BURDOCK GROUP
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December 13, 2017

Nadine Bewry, Ph.D., MPH
Consumer Safety Officer/Toxicology Reviewer
U.S. Food and Drug Administration
Office of Food Additive Safety
Division of Biotechnology and GRAS Notice Review
P: 240-402-1007
Nadine.bewry@fda.hhs.gov

Re: Request to Cease to Evaluate GRN000716

Dear Dr. Bewry,

Two conference calls were conducted in November 2017 between FDA's Office of Food Additive Safety (OFAS) and Burdock Group (agent for Arla Foods Ingredients Group P/S, sponsor of GRN000716). The subject of both of these calls was the notification of the conclusion of GRAS status completed for the bovine whey-derived osteopontin-based ingredient Lacprodan OPN-10, an ingredient to be added to term infant formula and powdered beverages targeted for children 1-3 years of age. The GRAS notification was received by FDA on July 10, 2017 and was accepted for filing by FDA on August 3, 2017. FDA provided questions on October 3, 2017 requesting clarification (generally) on methodology, safety factors, the levels of osteopontin in breast milk, breast milk osteopontin cleavage variations stated in the literature, and small but significant plasma threonine levels found in a clinical trial evaluating the ingredient in infant formula. None of these questions indicated significant toxicological concerns, and the notifier addressed these questions in a timely fashion (response provided by October 20, 2017). During the teleconferences it was requested by FDA that the notification be withdrawn. While an earlier in-person conference (February 24, 2015) with FDA did not indicate that FDA had safety concerns and the questions provided by FDA did not suggest significant concerns of the safety of Lacprodan OPN-10 under the intended conditions of use, during the recent conference calls it was indicated that there were inadequacies in the notification, not otherwise identified in your letter of April 16, 2015 or the request for additional clarification dated October 3, 2017.

Although the extent or specific types of newly announced inadequacies was not made clear during the November conference calls, FDA indicated it would deliver a clarifying memo and/or memorialized minutes of the call to identify the Agency's newly discovered inadequacies with the notification; unfortunately, we still await this written information. We have great expectations that this written memorandum will be provided in the near future. Nonetheless, in

the spirit of cooperation with the Agency, we will comply with your request and ask that your office cease to evaluate GRAS notification 000716.

Sincerely,

Ray A. Matulka

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

From:	Ray Matulka
To:	Bewry, Nadine
Cc:	Carrie Kennedy
Subject:	RE: GRN 000716 (bovine whey-derived osteopontin (bOPN)): Meeting memorandum
Date:	Thursday, December 21, 2017 12:10:34 PM
Attachments:	image008.png

Dear Dr. Bewry,

We have reviewed the memorandum that you provided yesterday, and the client has requested that a statement made within the memorandum be modified, as we do not feel that the statement completely reflects the information that has been provided within the notification documents.

The statement that requires clarification is the following:

"There is no evidence provided in GRN 000716 or in the October 20, 2017 amendment to GRN 000716 that infants have been safety exposed to higher levels of bOPN from the intended use."

We believe that the 6-month clinical trial conducted by Lonnerdal et al (2016) that was referenced in GRN 0000716 provides growth (i.e., anthropometry), formula intake and adverse event evaluations, consistent with evaluating safety of infants. This study indicates no serious adverse events when infants consume bOPN (i.e., Lacprodan OPN-10, the new ingredient) at levels at the intended use level.

We would appreciate it that the above-stated sentence be removed from the memorandum, as we do not feel that it accurately reflects the scientific research that has been completed on OPN-10, and the lack of concern from the pediatric community on the results provided in this peer-reviewed, published (Journal of Pediatric Gastroenterology and Nutrition) clinical trial.

Please contact me if you have any questions concerning this request.

Sincerely,

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

Burdock Group Consultants
Fusing Science & Compliance. Worldwide.

859 Outer Road
Orlando, FL 32814
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Memorandum of Meeting

Type	Teleconference
Dates & Times	November 9, 2017, 11:00 a.m. – 12:00 p.m. November 20, 2017, 11:00 a.m. – 11:30 a.m.
Location	FDA, Center for Food Safety and Applied Nutrition, Office of Food Additive Safety, 4300 River Road, College Park, MD 20740

Subject: GRN 000716 (Bovine whey-derived osteopontin (bOPN)) for use as a source of protein in milk-based, non-exempt infant formulas for term infants and in powdered beverages at levels up to 138 mg/L as consumed.

