashley, Michael Yeiser, Cheryl L. Harri, Suzanne I. Stolz, Jennifer L. Wampler, Anja Wittke and Timothy R. Cooper. Growth and tolerance of formula with lactoferrin in infants through one year of age: double-blind, randomized, controlled trial *BMC Pediatrics* (2015) 15:173) 480 infants were randomized to receive a marketed routine cow's milk-based infant formula (Control; n= 155) or one of two investigational formulas with bLf at 0.6 g/L (LF-0.6;n= 165) or 1.0 g/L (LF-1.0;n= 160) from 14–365 days of age. Investigational formulas also had a prebiotic blend of polydextrose (PDX) and galactooligosaccharides (GOS) and adjusted arachidonic acid (ARA). The primary outcome was weight growth rate from 14–120 days of age. Anthropometric measurements were taken at 14, 30, 60, 90, 120, 180, 275, and 365 days of age. Parental recall of formula intake, tolerance, and stool characteristics was collected at each time point. Medically-confirmed adverse events were collected throughout the study period. The concentrations of bLf in the test formulas are within the range of LTF concentration in human milk.

In conclusion, there were no group differences in growth rate (g/day) from 14–120 days of age; 353 infants completed the study through 365 days of age (Control: 110; LF-0.6: 127; LF-1.0: 116). Few differences in growth, formula intake, and infant fussiness or gassiness were observed through 365 day of age. Group discontinuation rates and the overall group incidence of medically-confirmed adverse events were not significantly different. From 30 through 180 days of age, group differences in stool consistency ( $P < 0.005$ ) were detected with softer stools for infants in the LF-0.6 and LF-1.0 groups versus Control. Compared to the Control, infants who received investigational formulas with bLf and the prebiotic blend of PDX and GOS experienced a softer stooling pattern similar to that reported in breastfed infants.

This study demonstrated that routine infant formulas with bLf were safe, well-tolerated, and associated with normal growth when fed to healthy term infants through 365 days of age.

### **Additional Supporting Publications**

In addition to the above, we have undertaken to review other documents and reviews that speak to the use of bLf in IF. Below, we have inserted excerpts and conclusions from these papers to further support the fact that bLf at levels up to 1000 mg/L is safe and acceptable for use in IF.

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In a review paper entitled “Structure and Functions of Lactoferrin as Ingredient in Infant Formulas”, Aly et al. [J Food Research, Vol 2, 25 – 36 (2013); Available at: [https://www.researchgate.net/publication/263991247\\_Structure\\_and\\_Functions\\_of\\_Lactoferrin\\_as\\_Ingredient\\_in\\_Infant\\_formulas/download](https://www.researchgate.net/publication/263991247_Structure_and_Functions_of_Lactoferrin_as_Ingredient_in_Infant_formulas/download)], reviewed the current status of lactoferrin in infant formulas. We have copied salient points from their discussion and conclusion below.

“In general, infant formulas have been designed to provide infants with all the required nutrients, being an adequate nutritional formula. For that purpose, infants with an age of 0-6 months, it has estimated to be safe an intake of approximately 1.2 g bovine lactoferrin per day from infant formula containing 200 mg bovine lactoferrin /100 g (European Food Safety Authority, EFSA, 2012). However, research advances are focused on those substances in human milk, which serve other than traditional nutritional roles. Attempts are in progress to supplement infant formulas with protective and trophic factors so far unique only to human milk. The final aim is not necessarily to mimic the composition of human milk in every respect, but to achieve physiological effects as in breast fed infants (Gallego, Pérez-Conesa, Bernal Cava, Periago-Castón, & Ros, 2009). Since human milk contains a considerable amount of lactoferrin, special attention is paid to its functional role. Many of those functions are directly related to its ability to bind iron, that is, its effect on iron absorption and bacteriostatic and antioxidant activities. Based on this, the addition of lactoferrin to infant formulas seems to be reasonable; nevertheless, the supplementation of infant formulas should be discussed intensively because there has to be a scientifically proven advantage for the infant to get this protein by daily formula (Sawatzki, 1997). Recently, EFSA (2012) accepted and approved bovine lactoferrin as a new food ingredient. Nowadays, there are many infant formulas supplemented with lactoferrin available in the market (Mulder, Connellan, Oliver, Morris, & Stevenson, 2008). From results obtained by different authors, it can be concluded that the addition of lactoferrin, usually bovine, to infant formulas, does not affect iron absorption. However, given its ability to bind iron, its use in infant formulas could be useful for protecting the gut of infants against infections from microbial-requiring iron, its ability to reduce interelemental interactions and especially to protect infant formulas supplemented with iron and ascorbic acid against free radical formation.

In this context, Raiten, Talbot and Waters (1998) and Wakabayashi et al. (2006) reported that it is possible to enrich infant formulas with bovine or recombinant human lactoferrin, although the former does not seem to affect iron absorption, probably because of an incompatibility with the intestinal receptors, and in the latter, there is not enough available information to evaluate toxicity. In this regard, it must be taken into account that the enrichment of infant formulas with human lactoferrin would probably lead to an improvement in their amino acidic profile, making it more similar to that of human milk (Jovani, Barberá, & Farré, 2001). The EFSA (2012) considered that the bovine lactoferrin, is essentially protein constituent of cow milk and is considered a novel food ingredient. Bovine lactoferrin is present in the novel food ingredient mostly as non-denatured lactoferrin. It must be noted that lactoferrin is a normal constituent of human milk, and that the intended consumption of the bovine lactoferrin is within the levels of human lactoferrin consumed in breast milk by infants; human lactoferrin is also non-denatured.”

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#### “Conclusion

The present review directs the attention towards some of the functional roles of lactoferrin and its roles in increasing the functional benefits of infant formulas. Lactoferrin is a new strategy for overcome some disease whether by orally administration or by food supplementation. Now is authorized and recommended using of lactoferrin as a new bioactive ingredient in the manufacturing of infant formulas to provide infants with nutritional and healthy effects especially for formula-fed infants and also after first 4-6 months. Many studies are required to study the effect of manufacturing and storage of infant formulas on lactoferrin. Also, it is possible using lactoferrin-derived functionally peptides for enrichment the infant formulas and this may be one of the growing and promising field of research.”

Even though the values sited in the paper are somewhat different than the EFSA doc, we concur with the authors’ assessment of the status of bLf.

We also call to your attention the research paper by Johnston et al. (Johnston, W.H., Ashley, C., Yeiser, M., Harris, C.L., Stolz, S.I., Wampler, J., Cooper, T.R. 2015. Growth and tolerance of formula with lactoferrin in infants through one year of age: Double-blind, randomized, controlled trial. BMC Pediatr 15(1):173. doi:10.1186/s12887-015-0488-3) where they concluded as follows:

#### “Conclusions

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Routine intact cow’s milk protein infant formulas with bLf at 0.6 and 1.0 g/L were associated with age-appropriate growth throughout the first year of life. This was the first large-scale pediatric nutrition trial in which formulas used concentrations of bLf that are within the range of Lf reported for mature human milk and included the prebiotic blend of PDX and GOS. Compared to infants who received the Control formula, infants who received investigational formulas with the prebiotic blend of PDX and GOS and bLf at 0.6 or 1.0 g/L experienced a softer stooling pattern similar to that reported in breastfed infants. Consequently, this study demonstrated that routine infant formulas with bLf, a blend of PDX and GOS, and adjusted ARA were safe, well-tolerated, and associated with normal growth when fed to healthy term infants throughout the first year of life.”

We concur with the authors’ assessment.

In a publication entitled “Lactoferrin: A Critical Player in Neonatal Host Defense” (Telang S, 2018, Nutrients 10: 1228) reported on the role of lactoferrin infant nutrition. This paper discussed anti-microbial effects, immunomodulatory functions, efficacy, etc. of lactoferrin. The research is summarized as follows:

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“Abstract: Newborn infants are at a high risk for infection due to an under-developed immune system, and human milk has been shown to exhibit substantial anti-infective properties that serve to bolster neonatal defenses against multiple infections. Lactoferrin is the dominant whey protein in human milk and has been demonstrated to perform a wide array of antimicrobial and immunomodulatory functions and play a critical role in protecting the newborn infant from infection. This review summarizes data describing the structure and important functions performed by lactoferrin in protecting the neonate from infection and contributing to the maturation of the newborn innate and adaptive immune systems. We also briefly discuss clinical trials examining the utility of lactoferrin supplementation in the prevention of sepsis and necrotizing enterocolitis in newborn infants. The data reviewed provide rationale for the continuation of studies to examine the effects of lactoferrin administration on the prevention of sepsis in the neonate.”

The author’s conclusion supports the use of lactoferrin as stated below:

“Conclusions

Taken together, the experimental and pre-clinical studies examining the functions of Lf present overwhelming evidence, supporting a pivotal role for this multifaceted glycoprotein in preventing infection, in immunomodulation, and bolstering host defense. Many questions remain to be answered regarding the function of this glycoprotein at the molecular level and the extent of direct and immune modulatory effects caused by supplementation of Lf in the diet. Several of these questions are best addressed by in vivo studies in patients. These are challenging studies, particularly as they are targeted towards the critical VLBW infant. However, the clinical data obtained thus far have been promising and certainly support the utility of continuation of studies to examine the effects of Lf supplementation on modulating the immune response and decreasing life-threatening infections in the highly vulnerable neonatal population. Several studies are currently underway, and their results will serve to clarify the benefits of Lf supplementation in the diet of the term and preterm infant, and potentially pave the way to using Lf in the clinical setting.”

We concur with the author’s conclusion.

In a publication entitled “Lactoferrin in a Context of Inflammation-Induced Pathology”, Kruzel et al. (Kruzel ML, Zimecki M, Actor JK. 2017. *Frontiers in Immunology* (8): Article 1438; doi: 10.3389/fimmu.2017.01438), the authors discussed the role of lactoferrin and concluded as follows:

“Conclusion

In conclusion, LTF plays a major functional role in physiologic homeostasis as related to development of disease and associated pathology. In many cases, LTF fulfills its anti-inflammatory roles *via* different cell receptors and activation of various cell signalling pathways, often through iron-dependent mechanisms. In fact, the ability of LTF to both sequester iron and to direct reactive oxygen intermediates is a major factor in

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lessening damage due to excessive inflammatory responses. The immunomodulatory nature of this protein derives from its unique ability to sense the immune activation status of an organism and act accordingly.