Summary: This memorandum summarizes the discussion points of OFAS' teleconference meetings with Burdock Group Consultants (agent) on November 9, 2017 and with Burdock Group Consultants and Arla Foods Ingredients Group PIS (AFI, notifier) on November 20, 2017 regarding GRN000716. OFAS has outstanding regarding the evidence for general recognition that bOPN is safe for use in infant formulas. The overarching question is whether the data provided by AFI are adequate to demonstrate general recognition of safety (reasonable certainty of no harm) by scientists with the appropriate expertise to evaluate the significance of bOPN activity at the intended consumption level by infants. OFAS considers this question to be important given the rapid development of the infant immune system, the poorly characterized modes of action for bOPN, the potential involvement of human OPN (hOPN) in infant immune maturation, lack of clarity regarding substantial equivalence of biological activities between bOPN and hOPN, and the large variation of the levels of hOPN in human milk.

On October 3, 2017, OFAS sent questions to AFI (see FDA Questions and Comments). On October 20, OFAS received AFI's responses. The amendment included information on the intended use level and resulting exposure to bOPN in infants. The amendment also included information on the bioequivalence of hOPN and bOPN. After reviewing AFI's responses, OFAS continues to question the general recognition of the safety of the intended use of bOPN in infant formulas.

On November 9, 2017 OFAS held a teleconference meeting with AFI's agents, Drs. Burdock and Matulka, to discuss the review team's outstanding questions regarding the GRAS status of the intended use of bOPN.

U.S. Food & Drug Administration
Center for Food Safety & Applied Nutrition
5001 Campus Drive
College Park, MD 20740

Participants: November 9, 2017 Meeting

Notifier: Arla Foods Ingredients Group PIS (AFI)'| Agent: Burdock Group Consultants *(phone)*

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

FDA/CFSAN/ OFAS/DBGNR (HFS-255)

Nadine Bewry, Ph.D., MPH	Consumer Safety Officer (CSO)
Jeremiah Fasano, Ph.D.	Acting Supervisory CSO
Romina Shah, Ph.D.	Chemist
Michael DiNovi, Ph.D. (*phone*)	Supervisory Chemist
Kotaro Kaneko, Ph.D.	Toxicologist
Ronald Chanderbhan, Ph.D.	Supervisory Toxicologist

FDA/CFSAN/ ONFL/ IFMS (HFS-850) (*phone*)

Linda Tonucci, Ph.D.	CSO
Suzanne Wolcoff, PhD.	Senior Dietitian-Nutritionist
Carrie Assar, Ph.D.	Lead Nutritionist

OFAS discussed three main lines of evidence contained in the notice and in the notifier's response to one of OFAS' questions, including the absence of evidence of toxicity in toxicological and clinical studies, the similarity of bOPN and hOPN, and existing infant exposure to both bOPN and hOPN.

OFAS noted that:

- OPN has multiple modes of action, including immunomodulatory and pro-inflammatory effects. None of the published safety studies discussed in GRN 000716 evaluated potential adverse effects of bOPN at the intended use level related to these modes of action.
- The available evidence indicates that hOPN and bOPN are not substantially bioequivalent in humans from a physiological perspective.
- The intended use level of bOPN in infant formula is higher than the basal level found in milk or milk-based formula.
    - Based on the literature, the levels of bOPN in cow's milk and cow's milk-derived infant formulas are 18 µg/ml and 5.3-13.0 µg/ml, respectively. However, levels of bOPN from intended use is ~138 µg/ml.
    - There is no evidence provided in GRN 000716 or in the October 20, 2017 amendment to GRN 000716 that infants have historically been safely exposed to bOPN containing dairy products at levels as high as the intended use level.

OFAS considers it important that AFI provide evidence that the existing data and information are adequate to demonstrate general recognition of safety (reasonable certainty of no harm) by scientists with the appropriate expertise to evaluate the significance of bOPN activity at the intended use level in infants. OFAS further noted that AFI's GRAS Panel appears to lack

expertise in neonatal immunology, which is relevant to the intended population and the known modes of action of bOPN. OFAS explained that the importance of including such expertise would be to develop insight into the views of scientists in this area about the significance of the intended use of bOPN, including some basis for concluding that these views are broadly shared. OFAS informed AFI that simply adding a neonatal immunologist to their current GRAS panel would not be sufficient to provide evidence of general recognition. The introduction of an additional individual without reanalysis and interpretation of existing data or reference to current thinking in their field would not be sufficient to resolve our questions about general recognition. Furthermore, OFAS noted that other regulatory authorities, including EFSA, JECFA, FSANZ, and Health Canada, have not approved the use of bOPN in infant formula, indicating a lack of general recognition of bOPN's safety in infant formulas. Given these outstanding questions regarding general recognition, OFAS advised AFI to request that OFAS cease to evaluate GRN 000716.

On November 15, 2017, Dr. Matulka requested a second teleconference meeting with OFAS because AFI stated that they would like to address OFAS' questions regarding the notice rather than request that OFAS cease to evaluate the notice. The purpose of the meeting was: (1) to share the steps that AFI will take to address our questions, and (2) to gain clarification on some of the discussion points from the November 9, 2017 teleconference meeting.

On November 20, 2017, OFAS held a second teleconference meeting with AFI and AFI's agents.

Participants: November 20, 2017 Meeting

Notifier: Arla Foods Ingredients Group PIS (AFI)'| Agent: Burdock Group Consultants *(Phone)*

George A. Burdock, Ph.D.	Owner, Burdock Group Consultants
Ray A. Matulka, Ph.D.	Director of Toxicology, Burdock Group Consultants
Carrie Kennedy, PMP, RAC	Project Leader, Burdock Group Consultants
Kal Ramanujam, Ph.D.	Senior Scientific Advisor, Arla Foods
Anders Steen Jorgensen	Head, Business Unit Pediatrics, Arla Foods
Anne Staudt Kvistgaard	Senior Manager, Documentation Pediatrics, Arla Foods

FDA/CFSAN/ OFAS/DBGNR (HFS-255)

Nadine Bewry, Ph.D., MPH	Consumer Safety Officer (CSO)
Shayla West-Barnette, Ph.D.	Supervisory CSO
Jeremiah Fasano, Ph.D.	Acting Supervisory CSO
Rachel Morissette, Ph.D.	CSO
Kotaro Kaneko, Ph.D. (*phone*)	Toxicologist
Supratim Choudhuri, Ph.D.	Acting Supervisory Toxicologist

FDA/CFSAN/ ONFL/ IFMS (HFS-850) (*Phone*)

Carrie Assar, Ph.D.	Lead Nutritionist
Andrea Lotze, M.D.	Medical Officer

OFAS' discussion points included some of those already covered during the November 9, 2017 teleconference meeting, as well as the following discussion points:

- OFAS considers that it would not be possible for AFI to address the unresolved questions regarding general recognition of the safety of the intended use of bOPN in the short term simply by locating or conducting additional studies.
- OFAS emphasized the office's view that the unresolved questions rest on the issue of whether scientists trained to evaluate immune function and development in infants would accept the existing evidence as adequate to show reasonable certainty of no harm.
- OFAS noted that there are several potential strategies to develop evidence for general recognition of the safety of the intended use of bOPN, which would involve recruiting additional expertise and re-engaging with OFAS on the issues we identified.
- OFAS noted that any future consideration of the GRAS status of AFI's intended use of bOPN by the office would include consulting FDA staff with appropriate training and expertise relevant to neonatal immune development.

In conclusion, OFAS still has outstanding questions regarding the evidence for general recognition that bOPN is safe for use in infant formulas. OFAS recommends that AFI request that we cease to evaluate GRN 000716.

Attachment:

GRN 716 2017-10-03 Email_FDA Questions and Comments

Nadine N. Bewry -S

Digitally signed by Nadine N. Bewry -S
DN: c=US, o=U.S. Government, ou=HHS, ou=FDA, ou=People, 0.9.2342.19200300.100.1.1=0014360008, cn=Nadine N. Bewry -S
Date: 2018.01.25 13:49:49 -05'00'

Nadine Bewry, Ph.D., MPH

R/D:HFS-255:NNBewry: 11/20/2017
Edit/Comments/Init: HFS-255: RShah:12/5/2017
Edit/Comments/Init: HFS-255: KKaneko:12/4/2017, 12/7/2017, 12/22/2017
Edit/Comments/Init: HFS-255: JFasano:12/4/2017, 12/7/2017
Edit/Commens:HFS-255: SWestBarnette:12/19/2017, 12/20/2017
Init:HFS-255: SWestBarnette:12/20/2017
F/T: HFS-255:NNBewry: 12/20/2017

Ray A. Matulka, Ph.D.
Director of Toxicology
Burdock Group Consultants
859 Outer Road
Orlando, FL 32814

Re: GRAS Notice No. GRN 00716

Dear Dr. Matulka:

This letter corrects our letter in response to GRN 000716 dated January 30, 2018. The purpose of this revised letter is to correct the date on which you asked FDA to cease to evaluate GRN 000716.