The interaction of LTF with its receptors can trigger “redundant” protective effects as reflected by (1) regulation of enzyme activities and ROS production; (2) immune deviation and modulation; (3) change of cell phenotype and cytokine profile; (4) binding to LPS or competition with its receptors, and (5) prevention of cell apoptosis. Many additional immune pathways are also affected, which culminate in the consequence of attenuated pathological changes as tissue repair processes are initiated.”

We concur with the authors’ assessment of the current state of the art regarding lactoferrin.

There is a large volume of scientific literature on lactoferrin with reliable evidence for the safe use of bLf in IF . While we note that active research is ongoing in the area of bLf in IF, the prevailing consensus is that, based on the totality of the evidence currently available, the use of bLf in IF is safe when used as intended.

Therefore, there is consensus, among scientists qualified by training and experience, including those with years of experience investigating lactoferrin, to assess the properties and activities of bLf in the context of the infant immune system that no adverse effects will result from the use of bLf in the general infant population at the intended use level in infant formula. There are no robust studies that show that there are any effects on the immune system or iron status. Clinical studies repeatedly show that bLf has beneficial effects on infants throughout. **Therefore, considering the totality of the existing evidence, we conclude that bLf is safe and GRAS for the uses intended by Glanbia. We also conclude that other scientists would reach the same conclusion.**

We trust that our responses are satisfactory to your questions/concerns. Thank you for your cooperation and assistance.

Sincerely,

A handwritten signature in black ink, appearing to read "Angela Walter", is written over a light gray rectangular background.

Angela Walter

Sr. Product Manager, Bioactive Dairy Fractions

Glanbia Nutritionals

**From:** [West-Barnette, Shayla](#)  
**To:** [Morissette, Rachel](#); [Fasano, Jeremiah](#); [Mihalov, Jeremy J.](#); [Kaneko, Kotaro](#); [Wang, Perry G](#)  
**Cc:** [Carlson, Susan](#); [Honigfort, Mica](#)  
**Subject:** FW: EAS/Glanbia meeting with FDA 11/29/18  
**Date:** Wednesday, April 10, 2019 3:27:20 PM  
**Attachments:** [FINAL - GLANBIA RESPONSE TO FDA GRAS 9APR2019.pdf](#)  
[Lactoferrin Pre-Submission Meeting\\_11-29-18\\_Final\\_Transmittal\\_Signed.pdf](#)  
**Importance:** High

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Hello Everyone,

Glanbia provided responses to the questions we posed during our meeting with them last November (please see attached). They request that we review their responses and then meet with them again.

(b) (5)  
[Redacted]

[Redacted]

[Redacted]

Thank you,

Shayla

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**From:** Cathryn Sacra <csacra@easconsultinggroup.com>  
**Sent:** Wednesday, April 10, 2019 1:45 PM  
**To:** West-Barnette, Shayla <Shayla.WestBarnette@fda.hhs.gov>  
**Cc:** Robert Martin <rmartin@easconsultinggroup.com>; Robin Guy <RGuy@easconsultinggroup.com>; Walter, Angela <awalter@glanbia.com>  
**Subject:** FW: EAS/Glanbia meeting with FDA 11/29/18  
**Importance:** High

Dear Shayla,

I am attaching our response to the memorandum of meeting held on 11-29-18. During that meeting, your team offered to meeting with us following our response to your questions. We are available to meeting with FDA either by telephone or in person as soon as you have completed the review of our response.

Best regards,

Cathryn

Cathryn W. Sacra  
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[www.easconsultinggroup.com](http://www.easconsultinggroup.com)

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**From:** West-Barnette, Shayla <[Shayla.WestBarnette@fda.hhs.gov](mailto:Shayla.WestBarnette@fda.hhs.gov)>  
**Sent:** Tuesday, February 26, 2019 1:29 PM  
**To:** Cathryn Sacra <[csacra@easconsultinggroup.com](mailto:csacra@easconsultinggroup.com)>  
**Subject:** RE: EAS/Glanbia meeting with FDA 11/29/18

Hello Catherine,

By way of this message, I am sharing our memorandum from the meeting held on 11-29-18. I am also sharing a document entitled, "Illustrative References", which is an attachment to this memorandum.

Thank you kindly for your patience with us.

Regards,

Shayla

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**From:** Cathryn Sacra <[csacra@easconsultinggroup.com](mailto:csacra@easconsultinggroup.com)>  
**Sent:** Monday, February 4, 2019 1:35 PM  
**To:** West-Barnette, Shayla <[Shayla.WestBarnette@fda.hhs.gov](mailto:Shayla.WestBarnette@fda.hhs.gov)>  
**Subject:** EAS/Glanbia meeting with FDA 11/29/18

Dear Shayla,

I hope you are well. I can't imagine the state of your email inbox after 5 weeks, along with all the projects that had to be put on hold and am sure that you are extremely busy. I did want to reach out this week to see if there was any update to the memorandum of meeting from our meeting on 11/29/18. Our client, Glanbia, is very anxious to see the questions raised at the meeting so they can be addressed and incorporated into the GRAS Notification on bovine lactoferrin.

Thanks so much,  
Cathryn

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**From:** West-Barnette, Shayla <[Shayla.WestBarnette@fda.hhs.gov](mailto:Shayla.WestBarnette@fda.hhs.gov)>  
**Sent:** Wednesday, December 12, 2018 11:53 AM  
**To:** Cathryn Sacra <[csacra@easconsultinggroup.com](mailto:csacra@easconsultinggroup.com)>  
**Cc:** Walter, Angela <[awalter@glanbia.com](mailto:awalter@glanbia.com)>  
**Subject:** RE: Please Confirm Phone Participants from Yesterday's Meeting

Hello Cathryn,

We are writing our memorandum of the meeting and I plan to share this with you once it is complete. The memorandum will include FDA's questions for Glanbia.

Regards,

Shayla

---

**From:** Cathryn Sacra <[csacra@easconsultinggroup.com](mailto:csacra@easconsultinggroup.com)>  
**Sent:** Wednesday, December 12, 2018 10:23 AM  
**To:** West-Barnette, Shayla <[Shayla.WestBarnette@fda.hhs.gov](mailto:Shayla.WestBarnette@fda.hhs.gov)>  
**Cc:** Walter, Angela <[awalter@glanbia.com](mailto:awalter@glanbia.com)>  
**Subject:** FW: Please Confirm Phone Participants from Yesterday's Meeting

Dear Shayla,

Thank you again for the meeting we had a couple of weeks ago. I wanted to follow up the questions that FDA was putting together for the Glanbia team.

Best regards,  
Cathryn

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**From:** Cathryn Sacra <[csacra@easconsultinggroup.com](mailto:csacra@easconsultinggroup.com)>  
**Sent:** Friday, November 30, 2018 2:51 PM  
**To:** 'West-Barnette, Shayla' <[Shayla.WestBarnette@fda.hhs.gov](mailto:Shayla.WestBarnette@fda.hhs.gov)>  
**Subject:** RE: Please Confirm Phone Participants from Yesterday's Meeting

Dear Shayla,

The phone participants from Glanbia Nutritionals were:  
Noreen Hobayan  
Brent Peterson  
Peter Budde

Cathryn W. Sacra  
Director of Labeling and Cosmetic Services  
EAS Consulting Group, LLC  
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---

**From:** Cathryn Sacra  
**Sent:** Friday, November 30, 2018 2:18 PM  
**To:** 'West-Barnette, Shayla' <[Shayla.WestBarnette@fda.hhs.gov](mailto:Shayla.WestBarnette@fda.hhs.gov)>  
**Subject:** RE: Please Confirm Phone Participants from Yesterday's Meeting

Dear Shayla,

Thank you again for the meeting. I will verify who participated via conference call and get back to you shortly.

Best regards,  
Cathryn

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Director of Labeling and Cosmetic Services  
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[www.easconsultinggroup.com](http://www.easconsultinggroup.com)

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**From:** West-Barnette, Shayla <[Shayla.WestBarnette@fda.hhs.gov](mailto:Shayla.WestBarnette@fda.hhs.gov)>  
**Sent:** Friday, November 30, 2018 12:18 PM  
**To:** Cathryn Sacra <[csacra@easconsultinggroup.com](mailto:csacra@easconsultinggroup.com)>  
**Subject:** Please Confirm Phone Participants from Yesterday's Meeting

Hello Cathryn,

Thank you for coming to speak with us for yesterday's meeting. I hope you found our discussion to be helpful.

I am preparing the memorandum of meeting, and I want to be sure that I have the correct attendee list. I have the following individuals listed as phone participants:

**Noreen Hobayan**  
**Brent Peterson**

In the attendee list you sent to me by email before our meeting, you indicated that additional participants would join by phone. It is possible that they joined, and that I did not see their names on the WebEx list.

Can you confirm whether the names above were the only phone participants, or let me know of any others who joined in by phone?

Thank you,

Shayla



## *Specializing in FDA Regulatory Matters*

### **Memorandum of Meeting**

On Wednesday, September 19, 2018, representatives from Glanbia Nutritionals and EAS Consulting Group met with officials from CFSAN to discuss a draft GRAS notice for the use of bovine lactoferrin (bLf) in infant formula and toddler foods. The meeting took place at the CFSAN Office of Food Additive Safety, College Park, MD from 11:00 a.m. – 12 noon. The individuals attending the meeting are listed below.

#### **Visitors**

##### **Glanbia Nutritionals**

Angela Walter, Senior Product Manager, Lactoferrin  
Noreen Hobayan, Director of Quality Assurance, Specialties  
Peter Budde, Senior Director Product Management, Lactoferrin  
Brent Peterson, Senior Director, Ingredient/Bioactives R&D  
Ankur Jhanwar, Senior Technical Services Manager

##### **EAS Consulting Group**

Cathryn Sacra, Director of Labeling and Cosmetic Services  
Robin Guy, MS, DABT, RQAP-GLP

##### **CFSAN Attendees**

**NOTE:** A sign-in sheet was maintained. Regrettably, we did not get a copy of the sign-in sheet. Based on the introductions, we understood that the CFSAN representatives were from OFAS and the Infant Formula Group.