The Food and Drug Administration (FDA, we) is granting Arla Foods Ingredients Group PIS (AFI)'s request to cease our evaluation of GRN 000716, which we filed on August 3, 2017. We received your request on December 13, 2017.

The subject of the notice is bovine whey-derived osteopontin (bOPN) for use as an ingredient in milk-based, non-exempt infant formulas for term infants and in powdered beverages at levels up to 138 mg/L as consumed. The notice informs us of AFI's view that this use of bOPN is GRAS through scientific procedures.

On October 3, 2017, we sent questions to AFI. After reviewing AFI's responses, we have questions regarding the intended use of bOPN in infant formulas. In a telephone conversation with Burdock Group Consultants (Burdock Group) on November 9, 2017, and in a telephone conversation with Burdock Group and AFI on November 20, 2017, we discussed our outstanding questions. We explained that the amendment we received from AFI on October 20, 2017, did not fully address our questions. The amendment included information on the intended use level and resulting exposure to bOPN in infants. The amendment also included information on the bioequivalence of human OPN and bOPN. We also discussed the opportunity for AFI to ask us to cease our evaluation of GRN 000716.

U.S. Food & Drug Administration
Center for Food Safety & Applied Nutrition
5001 Campus Drive
College Park, MD 20740

In accordance with 21 CFR 170.275(b)(3), the text of this letter responding to GRN 000716 is accessible to the public at www.fda.gov/grasnoticeinventory.

Sincerely,

Susan J. Carlson, Ph.D.
Director
Division of Biotechnology
and GRAS Notice Review
Office of Food Additive Safety
Center for Food Safety
and Applied Nutrition

cc: **GRN 000716**
Electronic mail cc:
HFS-200 (DKeefe, MAdams)
HFS-255 (SCarlson, RFChanderbhan, MDiNovi, RIMerker, SWestBarnette, PMGaynor, RShah, KKaneko, RBonnette, LShepherd))
HFS-850 (ALotze, CAssar)

R/D:HFS-255:NNBewry:12/14/2017
Edit/Comment:HFS-255:SWestBarnette:12/19/17, 12/20/17
Init:HFS-255:SWestBarnette:12/20/17, 02/01/2018
Edit/Comment:PMGaynor:01/11/2018, 1/30/2018Comment:HFS-255:KKaneko:12/22/2017
Comment:HFS-255:JFasano:01/26/2018
Edit:HFS-255:NNBewry: 12/15/2017, 1/12/2018, 1/22/2018, 1/24/2017, 1/29/2017, 1/30/2018, 1/31/2018, 02/01/2018
Discussion and edits:HFS-255:SCarlson,SWestBarnette,PGaynor:01/30/2018
Init:HFS-255: SCarlson:01/30/2018, 01/31/2018
F/T:HFS-255: NNBewry:02/01/2018

NAME	ELECTRONIC SIGN-OFF	ACTING?
Nadine Bewry Consumer Safety Officer	Nadine N. Bewry -S Digitally signed by Nadine N. Bewry -S DN: c=US, o=U.S. Government, ou=HHS, ou=FDA, ou=People, 0.9.2342.19200300.100.1.1=0014360008, cn=Nadine N. Bewry -S Date: 2018.02.01 10:40:01 -05'00'	☐
Susan Carlson Director, Division of Biotechnology & GRAS Notice Review	Susan J. Carlson -S Digitally signed by Susan J. Carlson -S DN: c=US, o=U.S. Government, ou=HHS, ou=FDA, ou=People, 0.9.2342.19200300.100.1.1=2000419015, cn=Susan J. Carlson -S Date: 2018.02.01 12:01:51 -05'00'	☐

Ray A. Matulka, Ph.D.
Director of Toxicology
Burdock Group Consultants
859 Outer Road
Orlando, FL 32814

Re: GRAS Notice No. GRN 000716

Dear Dr. Matulka:

Enclosed is a revised copy of the response letter for GRN 000716. The original letter for this GRAS notice was signed on January 30, 2018. In an electronic mail message dated January 30, 2018, you informed us that Arla Foods Ingredients Group PIS (AFI) requested that FDA cease to evaluate GRN 000716 on December 13, 2017, not December 13, 2016.