After introductions of the meeting attendees, Ms. Hobayan thanked the CFSAN representatives for arranging this meeting to allow Glanbia Nutritionals (Glanbia) and EAS to discuss their draft GRAS Notice for its use in infant formula and toddler foods and to seek advice and input from CFSAN as Glanbia planned to go forward on this project. This was followed by a brief introduction of Glanbia by Ms. Hobayan. Glanbia's bLf (Bioferrin) was then discussed by Glanbia and EAS representatives to include: its formula, regulatory status, chemistry, exposure, and safety information.

The meeting revolved around a PowerPoint presentation prepared by Glanbia and EAS that had been submitted to CFSAN in advance of the meeting. In advance of the meeting, Glanbia and EAS had indicated to CFSAN that there were two questions that they hoped to resolve and confirm at the meeting:

1. Bioferrin is substantially equivalent to the bovine Lf's that were previously reviewed by FDA for use in IF. Comparison of the specifications in table II-14 and infrared spectra in figures II-3a - II-3f listed on pages 34-38 of the draft GRN establish that Bioferrin is identical to the bLfs that have been approved by FDA.

2. That use levels up to 1000 mg/L is safe for use in infant formula as established by the opinions of other international regulatory bodies and FDA's reviews of other bLf GRNs and is supported by the draft GRAS dossier.

There was good discussion related to the product. The CFSAN representatives essentially agreed that Bioferrin is equivalent to the bLfs that FDA had previously evaluated. Among the questions/issues raised by an OFAS toxicologist were:

- The FDA Toxicologist stated that the Agency has changed the way that they consider GRAS since GRN 669 for bioactive molecules. The Toxicologist stated that they want to make sure that there is a reasonable certainty of no harm, especially since this is for infants who inherently have an underdeveloped immune system. We note that Bioferrin in this GRN is intended for use in term infants who are presumed to be healthy at birth. Clarification is needed as to exactly what the toxicologist was referring to here.
- Most of the studies conducted have specific normal toxicological endpoints, especially with the studies conducted in adult rats. He implied that he didn't know how these types of studies would be appropriate with regard to infant endpoints. He stated that he had not read the dossier; however, since the clinical trials were set up to address specific endpoints, we need to address how this relates to the infant, and how does this effect the preterm and VLBW infants. Again, as noted above, this is confusing as the GRN use is intended for term infants and not VLBW infants or premature infants, etc.
- He stated that even if EFSA found this use to be okay, we don't know if they did the correct evaluations.
- He wants the GRAS document to address:

The functionality of the protein  
Intended population  
Studies not looking at more subtle safety endpoints such as immunological effects.

As this was not clear to us, it was suggested that we contact the toxicologist and request any pertinent references that may have led to his concerns in this area.

The meeting ended at 12 noon. The visitors thanked the CFSAN representatives for their input.

Cathryn W. Sacra  
Director, Labeling and Cosmetics  
EAS Consulting Group



## Memorandum

|                |   |
|----------------|---|
| <b>Date</b>    | March 21, 2019  |
| <b>From</b>    | Jeremiah Fasano (HFS-255)   |
| <b>Through</b> | <p>Shayla West-Barnette (HFS-255)  Digitally signed by Shayla West-barnette -S<br/>Date: 2019.03.25 12:42:13 -04'00'</p> <p>Romina Shah (HFS-255)  Digitally signed by Romina Shah -S<br/>Date: 2019.03.25 11:20:29 -04'00'</p> <p>Kotaro Kaneko (HFS-255)  Digitally signed by Kotaro J. Kaneko -S<br/>Date: 2019.03.25 07:59:19 -04'00'</p> |
| <b>Subject</b> | GRN 000716, Policy Memo   |
| <b>To</b>      | Administrative File, GRN 000716   |

GRAS Notice GRN No. 000716 (GRN 716) informs the Food and Drug Administration (FDA, we) of Arla Foods Ingredients Group P/S's (the notifier) view that use of bovine osteopontin (bOPN) in formulas for term infants is generally recognized as safe. On its face, this view seems plausible, in part due to the widespread consumption of both human osteopontin (hOPN<sup>1</sup>) in human milk and bOPN<sup>2</sup> in bovine milk and milk-derived products. However, our evaluation identified a number of questions involving potential adverse consequences of bOPN exposure

<sup>1</sup> hOPN is the product of the human *SPP1* gene.

<sup>2</sup> bOPN is the product of the bovine *SPP1* gene.

at the intended use level. These questions were prompted by what is currently known about the properties of hOPN, bOPN, and the infant immune system. In summary, the state of the science of the safety of bOPN as an ingredient is currently unsettled. We describe in detail these questions in the context of our interpretation of the current research literature in a science memo to the GRN 000716 file. The purpose of this policy memo is to detail our findings regarding the second element of GRAS, general recognition, as it pertains to the use of OPN as an infant formula ingredient.

The notifier's basis for concluding that the intended use of bOPN is safe is described in the notice. None of the existing data and information contained in the notice, including traditional toxicology and safety studies ordinarily used to support food ingredient safety assessments, indicates toxicity. No adverse effects were reported in growth studies involving infants exposed to bOPN. Furthermore, given our understanding of protein-based toxicity, no such toxicity would be predicted for bOPN. In addition, bOPN is a constituent of bovine milk, and is present in bovine milk-based infant formulas. Thus, there is an extensive history of exposure to this protein in this population, as well as adult exposure in a wide variety of milk-based products, without any reported adverse effects in either population. Finally, human milk contains an orthologous hOPN with some structural and functional similarity to bOPN. Infants are exposed to this protein in the course of routine human milk consumption.

However, our review of the existing scientific literature generated a number of questions (documented in the GRN 000716 science memo) about the intended use of bOPN that complicate the notifier's narrative.<sup>3</sup> First, we note that the intended use level of bOPN is roughly five to ten times higher than levels naturally present in bovine milk; and thus represents a significantly higher level of exposure than currently occurs in this population. We also note that the postnatal infant immune system is rapidly developing and that its typical developmental trajectory is susceptible to perturbation with unknown long-term consequences. OPNs have been reported to possess a variety of modes of action (MoAs), including immunomodulatory MoAs, with correlative and associative evidence indicating its potential roles in the etiology of certain adverse physiological conditions. Our review of the scientific literature indicates that while many studies have been conducted on bOPNs and hOPNs, these MoAs are still poorly and incompletely understood, especially in the context of exposure to infants. Furthermore, the functional differences and similarities between bOPN and hOPN, their significance for short-term immune status, as well as long-term health outcomes (if any) in this population are not well understood. Finally, the significance and consequences of observed interindividual variations in hOPN expression and exposure in the context of genetic, dietary, and environmental impacts on maternal-infant dyad are not known. This is of particular importance because of the notifier's reliance on the mean hOPN level from a single published study (Schenk et al., 2009) as the basis for a general use level in infant

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<sup>3</sup> OFAS staff consulted colleagues elsewhere in CFSAN with relevant expertise, including a board-certified neonatologist, as well as colleagues at FDA's Center for Biologics Evaluation and Research with expertise in assessment of biologically active proteins and immunomodulation, as described in Attachment 1 ("Intercenter Discussion on Osteopontin"). The focus of discussion was not on developing answers to the questions we had identified, but rather on whether such questions were reasonable when considering the basis for a conclusion of general recognition. The results of our discussion suggest that there may not be a robust consensus at this time about the appropriate and sufficient data needed to establish safety for ingredient uses of this kind.

formula. We note that the number of subjects who donated milk in the study was not large and was seemingly from a relatively homogeneous population. At this juncture, it is not clear from the literature whether or not derivation of a target value for intended use can be justifiably inferred for the entire population by estimating a single arithmetic mean from one or several studies with potentially unrepresentative sampling, or indeed whether a single mean value is appropriate given the observed variation in expression and anticipated activity of OPN.

In our view, these questions are consistent with the recommendations of the Institute of Medicine<sup>4</sup> who suggested that, in addition to traditional toxicological endpoints, the “appropriate level of [safety] assessment” for a new ingredient for infant formula use consider:

- The reversibility of potential harmful effects,
- The severity and consequences of adverse effects,
- The time of onset of manifestations of the adverse effects,
- The likelihood that a new ingredient could adversely affect a specific system, and
- Whether the effect would be common or rare.

We initially attempted to resolve these questions by the routine approach of asking clarifying questions of the notifier. However, our review of the responses to these questions convinced us that they could not be resolved by additional data in any reasonable time frame. Further consideration indicated a basic question underlying our earlier inquiries; namely, what is the *significance* of the questions we identified? Complete mechanistic characterization of a substance in the organism is rarely, if ever, necessary to reach a conclusion of safety. On the other hand, advances in scientific disciplines, technologies, and risk assessment practices can shift expectations about the data and information appropriate to establish reasonable certainty of no harm. Ultimately, we were led to ask:

Given the current state of our knowledge about the infant immune system and the known and anticipated properties of both bOPN and hOPN, is the existing data and prior experience with bOPN and hOPN:

- generally recognized at this time
- by experts qualified by scientific training and experience<sup>5</sup>
- as adequately demonstrating the safety of the intended use?

It became clear that the fundamental issue was whether the currently available data and information presented by Arla were generally recognized as appropriate and sufficient, or whether a more robust discussion among qualified experts would identify substantive disagreement about the adequacy of the data, indicating lack of consensus and a potential need for additional data and information. In the case of OPN, given what is known about its MoAs and the intended infant population, we conclude that there is a strong need for experts to

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<sup>4</sup> Institute of Medicine (2004), “Strengthening the Current Process to Evaluate New Ingredients for Infant Formula” in *Infant Formula: Evaluating the Safety of New Ingredients* (Washington, DC: The National Academies Press), pp. 55-69.

<sup>5</sup> In this case, including experts in pediatric allergy and immunology.

publicly debate the relevance and adequacy of the existing body of evidence for a safety determination. In other words, we have identified many questions potentially relevant to the possibility of immediate or long-term perturbation in the developmental trajectory of infant organ systems such as the immune system. Our preliminary consultations with FDA colleagues with relevant expertise, as noted above, suggest that these questions are reasonable given the available information and intended use. However, we have not seen any evidence that these questions have been considered and addressed from an appropriately diverse set of viewpoints among the scientific community of qualified experts.

For GRN 000716, the notifier chose to provide evidence of general recognition by citing a GRAS panel's views on the data and information in the notice. The panel considered that this data and information was adequate to show safety. However, the panel's conclusions do not provide, in our view, compelling evidence of **general** recognition by qualified experts. We have identified two specific issues that limit the panel's ability to generate this evidence. These issues collectively undermine the panel's ability to serve as an effective proxy for the views of the broader scientific community.

First, there is no evidence in the notice that the GRAS panel's deliberations included the questions we describe in the GRN 000716 science memo. In our view, deliberations about the safety of the intended use did not include sufficiently detailed consideration of the known immunomodulatory properties of bOPN and their potential significance for this intended population. For this reason, we concluded that these deliberations were incomplete and of limited use in inferring consensus views of the totality of the evidence. Second, the panel appears to lack members with specialized expertise related to the known properties of bOPN and the intended population. Without the contributions and insights of this expertise to the panel's deliberations, conclusions on the adequacy of the safety data are unlikely to be representative of views of relevant expert communities.

It may be possible to address the issues discussed above, although there is no guarantee that efforts to elicit evidence of general recognition will be successful at this time. For example, even a well-constructed GRAS panel with appropriate and representative expertise to consider a particular intended use could ultimately conclude that there is not consensus among their peers about the usefulness and adequacy of the data available to support that use. Given the nature of the questions we have raised, generating convincing evidence that the available data is generally recognized as appropriate and sufficient may require sustained, substantial effort. We do not expect that our questions could be resolved without evidence of a robust and expansive discussion of these issues. However, any sources of potential bias<sup>6</sup> present would need to be identified and adequately addressed. Both the transparency of the discussion as well as the credibility of the discussants with respect to expertise and sources of potential bias are critical factors in any approach to generate compelling evidence of general recognition. The more open and inclusive the discussion (for example, a public symposium at a professional meeting with appropriate expertise in attendance, compared to a GRAS panel), the easier it would be to make the case that issues of bias, expertise, and representation of viewpoints had

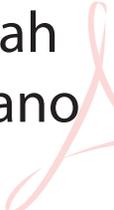
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<sup>6</sup> Although the guidance "Best Practices in Convening GRAS Panels" (Ref. 1) is currently in draft, it may be helpful in illustrating the concept of sources of potential bias and some potential strategies for addressing them.

been adequately addressed.

Jeremiah  
M. Fasano  
-S

Digitally signed  
by Jeremiah M.  
Fasano -S  
Date: 2019.03.25  
12:46:25 -04'00'



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Jeremiah Fasano

Reference:

1. FDA, "Draft Guidance for Industry: Best Practices for Convening a GRAS Panel," (<http://www.regulations.gov> in Docket No. FDA-2017-D-0085), 2017.

cc: **GRN 000716**

E-mail cc: HFS-255 (SCarlson, RChanderbahn, MJDiNovi, RIMerker, SWestBarnette, PMGaynor, RShah, KKaneko, , RBonnette, LShepherd) HFS-850 (ALotze, Cassar)

R/D:HFS-255:Jeremiah Fasano:02/27/2018  
Comments:HFS-255:KKaneko:02/28/2018  
Edit:HFS-255:JMFasano:03/29/2018  
Comment/Edit:HFS-255:PGaynor:04/16/2018  
Edit/Init:HFS-255:SCarlson:06/19/18  
Edit:HFS-255JMFasano:07/05/2018  
Comments:HFS-255:KKaneko:07/05/2018  
Init:HFS-255:NBewry:07/11/2018  
Edit:HFS:255:JMFasano:03/10/2019  
Comments:HFS-255:RIMerker:03/11/2019  
Comments:HFS-255:SChoudhuri:03/11/2019  
Comments:HFS-255:PGaynor:03/13/2019  
Comments:HFS-850:ALotze:03/13/2019  
Comments:HFS-255:SCarlson:03/18/2019  
F/T:HFS-255:JMFasano:03/21/2019

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## GRN000716 (Bovine whey-derived osteopontin)

### - FDA Questions and Comments -

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**Date:** October 3, 2017

**Notifier:** Arla Foods Ingredients Group PIS (AFI)

#### **Chemistry**

1. The notifier describes a nitrogen quantification method to quantify the protein content of their ingredient. However, the notifier also described other methods extensively within the notice, including the ELISA method.

Please clarify what methods were used to quantify the protein content of their ingredient.

#### **Toxicology**

2. On page 16, Table 4, the notifier states that the predicted bovine whey-derived osteopontin (OPN) exposure to infants <1 month of age from the intended use at the 90<sup>th</sup> percentile is 39.5 mg/kg bw/day. On Pg. 86, the notifier states that the Acceptable Daily Intake (ADI) for bovine whey-derived OPN is 50 mg/kg bw/day based on No-Observed-Adverse-Effect-Level (NOAEL) of 2500 mg/kg bw/day from a published teratogenicity study in rats. However, traditionally, the safety factor for interspecies and intraspecies extrapolations using rodent studies is 100 (Benford, 2000). Thus, the NOAEL of 2500 mg/kg bw/day would be extrapolated to ADI of 25 mg/kg bw/day.

Please provide a rationale for:

- Why the safety factor of 50, instead of 100, is appropriate.
  - Why the estimated exposure of 39.5 mg/kg bw/day at the 90<sup>th</sup> percentile in a sensitive and vulnerable population is not a safety concern.
3. OPN is similar to lactoferrin in that they both possess immunomodulatory bioactive properties. It has been previously reported that lactoferrin binds OPN at approximately 3:1 ratio (Yamniuk et al., 2009). Lactoferrin is considered lower in non-supplemented infant formulas compared to breastmilk.  
  
Given that many infant formulas do not supplement the formula with lactoferrin to levels normally observed in breastmilk, please provide a rationale as to why increasing the levels of OPN does not negatively impact the bioavailability of lactoferrin in bovine whey-derived OPN-supplemented infant formulas.
  4. Estimation of the level of human OPN (hOPN) in breastmilk was based on a single study (Schack et al., 2009) of 29 samples from Denmark, a country considered to have relatively homogeneous population (Athanasiadis et al., 2016). As stated by the

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## GRN000716 (Bovine whey-derived osteopontin)

### - FDA Questions and Comments -

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study authors as well as the notifier, there is also a considerable large variation in the level of OPN detected.

Please address the following:

- Given the difference in demographics between nursing mothers in Denmark and the United States as well as the existence of large variations obtained from a small sample size, elaborate on why ~138 mg/L of OPN was chosen with respect to its level being generally recognized as safe. In your answer, elaborate on why the concentration of OPN (i.e. mg/L of breastmilk) was chosen rather than %OPN/total protein in breastmilk for the estimation of appropriate amount of OPN to be added to infant formula.
  - Given that one of the components in your safety narrative relies on the assumption that ~138 mg/L of OPN is the “normal” level of OPN found in all breastmilk across demographics and days post- parturition, it appears that the reliability of this information is vital to your assessment. If this is not the case, please elaborate.
5. Although ELISA quantitation described in Nagatomo et al. (2004) may be considered an overestimation, it appears that majority of hOPN in whey protein (presumably from crude preparations) in transitional and mature human milk is in the full-length form as assayed by Western blotting analysis using 10A16 monoclonal antibody (Fig. 2 of the manuscript). In fact, Bissonnette et al. (2012) confirmed the absence of cleaved hOPN form in breastmilk. However, the purified bovine whey-derived OPN in the notice consists mainly of cleaved peptides (80% C-terminal truncated vs. 20% full-length, pg. 9 of notice). Furthermore, as stated by Christensen and Sorensen (2014), “... the cleavage pattern observed for hOPN in milk is not necessarily identical to that for bOPN ... [k]nowledge of the exact cleavage sites is important, as ***small differences in the C-terminal of the fragments*** may have significant effects on the interaction between these and integrins. (emphasis added)”

Please discuss why the potential differences in the proportion of full-length vs. cleaved peptide(s) between hOPN in human milk and bovine OPN (bOPN) in bovine milk are not a safety concern.

6. On page 23, paragraph 4, and page 57, paragraph 3, there are blank parentheses after the citations.

Please indicate whether this is a typo or missing references.

7. On page 79, in discussing findings of Lonnerdal et al. (2016), the notifier states:

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## GRN000716 (Bovine whey-derived osteopontin)

### - FDA Questions and Comments -

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*“The decrease ( $P < 0.05$ ) plasma threonine concentration in the F130 group compared to the F65 group was not expected by the authors. The authors did not speculate on a reason for this slight, but significant change.”*

Since the GRAS conclusion is made by the notifier, not the study authors, please clarify whether this “slight, but significant change” is a safety concern.

8. On page 22, in discussing the association of variant splice forms of OPN to cancer, the notifier states that the OPN-a form, a full-length native OPN present in human bovine milk, “has never been associated with such malignant properties.”

However, FDA’s literature search has identified two published reports (Blasberg et al., 2010; Hao et al., 2017) in which OPN-a form has been associated with non-small cell lung cancer:

Blasberg et al. concludes:

“OPNa overexpression was associated with increased bovine capillary endothelial tubule length and vascular endothelial growth factor secretion ... These findings may lead to therapeutic strategies for selective isoform inhibition in non-small cell lung cancer.”

Hao et al. state:

“Collectively, our results have clearly demonstrated the clinical value of OPN-a in human non-small cell lung cancer as a potential target for therapy and a potential prognostic factor. The study has also revealed the importance of OPN-a in the aggressiveness of lung cancer cells with a particular relevance to bone metastasis related cell function of lung cancer cells.”

Please provide a brief explanation of why this information does not impact the notifier’s safety assessment.

### **References**

Athanasiadis, G., Cheng, J.Y., Vilhjalmsón, B.J., Jorgensen, F.G., Als, T.D., Le Hellard, S., Espeseth, T., Sullivan, P.F., Hultman, C.M., Kjaergaard, P.C., *et al.* (2016). Nationwide Genomic Study in Denmark Reveals Remarkable Population Homogeneity. *Genetics* 204, 711-722.

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## GRN000716 (Bovine whey-derived osteopontin)

### - FDA Questions and Comments -

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Benford, D. (2000). The Acceptable Daily Intake: A Tool for Ensuring Food Safety (ILSI Europe).