In response to your electronic mail message, we have revised the response letter to reflect the correct date of the cease-to-evaluate request. We regret any inconvenience that our error may have caused. If you have any questions, please contact me by electronic mail at Nadine.Bewry@fda.hhs.gov or by telephone at 240-402-1007.

Sincerely,

Nadine N. Bewry -S

Digitally signed by Nadine N. Bewry -S
DN: c=US, o=U.S. Government, ou=HHS, ou=FDA, ou=People, 0.9.2342.19200300.100.1.1=001436000 8, cn=Nadine N. Bewry -S
Date: 2018.02.01 16:40:11 -05'00'

Nadine Bewry, Ph.D., MPH
Division of Biotechnology
and GRAS Notice Review
Center for Food Safety
and Applied Nutrition

U.S. Food & Drug Administration
Center for Food Safety & Applied Nutrition
5001 Campus Drive
College Park, MD 20740

cc: **GRN 000716**

R/D:HFS-255:NNBewry:01/31/2018
Edit/Comment:HFS-255:SWestBarnette: 2/1/18
Init:HFS-255:SWestBarnette: 2/1/18

Ray A. Matulka, Ph.D.
Director of Toxicology
Burdock Group Consultants
859 Outer Road
Orlando, FL 32814

Re: GRAS Notice No. GRN 000716

Dear Dr. Matulka:

Enclosed is a revised copy of the response letter for GRN 000716. The original letter for this GRAS notice was signed on January 30, 2018. In an electronic mail message dated January 30, 2018, you informed us that Arla Foods Ingredients Group PIS (AFI) requested that FDA cease to evaluate GRN 000716 on December 13, 2017, not December 13, 2016.

In response to your electronic mail message, we have revised the response letter to reflect the correct date of the cease-to-evaluate request. We regret any inconvenience that our error may have caused. If you have any questions, please contact me by electronic mail at Nadine.Bewry@fda.hhs.gov or by telephone at 240-402-1007.

Sincerely,

Nadine N. Bewry -S

Digitally signed by Nadine N. Bewry -S
DN: c=US, o=U.S. Government, ou=HHS, ou=FDA, ou=People, 0.9.2342.19200300.100.1.1=0014360008, cn=Nadine N. Bewry -S
Date: 2018.02.01 16:54:30 -05'00'

Nadine Bewry, Ph.D., MPH
Division of Biotechnology
and GRAS Notice Review
Center for Food Safety
and Applied Nutrition

U.S. Food & Drug Administration
Center for Food Safety & Applied Nutrition
5001 Campus Drive
College Park, MD 20740

From:	Bewry, Nadine
To:	Ray Matulka (RMatulka@burdockgroup.com)
Subject:	GRN 000716 (bovine OPN) Corrected Response Letter
Date:	Thursday, February 1, 2018 5:02:20 PM
Attachments:	GRN 716 Correction Letter.pdf GRN 716 2018-01-31 Corrected Cease to Evaluate Response Letter.pdf image001.png

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Dear Dr. Matulka,
FDA's corrected "cease-to-evaluate" response letter to GRN 000716 was signed today by our Division Director, Dr. Susan Carlson. I have also attached a letter that describes the correction. Both letters are attached.
To address your question, the information that will be available publicly on our website are the following:

1. GRN 000716,
2. The 10-20-2017 amendment to GRN 000716 (response to FDA's questions and comments), and
3. FDA's cease-to-evaluate response letters (the original and the corrected versions).

Please let me know if you have any questions.

Best regards,
Nadine

Nadine Bewry, PhD, MPH
Consumer Safety Officer | Toxicology Reviewer

Division of Biotechnology and GRAS Notice Review
Office of Food Additive Safety
Center for Food Safety and Applied Nutrition

U.S. FOOD & DRUG ADMINISTRATION

5001 Campus Drive, HFS-255
College Park, MD 20740

From:	Ray Matulka
To:	Carlson, Susan
Cc:	Bewry, Nadine; Carrie Kennedy
Subject:	RE: Burdock checking on scientific memorandum for OPN
Date:	Monday, June 18, 2018 5:12:01 PM
Attachments:	image001.png image032.png image038.png image039.png image040.png image041.png Arla-Letter to FDA-actions forward-signed-2018JUN18.pdf

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Dear Dr. Carlson,

First, I wanted to thank you for your previous update on the status of the bovine osteopontin memoranda that are being prepared as part of the record for GRN 000716, if I did not previously.