Blasberg, J.D., Goparaju, C.M., Pass, H.I., and Donington, J.S. (2010). Lung cancer osteopontin isoforms exhibit angiogenic functional heterogeneity. *J Thorac Cardiovasc Surg* 139, 1587-1593.

Christensen, B., and Sorensen, E.S. (2014). Osteopontin is highly susceptible to cleavage in bovine milk and the proteolytic fragments bind the alphaVbeta(3)-integrin receptor. *J Dairy Sci* 97, 136-146.

Hao, C., Cui, Y., Hu, M.U., Zhi, X., Zhang, L., Li, W., Wu, W., Cheng, S., and Jiang, W.G. (2017). bovine whey-derived osteopontin-a Splicing Variant Expression in Non-small Cell Lung Cancer and its Effects on the Bone Metastatic Abilities of Lung Cancer Cells In Vitro. *Anticancer Res* 37, 2245-2254.

Nagatomo, T., Ohga, S., Takada, H., Nomura, A., Hikino, S., Imura, M., Ohshima, K., and Hara, T. (2004). Microarray analysis of human milk cells: persistent high expression of osteopontin during the lactation period. *Clin Exp Immunol* 138, 47-53.

Schack, L., Lange, A., Kelsen, J., Agnholt, J., Christensen, B., Petersen, T.E., and Sorensen, E.S. (2009). Considerable variation in the concentration of osteopontin in human milk, bovine milk, and infant formulas. *J Dairy Sci* 92, 5378-5385.

Yamniuk, A.P., Burling, H., and Vogel, H.J. (2009). Thermodynamic characterization of the interactions between the immunoregulatory proteins osteopontin and lactoferrin. *Mol Immunol* 46, 2395-2402.

### **Additional Questions and Comments**

1. On page 18, the notifier states, “... *would only be used in the wet blending-spray drying process of the production of infant formula, where ingredients are blended in water, homogenized, pumped to a heat exchanger for pasteurization, and then spray dried into a powdered product; for full- or near-full-term infants...*”

Please clarify the meaning of “near-full term infants.” The notifier states that this ingredient is not intended for use in products that are preterm focused or exempt.

2. On page 5 (A.2), the notifier states “OPN-10 contains at least 78% protein (N\*6.38), greater than 95% of which is bovine whey-isolated OPN.”

Please clarify what is the other 5% of protein.

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## GRN000716 (Bovine whey-derived osteopontin)

### - FDA Questions and Comments -

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3. On page 5, the second paragraph, the notifier states that “...OPN is safe for human consumption as a food ingredient in term nonexempt milk-based infant formula (which includes formula for infants 6-12 month of age)...”

Please clarify whether the ingredient will be added to non-exempt term infant formula for infants 0-12 months of age or only to non-exempt term formula for infants 6-12 months of age.

4. On page 45, first paragraph: Some of the cited references do not appear to support the statements in this paragraph. The Greer reference only concerns premature infants; there is no information in this reference that addresses the amount of human milk that a term infant will consume daily. The information on the American Academy of Pediatrics (accessed September 1, 2015) website does not support the information provided in this paragraph. Additionally, we are unable to find the stated information in the US Environmental Protection Agency 2011 reference. The Butte 2005 reference appears valid.

Please provide an accurate statement on the daily consumption of infant formula/human milk for term infants with appropriate references.



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October 20, 2017

Nadine Bewry, Ph.D., MPH  
Consumer Safety Officer/Toxicology Reviewer  
U.S. Food and Drug Administration  
Center for Food Safety and Applied Nutrition  
Office of Food Additive Safety  
Division of Biotechnology and GRAS Notice Review  
Phone: 240-402-1007  
Email: [Nadine.Bewry@fda.hhs.gov](mailto:Nadine.Bewry@fda.hhs.gov)

**Re: Questions concerning GRAS notification (GRN) 000716**

Dear Dr. Bewry,

We received a request from you for Burdock Group to provide responses to several different questions posed by your division concerning the notification of the conclusion of GRAS status for bovine whey-derived osteopontin (designated as GRN 000716). The specific questions you have provided are indicated below, numbered and in italics; we have addressed your questions as indicated by a "REPLY" statement following the numbered question.

Please let me know if you need any additional information for this notification of GRAS status.

Sincerely,

A handwritten signature in blue ink that reads 'Ray A. Matulka'.

Ray A. Matulka, Ph.D.  
Director of Toxicology  
Burdock Group

## **Chemistry**

1. *The notifier describes a nitrogen quantification method to quantify the protein content of their ingredient. However, the notifier also described other methods extensively within the notice, including the ELISA method.*

*Please clarify what methods were used to quantify the protein content of their ingredient.*

**REPLY:** The total nitrogen content of the Lacprodan OPN-10 raw material is determined under ISO 8968-3/ IDF 20-3, the determination of nitrogen content in milk and milk products. Two different factors were used, either nitrogen multiplied by a factor of 6.38, commonly used to determine protein levels in dairy raw materials (WHO-FAO, 2008), or nitrogen multiplied by a factor of 7.17, a more accurate factor based on the purity of the ingredient, its post translational modifications and other factors (to accurately reflect protein content in raw material contributed by OPN) to determine the protein content of the ingredient (de Boer, 2014). The Lacprodan OPN-10 specifications lists the total nitrogen content calculated with the factor of 6.38.

## **Toxicology**

2. *On page 16, Table 4, the notifier states that the predicted bovine whey-derived osteopontin (OPN) exposure to infants <1 month of age from the intended use at the 90th percentile is 39.5 mg/kg bw/day. On Pg. 86, the notifier states that the Acceptable Daily Intake (ADI) for bovine whey-derived OPN is 50 mg/kg bw/day based on No-Observed-Adverse-Effect-Level (NOAEL) of 2500 mg/kg bw/day from a published teratogenicity study in rats. However, traditionally, the safety factor for interspecies and intraspecies extrapolations using rodent studies is 100 (Benford, 2000). Thus, the NOAEL of 2500 mg/kg bw/day would be extrapolated to ADI of 25 mg/kg bw/day.*

*Please provide a rationale for:*

- Why the safety factor of 50, instead of 100, is appropriate.*
- Why the estimated exposure of 39.5 mg/kg bw/day at the 90th percentile in a sensitive and vulnerable population is not a safety concern.*

**REPLY:** The OPN molecule contained in the Lacprodan OPN-10 product is substantially similar to the OPN molecule that is naturally found in human breast milk, and is being added to infant formula at a level not exceeding that found in breast milk (Schack et al., 2009). The NOAEL from the 90-day toxicity study was 2000 mg/kg bw/day, the highest dose evaluated, and the NOAEL of 2500 mg/kg bw/day from the published teratogenicity study was also the highest dose evaluated in the study (Kvistgaard et al., 2014). The evaluation by the Expert Panel was that, based on the lack of toxicologically relevant adverse events in any of the safety studies conducted on OPN-10, the level of estimated intake at 50 mg/kg bw/day was safe for the intended consumers (i.e., infants). In addition, OPN from bovine sources has been consumed for centuries at low levels when contained in dairy products.

Further, the estimated exposure of 39.5 mg/kg bw/day at the 90<sup>th</sup> percentile is not a safety concern for the same reasons that were provided above, as the safety studies did not indicate adverse effects at any level, and the amount of OPN from OPN-10 when added to infant formula is at or below the levels of OPN found in human breast milk as cited in the published literature (Schack et al., 2009).

Lastly, with regard to the 100-fold safety factor, this is an arbitrary, one-size-fits-all rule of thumb and not part of any regulation pertaining to a GRAS conclusion. Publications on determining the safety of novel foods and food ingredients stresses that safety assessments for such products should take account of the characteristics of the individual ingredient, including in the assessment an understanding of the origin, production, compositional analysis, nutritional characteristics, previous human exposure and anticipated use of the product (Edwards, 2005; Jones, 2007). The information indicated above and provided in the notification confirms that a 100-fold safety factor is not necessary for Lacprodan OPN-10. The 100-fold safety factor, is however, recommended in U.S. regulations, but specifically for food and color additive petitions and, is not required for a conclusion of GRAS status. In this instance, OPN-10, under the intended conditions of use, was concluded, among qualified experts, to be safe under the conditions of its intended use, and to meet the statutory and regulatory safety standard of “a reasonable certainty of no harm” and, is therefore GRAS.

*3. OPN is similar to lactoferrin in that they both possess immunomodulatory bioactive properties. It has been previously reported that lactoferrin binds OPN at approximately 3:1 ratio (Yamniuk et al., 2009). Lactoferrin is considered lower in non-supplemented infant formulas compared to breastmilk.*

*Given that many infant formulas do not supplement the formula with lactoferrin to levels normally observed in breastmilk, please provide a rationale as to why increasing the levels of OPN does not negatively impact the bioavailability of lactoferrin in bovine whey-derived OPN-supplemented infant formulas.*

**REPLY:** Yamniuk et al. (2009) described the in vitro binding of osteopontin to lactoferrin in titration experiments performed in HEPES buffers. The pH conditions under which the experiments are performed are not given in the paper, but HEPES buffer is usually used at neutral pH (6-8). The highly acidic environment of the stomach is expected to protonate acidic groups of the amino acid side chains in osteopontin and hence, eliminates potential electrostatic interactions the protein may have with other proteins, including lactoferrin. Likewise, the action of pepsin in the stomach on both lactoferrin and osteopontin is also expected to contribute to dissociation of whatever complexes they may have formed. It is therefore highly unlikely that osteopontin influences the bioavailability of lactoferrin in milk or infant formulas in any significant way.

In general, in vitro studies of protein-protein interactions can only to a limited extent be used as indications for binding under in vivo conditions, especially at very high protein concentrations as in milk. There are few proteins from milk that have been shown, under carefully designed in vitro conditions, can interact with each other. Examples of this include lactoferrin electrostatic binding to milk beta-lactoglobulin and of lactoferrin binding to albumin (Lampreave et al., 1990); both of which are present in milk and infant formulas in significantly higher concentrations than OPN. Even if such a complex between OPN and lactoferrin may be found in vitro, the low pH of the stomach and bile emulsifiers and pancreatic enzymes make it very unlikely that it would even exist in vivo. It is also noted that Yamniuk et al. (2009) concluded that a complex between OPN and lactoferrin would likely be considered a benefit to the consumption of both OPN and lactoferrin, not a detriment; the authors suggested that “OPN may act as a carrier protein for LF [lactoferrin] in milk”.

4. Estimation of the level of human OPN (hOPN) in breastmilk was based on a single study (Schack et al., 2009) of 29 samples from Denmark, a country considered to have relatively homogeneous population (Athanasiadis et al., 2016). As stated by the study authors as well as the notifier, there is also a considerable large variation in the level of OPN detected.

Please address the following:

- Given the difference in demographics between nursing mothers in Denmark and the United States as well as the existence of large variations obtained from a small sample size, elaborate on why ~138 mg/L of OPN was chosen with respect to its level being generally recognized as safe. In your answer, elaborate on why the concentration of OPN (i.e. mg/L of breastmilk) was chosen rather than %OPN/total protein in breastmilk for the estimation of appropriate amount of OPN to be added to infant formula.
- Given that one of the components in your safety narrative relies on the assumption that ~138 mg/L of OPN is the “normal” level of OPN found in all breastmilk across demographics and days post- parturition, it appears that the reliability of this information is vital to your assessment. If this is not the case, please elaborate.

**REPLY:** While the presence of OPN in milk was initially reported in a published paper in 1989 (Senger et al), there have been few published papers since that time that determine the concentration of OPN in human breastmilk. Among those that analyzed human breast milk levels of OPN, Schack et al. (2009) utilized a method that has since been evaluated as being specific for human OPN and accounted for potential confounding factors in the quantification of OPN in breast milk. Schack et al. (2009) provided the concentration of OPN in human and bovine milk in both a “mg/L” and “%OPN/total protein” basis, but conducted most comparisons with infant formula utilizing the “mg/L” data. Utilizing the “mg/L” dataset, along with the amount of formula consumed per day allowed for a more direct comparison with available consumption data and safety study intake data. In addition, previous notifications of GRAS status indicated the addition of an infant formula ingredient on a “mg/100 ml formula” basis (Morinaga, 2014) and therefore considered acceptable for analysis of an infant formula ingredient.

Recently, a multicenter study comprising 629 mothers from China, Denmark, Japan and South Korea was conducted. The data from the study is not published, but may be viewed as corroborative and will soon be submitted for publication. In brief, the median OPN content across sites, based on the first sample from each of the 629 mothers was 157.00 mg/L (IQR 95.40-229.50, min-max 2.19-474.84). Based on the first sample from the 495 mothers with a corresponding protein concentration available, the median OPN concentration was 172.04 mg/L (IQR 114.36-240.76, min-max 11.99-474.84), and the median OPN/protein% was 1.79% (IQR 1.25-2.56, min-max 0.14-16.47).

Some variation among study sites was observed. In China the median OPN concentration was 266.22 mg/L, (IQR 210.82-323.92, min-max 100.52-455.68), corresponding to 2.69% (IQR 2.18-3.58, min-max 0.84-16.47) of the protein concentration. In South Korea the mean OPN concentration was 216.20 mg/L (IQR 160.56-268.80, min-max 35.56-474.84), corresponding to 1.76% (IQR 1.27-2.09, min-max 0.27-3.52) of the protein concentration (high protein concentrations were observed in the South Korean milk). In the Japanese milk the median OPN concentration was 185.00 mg/L (IQR 151.00-229.50, min-max 60.00-358.00), corresponding to 2.39% (IQR 1.77-2.90, min-max 0.71-6.24) of the protein concentration. In the Danish breast

milk the median OPN concentration was 99.68 mg/L (IQR 67.45-149.10, min-max 2.19-355.40). Regarding the 185 samples with enough material for macronutrients analysis, the OPN concentration was 107.40 mg/L (IQR 68.19-156.20, min-max 11.99-355.40), corresponding to 1.32% (IQR 0.88-1.71, min-max 0.14-8.70) of the protein concentration.

Variation in the OPN content do exists among mothers and among different geographical populations. As the Danish mothers' milk have the lowest OPN level among the populations analyzed, it reasonable to claim that a level of 138 mg/L can be regarded as safe. The data from the Asian populations indicate that much higher levels could also be regarded as normal and safe.

Several studies have shown that the composition of human milk vary geographically. Recently, it was shown that human milk oligosaccharide concentrations and profiles varied extensively among milk samples from 11 international cohorts from four different continents (McGuire et al., 2017). In an older study, the content of the bioactive milk protein lactoferrin was found to be significantly higher in milk from Ethiopian than Swedish mothers (Lönnerdal et al., 1976). Likewise, a recent study showed that the levels of both lactoferrin and lactadherin in breastmilk of both Indian and South African women were significantly higher than those from women in the United States (Moon et al., 2013).

The soon to be published data provided to Arla reported the content of OPN in breast milk that was analyzed from 629 mothers from China, Denmark, Japan and South Korea (Table 1). This multicenter study obtained a total of 829 breast milk samples from the subjects (521 mothers provided one sample, 16 provided 2 samples, and 92 mother delivered 3 samples at different visits). This corroborative data found that, across all sites and when delivering the first sample, the median OPN content was 157 mg OPN/L breast milk, which is slightly higher than the conservative 138 mg/L published by Schack et al (2009). The data also showed that there was a decrease in the OPN concentration with increasing infant age, but this inverse relationship was evident only within the first three months of life.

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**Table 1. Maternal and infant characteristics, absolute (mg/L) and relative (%) OPN concentration**

|  | <b>China</b>                    | <b>Denmark</b>                | <b>Japan</b>                             | <b>South Korea</b>          |
|--|---------------------------------|-------------------------------|--|-----------------------------|
| <b>Period of collection</b>                      | February 2016 –<br>October 2016 | May 2012 –<br>February 2014   | February 2015 –<br>September 2016        | March 2017 –<br>April 2017  |
| <b>Mothers (N)</b>                               | 76                              | 318                           | 118                                      | 117                         |
| <b>Number of samples (n)</b>                     | 225                             | 318                           | 169                                      | 117                         |
| <b>Number of visits</b>                          | 1-3                             | 1                             | 1-3                                      | 1                           |
| <b>Samples collected (n)</b>                     |                                 |                               |  |                             |
| Visit 1  | 75                              | 318                           | 79                                       | 117                         |
| Visit 2  | 76                              | -                             | 69                                       | -                           |
| Visit 3  | 74                              | -                             | 21                                       | -                           |
| <b>Number of mothers with</b>                    |                                 |                               |  |                             |
| One sample                                       | 1 <sup>1</sup>                  | 318                           | 85 <sup>6</sup>                          | 117                         |
| Two samples                                      | 1 <sup>2</sup>                  | -                             | 15 <sup>7</sup>                          | -                           |
| Three samples                                    | 74                              | -                             | 18                                       | -                           |
| <b>Infant age (weeks), median (IQR)</b>          |                                 |                               |  |                             |
| Visit 1  | 4.29 <sup>3</sup>               | 17.4 (14.9-19.3)              | 8.0 (6.0-9.1)                            | 3.9 (3.0-4.9)               |
| Visit 2  | 8.58                            | -                             | 16.9 (14.1-18.9)                         | -                           |
| Visit 3  | 12.87                           | -                             | 25.1 (22.7-26.3)                         | -                           |
| <b>Maternal age (years), mean ± SD (min-max)</b> |                                 |                               |  |                             |
| Visit 1  | 29.8 ± 3.6<br>(20.33-41.41)     | 31.4 ± 4.0<br>(21.73-43.43)   | 32.6 ± 4.2<br>(24.12-41.87)              | 32.2 ± 3.6<br>(22.28-42.24) |
| Visit 2  | 29.9 ± 3.6<br>(20.40-41.49)     | -                             | 31.6 ± 4.2<br>(21.55-42.02) <sup>8</sup> | -                           |
| Visit 3  | 29.9 ± 3.6<br>(20.53-41.58)     | -                             | 33.5 ± 4.3<br>(26.69-42.19)              | -                           |
| <b>OPN (mg/L), median (IQR)</b>                  |                                 |                               |  |                             |
| Visit 1  | 266.24<br>(212.72-325.52)       | 99.68<br>(67.45-149.10)       | 182.50<br>(151.00-225.00)                | 216.20<br>(160.56-268.80)   |
| Visit 2  | 195.20<br>(147.50-237.46)       | -                             | 169.50<br>(118.00-229.50)                | -                           |
| Visit 3  | 175.92<br>(117.44-210.40)       | -                             | 119.00<br>(81.50-198.00)                 | -                           |
| <b>OPN/protein (%), median (IQR)</b>             |                                 |                               |  |                             |
| Visit 1  | 2.72 (2.18-3.58) <sup>4</sup>   | 1.32 (0.88-1.71) <sup>5</sup> | 2.39 (1.75-2.81)                         | 1.76 (1.27-2.09)            |
| Visit 2  | 2.24 (1.60-3.34)                | -                             | 2.20 (1.50-2.80)                         | -                           |
| Visit 3  | 2.05 (1.54-2.72)                | -                             | 1.80 (1.06-2.50)                         | -                           |

1) At visit 1; 2) At visits 1 and 2; 3) Corresponding to day 30, 60 and 90 respectively; 4) n = 74 due to macronutrients analysis device breakdown; 5) n = 185 due to lack of sample material; 6) At visit 1, n = 46; at visit 2, n = 39; 7) At visits 1 and 2, n = 12; at visits 1 and 3, n = 3; 8) Lower than the previous due to a number of mothers providing their only sample at the second visit

5. Although ELISA quantitation described in Nagatomo et al. (2004) may be considered an overestimation, it appears that majority of hOPN in whey protein (presumably from crude preparations) in transitional and mature human milk is in the full-length form as assayed by Western blotting analysis using 10A16 monoclonal antibody (Fig. 2 of the manuscript). In fact, Bissonnette et al. (2012) confirmed the absence of cleaved hOPN form in breastmilk. However, the purified bovine whey-derived OPN in the notice consists mainly of cleaved peptides (80% C-terminal truncated vs. 20% full-length, pg. 9 of notice). Furthermore, as stated by Christensen and Sorensen (2014), "... the cleavage pattern observed for hOPN in milk is not necessarily identical to that for bOPN ... [k]nowledge of the exact cleavage sites is important, as **small differences in the C-terminal of the fragments may have significant effects on the interaction between these and integrins. (emphasis added)**"

*Please discuss why the potential differences in the proportion of full-length vs. cleaved peptide(s) between hOPN in human milk and bovine OPN (bOPN) in bovine milk are not a safety concern.*

**REPLY:** We have reached out to Dr. Sorensen (Aarhus University, Denmark) who has +20 years of experiences with human and bovine milk OPN and has published extensively on OPN and other milk constituents, who has stated the following:

we do always observe several OPN fragments when analyzing human milk by SDS-PAGE or Western blotting. The degree of fragmentation is subject to large variation among individual mothers, which is most likely a reflection of the activities of proteases that cleave OPN in the most susceptible region around the thrombin/plasmin cleavage site. The staining and migration of the OPN fragments varies significantly from system to system and this could be some of the explanation to why some articles describes human milk OPN as less fragmented or not fragmented.

To emphasize the relatively rare occurrences of mothers milk without OPN fragmentation, we have a very few milk donors that we have designated "super-moms", to reflect that they have very little fragmentation of their milk OPN (approx. 90% full length OPN). Milk from these donors is used to purify the pure full length OPN form, which is used for structural OPN studies in our laboratory.

In Christensen et al. (2010), the fragmentation of OPN in human milk is thoroughly characterized by Western blotting, reverse-phase HPLC and mass spectrometry (MS) identification of sites of cleavage. The Western blotting (using polyclonal antibodies which recognizes several different epitopes and modification variants of OPN) of human milk OPN purified from pooled donor milk from several mothers showed significant fragmentation of OPN (Fig 1 in the Christensen article, provided below).

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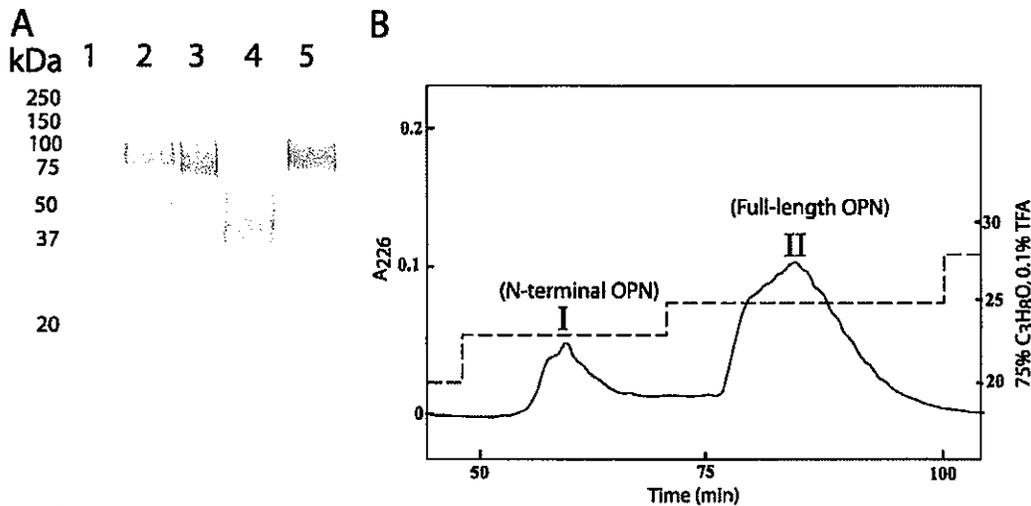


Figure 1. Analysis of human milk OPN (Christensen et al., 2010a). A, Western blot analysis using a polyclonal OPN antibody. Lane 1, molecular mass standards. Lane 2, skim milk. Lane 3, OPN purified from milk. Lane 4, fraction I from B. Lane 5, fraction II from B. B, RP-HPLC of purified human milk OPN.

The fragmented forms of human OPN were purified and characterized and six sites of cleavage were determined in the region close to the OPN integrin binding sites in OPN. Hence, it is clear that several truncated OPN forms exist in human milk. In Christensen and Sørensen (2014) the cleavage forms of OPN in bovine milk were determined and though they are not exactly the same as those observed in the human milk OPN, they are all located in the same region within a couple of amino acids from each other. A 100% match in proteolytic cleavage between species cannot be expected, as there are also large individual variations. Much of the variation in the cleavage sites is most likely due to trimming of the C-terminal by carboxypeptidases in the milk.

The antibodies used by Nagatomo et al. (2004) for Western blotting are the same antibodies used in the IBL OPN ELISA, which has been shown to overestimate the amount of OPN in milk quite significantly. This overestimation is most likely due to incorrect recognition of the OPN in milk and/or the OPN standard. Hence, it is also questionable whether the antibodies recognize the OPN milk forms correctly and quantitatively in Western analyses. It is not clear from the article how many individual mothers actually contributed to the milk, and how and why they use milk whey protein for Western analyses is also unclear. The normal procedure would be to apply a few microliter of fresh skimmed milk to the gel. The preparation of the milk whey protein (and perhaps drying?) could potentially include steps that resulted in a loss of some of the fragments of OPN. Though, I would claim that fragmentation is actually seen in Fig 2 in Nagatomo et al (2004). The “smear” observed to migrate at 50 kDa and faster in panel (a) represents N-terminal OPN fragments. In panel (b) using the 10A16 antibody this staining is not observed as this antibody recognizes the epitope KSKKFRRPDIQYPDATDE, present in the C-terminal part of OPN. In this panel C-terminal fragments are seen migrating at 33 kDa. These fragments are not observed in panel (a) as the antibody used here is O-17 which recognizes an N-terminal epitope in OPN. So in conclusion, Nagatomo et al. (2014) actually nicely show that human milk OPN is indeed fragmented.

In Bissonnette et al. (2012) it is noted that human milk OPN is not fragmented and no truncated form is present in milk. This conclusion is based on Western of a single human milk sample (the origin of this is not stated in the article) and of a commercial human OPN sample. This commercial human OPN is a recombinant protein, which of course is not fragmented. Based

on the current knowledge, it is known that in the majority of women OPN in milk is fragmented (except for a few super moms) and also cleavage patterns between bovine and humans are substantially similar.

6. *On page 23, paragraph 4, and page 57, paragraph 3, there are blank parentheses after the citations.*

*Please indicate whether this is a typo or missing references.*

**REPLY:** The blank parentheses after the citations were typographical errors. Please disregard.

7. *On page 79, in discussing findings of Lonnerdal et al. (2016), the notifier states: "The decrease (P<0.05) plasma threonine concentration in the F130 group compared to the F65 group was not expected by the authors. The authors did not speculate on a reason for this slight, but significant change."*

*Since the GRAS conclusion is made by the notifier, not the study authors, please clarify whether this "slight, but significant change" is a safety concern.*

**REPLY:** Formula-fed infants generally have considerably higher plasma threonine concentrations (due to the high protein content and threonine-rich whey protein) than breast fed infants (Sandstrom et al 2008; Haschke-Becher et al., 2016). It is generally considered that lowering the plasma concentrations of formula-fed infants, making them more similar to breast-fed infants, would be beneficial. The Lonnerdal et al (2016) reported that the F130 dose group had plasma threonine levels (at 6 months) similar to plasma threonine levels found in the breast fed infants. Achieving infant plasma amino acid levels similar to levels found in breast milk-fed infants is considered optimal, as breast milk is considered the gold standard of infant nutrition. Thus, this decrease in plasma threonine levels in the F130 dose group is NOT a safety concern.

8. *On page 22, in discussing the association of variant splice forms of OPN to cancer, the notifier states that the OPN-a form, a full-length native OPN present in human bovine milk, "has never been associated with such malignant properties."*

*However, FDA's literature search has identified two published reports (Blasberg et al., 2010; Hao et al., 2017) in which OPN-a form has been associated with non-small cell lung cancer: Blasberg et al. concludes:*

*"OPNa overexpression was associated with increased bovine capillary endothelial tubule length and vascular endothelial growth factor secretion ... These findings may lead to therapeutic strategies for selective isoform inhibition in non-small cell lung cancer."*

*Hao et al. state:*

*"Collectively, our results have clearly demonstrated the clinical value of OPN-a in human non-small cell lung cancer as a potential target for therapy and a potential prognostic factor. The study has also revealed the importance of OPN-a in the aggressiveness of lung cancer cells with a particular relevance to bone metastasis related cell function of lung cancer cells."*

*Please provide a brief explanation of why this information does not impact the notifier's safety assessment.*

**REPLY:** Blasberg et al (2010) analyzes the expression of the three genetic isoforms of OPN in non-small cell lung cancer cells and immortalized bronchial epithelial cells. In these in vitro experiments using cell lines, they find that the OPN-a isoform is expressed by the cancer cells and that OPN-a overexpression is associated with events that could be involved in tumorigenic events, such as angiogenic properties. By overexpressing the OPN isoforms the authors intentionally create unnatural conditions, and since OPN is involved in numerous physiological processes, including tissue transformation and growth, it is not surprising that overexpression of OPN increased VEGF expression. The new finding of the paper is that the authors have analyzed the three isoforms and now report different capabilities of those in their in vitro assay. As the title states this study concerns lung cancer osteopontin, which is expressed by the lung cancer cells. It is a mechanistic in vitro study and there is absolutely no evidence that ingestion of milk osteopontin should in any way be correlated with development or progression on non-small cell lung cancer cells.

Hao et al. (2017) claims that OPN-a could be used as a prognostic marker for non-small cell lung cancer. This is not to be disputed, as numerous biomarkers have been suggested for such tumor site biomarkers. This does not mean that OPN-a causes or promotes the cancer, simply that the protein is upregulated during tumorigenic events. OPN is also upregulated under infections (as part of the immunological process), bone growth and fractures (during bone remodeling), in the growing fetus blood and many other events in which the body is undergoing traumas, transformations and growth.

Hao et al. (2017) also show that OPN is capable of binding the  $\alpha V\beta 3$  integrin and links this to bone metastatic events. The  $\alpha V\beta 3$  integrin is one of the most prominent and studied receptors for OPN, so it is well known that OPN binds to this receptor, though, OPN also binds this receptor under normal non-malignant conditions, such as when the osteoclasts use this receptor to anchor to the mineralized matrix of bone, via OPN, during bone remodeling processes. Use of OPN as a prognostic marker for some cancer types does not imply that OPN is causing cancer, but implies that the processes involved in cancer progression could use endogenously expressed OPN forms as a mediator molecule in some of the cellular processes, like cell anchoring. Another "role" of OPN in many cancers (like it is in infections and inflammations) is that OPN is actually part of the immune response to the cancer, as OPN has been shown to be expressed by immune cells and to take part in the cellular immune response (Brown, 2012).

Overall, the in vitro studies of Hao *et al.* (2017) and Blasberg *et al.* (2010) using OPN expressed by cell lines do not in any way impact the safety assessment of milk OPN intended for ingestion.

#### **Additional Questions and Comments**

1. On page 18, the notifier states, "... *would only be used in the wet blending-spray drying process of the production of infant formula, where ingredients are blended in water, homogenized, pumped to a heat exchanger for pasteurization, and then spray dried into a powdered product; for full- or near-full-term infants...*"

*Please clarify the meaning of “near-full term infants.” The notifier states that this ingredient is not intended for use in products that are preterm focused or exempt.*

**REPLY:** OPN-10 is not intended for use in products that are preterm focused or exempt. The use of the term “near-full term infants” should be disregarded, as this term had been obtained from previous publications discussing infants and does not fit current definitions of “full-term infants. Please disregard use of the word “near” in this context.

*2. On page 5 (A.2), the notifier states “OPN-10 contains at least 78% protein (N\*6.38), greater than 95% of which is bovine whey-isolated OPN.”*

*Please clarify what is the other 5% of protein.*

**REPLY:** The enriched protein in Lacprodan OPN-10 is predominantly OPN as can be seen from the attached chromatogram (APPENDIX 1). We conservatively wrote the specification that the raw material contains greater than 95% OPN to account for any batch to batch variation and analytical uncertainty. Our production batches show greater 98 to 99% purity. Due to high purity observed, we have not taken the OPN fraction and conducted Mass Spectrometry analysis to see other minor proteins. A very small fragment of peptides of OPN or other dairy proteins cannot be ruled out. Pre-clinical safety (Kvistgaard et al 2014) trials and a clinical trial (Lonnerdal et al 2016) have been conducted with the Lacprodan OPN-10 material that includes any possible minor proteins that may be present in Lacprodan OPN-10.

*3. On page 5, the second paragraph, the notifier states that “...OPN is safe for human consumption as a food ingredient in term nonexempt milk-based infant formula (which includes formula for infants 6-12 month of age)...”*

*Please clarify whether the ingredient will be added to non-exempt term infant formula for infants 0-12 months of age or only to non-exempt term formula for infants 6-12 months of age.*

**REPLY:** The ingredient is to be added to non-exempt term infant formula for infants 0-12 months of age.

*4. On page 45, first paragraph: Some of the cited references do not appear to support the statements in this paragraph. The Greer reference only concerns premature infants; there is no information in this reference that addresses the amount of human milk that a term infant will consume daily. The information on the American Academy of Pediatrics (accessed September 1, 2015) website does not support the information provided in this paragraph. Additionally, we are unable to find the stated information in the US Environmental Protection Agency 2011 reference. The Butte 2005 reference appears valid.*

*Please provide an accurate statement on the daily consumption of infant formula/human milk for term infants with appropriate references.*

**REPLY:** The estimated daily consumption of OPN-10 was obtained utilizing the NHANES 2011-2012 national food survey data set. This information was the basis of the amount of OPN-10 that would be consumed when added to infant formula (mg/day). To determine “mg/kg bw/day”, the information obtained from the EPA (2011) reference (Chapter 8; Body Weight Studies) was utilized. The website link to Chapter 8 of the EPA (2011) reference, which contains the infant body weight information, is provided as follows:

[https://cfpub.epa.gov/si/si\\_public\\_file\\_download.cfm?p\\_download\\_id=526169](https://cfpub.epa.gov/si/si_public_file_download.cfm?p_download_id=526169)

Daily consumption of infant formula/human milk has been evaluated by Hester *et al.* (2012), who found (through a systematic review of the literature) that “although variation in breast milk intakes was apparent during the first few days of life, “breast milk intake tended to increase from 21.5±0.4 mL/day on day 1 to 495.3±33.4 mL/day on day 7 to 673.6±29 mL/day after 14 days.” When analyzing formula consumption, Hester *et al.* (2012) reported that “infant formula intake increased from 170.5 mL/day on day 1 to 265.0±67.7 mL/day on day 2 and to 761.8±18 mL/day after 14 days”. The United Kingdom’s Scientific Advisory Committee on Nutrition (SCAN, 2011) has published dietary reference values for energy, which when using an average energy content of infant formula (EU Directive, 2006), an estimated amount of infant formula (Table 2) was calculated (First Steps Nutrition Trust, 2017). The resources provided here, when used in determining the consumption of Lacprodan OPN-10 by term infants, indicates similar Lacprodan OPN-10 intake levels to the data provided by the NHANES consumption assessment (e.g., addition of Lacprodan OPN-10 at 160 mg/L formula consumed by the highest consumers/body weight (boys 1 month of age) would result in consumption of OPN-10 at 31.04 mg/kg/day; 160mg/L \* 0.194 L/kg/day).

**Table 2. Estimated amounts of infant formula required, using energy requirements from the SACN report Dietary Reference Values for Energy (2011).**

| Age (months) | Median weight (boys)  | Energy requirements (kcal) | EAR (kcal/kg/day) | mL formula/day | mL/kg/day |
|--------------|-----------------------|----------------------------|-------------------|----------------|-----------|
| 1            | 4.47                  | 563                        | 126               | 866            | 194       |
| 2            | 5.56                  | 646                        | 116               | 992            | 178       |
| 3            | 6.37                  | 657                        | 103               | 1,010          | 159       |
| 4            | 9.0                   | 607                        | 87                | 934            | 133       |
| 5            | 7.51                  | 639                        | 85                | 982            | 131       |
| 6            | 7.93                  | 665                        | 84                | 1,023          | 129       |
| Age (months) | Median weight (girls) |                            |                   |                |           |
| 1            | 4.19                  | 515                        | 123               | 792            | 189       |
| 2            | 5.13                  | 589                        | 115               | 741            | 144       |
| 3            | 5.84                  | 600                        | 103               | 923            | 158       |
| 4            | 6.42                  | 573                        | 89                | 881            | 137       |
| 5            | 6.9                   | 601                        | 87                | 924            | 134       |
| 6            | 7.3                   | 623                        | 85                | 958            | 131       |

# 50<sup>th</sup> percentile weight for age from the UK-WHO charts

\*Energy content of infant formula assumed to be 65 kcal/100 ml, the middle of the range stipulated in the EU Directive (2006)

## References

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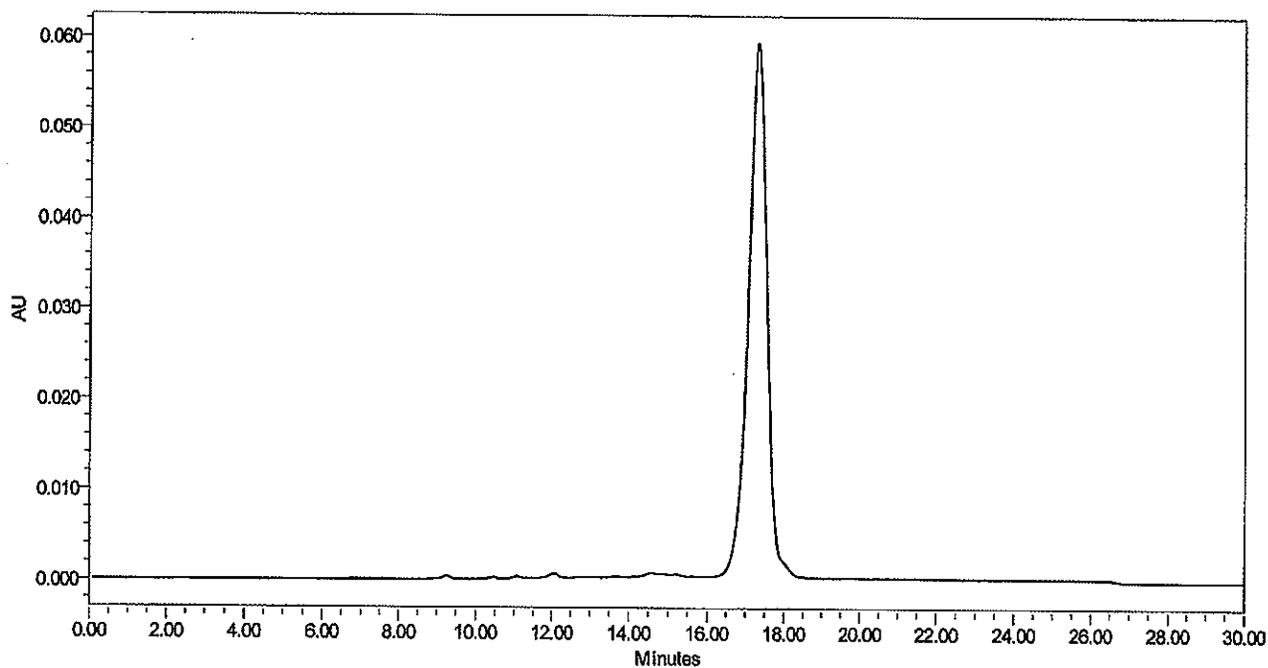
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**APPENDIX I. Chromatogram of Lacprodan OPN-10 purity (please see following page).**

**SAMPLE INFORMATION**

|                   |                           |                     |              |
|-------------------|---------------------------|---------------------|--------------|
| Sample Name:      | K. Hi                     | Acquired By:        | System       |
| Sample Type:      | Unknown                   | Sample Set Name     | OPN210917SNS |
| Vial:             | 13                        | Acq. Method Set:    | OPN271010    |
| Injection #:      | 1                         | Processing Method   | ****         |
| Injection Volume: | 25.00 ul                  | Channel Name:       | ****         |
| Run Time:         | 30.0 Minutes              | Proc. Chnl. Descr.: | ****         |
| Date Acquired:    | 9/22/2017 6:55:52 AM CEST |                     |              |
| Date Processed:   | ****                      |                     |              |

Error Log

Basic LC Peaks Table group contains information that doesn't match the data being reported.