However, Arla is anxious to move forward to address concerns within your group on the safety of this bovine osteopontin ingredient. Therefore, Arla requested that I provide your group the attached letter indicating the planned actions to address the concerns stated in the previous meetings with your group. I will follow this email with a certified mailing of the original letter, for your records.

Please let me know if you have any questions.

Sincerely,

Ray A. Matulka, Ph.D.
Director of Toxicology
PH: 407-802-1400, x 164

BURDOCK GROUP
CONSULTANTS

From: Carlson, Susan [mailto:Susan.Carlson@fda.hhs.gov]
Sent: Thursday, April 19, 2018 4:57 PM
To: Ray Matulka
Cc: Bewry, Nadine; Carrie Kennedy
Subject: RE: Burdock checking on scientific memorandum for OPN

Dear Dr. Matulka,

I want to give you an update on the status of the bovine osteopontin memoranda that we are preparing as part of the record for GRN 000716.

We are still working on the memoranda. I can assure you that these documents are at the forefront of our work. Indeed, we have been having numerous internal conversations with our colleagues

about the issues raised by the proposed use of this substance. We are working to further refine our thoughts and written record in a manner that will be useful to us and your client. I will share with you that there are drafts that are circulating for comment and there will need to be some further work on the documents by the team.

As you are well aware, the GRAS concept offers much in the way of regulatory flexibility; where we must take a pause is when we are asked to assess a substance like osteopontin in that it lacks a counterpart in our program. We believe that this substance warrants our best thinking and we hope that you and your client can be patient for a bit longer. I am sure that you are anxious for a date. I've learned over the years that there are too many variables in our work to accurately project completion dates. However, I invite you to continue to check in with us on a regular basis because it does help to keep the pressure on us. We don't like to leave projects undone—there isn't any satisfaction in that for anyone!

I would also like to take this opportunity to thank you for your continued participation in our GRAS Notification Program. We would not be able to do the work that we do without the dedicated efforts of our stakeholders like you.

Sincerely,

Susan J. Carlson, Ph.D.
Division Director
Center for Food Safety and Applied Nutrition
Office of Food Additive Safety, Division of Biotechnology and GRAS Notice Review
U.S. Food and Drug Administration
Tel: 240-402-1253
Susan.Carlson@fda.hhs.gov

From: Bewry, Nadine
Sent: Wednesday, April 18, 2018 5:13 PM
To: Carlson, Susan <Susan.Carlson@fda.hhs.gov>
Subject: RE: Burdock checking on scientific memorandum for OPN

From: Ray Matulka [mailto:RMatulka@burdockgroup.com]
Sent: Tuesday, April 17, 2018 9:43 AM
To: Bewry, Nadine <Nadine.Bewry@fda.hhs.gov>
Cc: Carrie Kennedy <CKennedy@burdockgroup.com>
Subject: RE: Memorandum of Meeting on 03/01/2018: bovine osteopontin (bOPN)

Dear Dr. Bewry,

I was wondering if there has been any indication on the possible timing of the release of the scientific memorandum for the Arla GRN (see below)?

Any information you can provide would be appreciated.

Sincerely,

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

From: Bewry, Nadine [mailto:Nadine.Bewry@fda.hhs.gov]
Sent: Tuesday, April 3, 2018 11:18 AM
To: Ray Matulka
Cc: Carrie Kennedy
Subject: RE: Memorandum of Meeting on 03/01/2018: bovine osteopontin (bOPN)

Good morning Dr. Matulka,

Thank you for checking-in on the memoranda and for your patience. I understand the notifier's desire to move forward. Please know that I share your correspondences with the review team and I check on the status of both memoranda regularly.

The reference to a "few days" that you mentioned in your email below was pertaining to the completion of the draft scientific memorandum. At the time of our meeting, both memoranda were in different stages of development.

Currently, the scientific and policy memoranda are undergoing the office's internal review and clearance process and the team is working diligently to complete that process.

Best regards,

Nadine Bewry, PhD, MPH
Consumer Safety Officer | Toxicology Reviewer

From: Ray Matulka [mailto:RMatulka@burdockgroup.com]
Sent: Monday, April 2, 2018 1:48 PM

To: Bewry, Nadine <Nadine.Bewry@fda.hhs.gov>
Cc: Carrie Kennedy <CKennedy@burdockgroup.com>
Subject: RE: Memorandum of Meeting on 03/01/2018: bovine osteopontin (bOPN)

Dear Dr. Bewry,

I am inquiring concerning the completion of the scientific and policy memorandum for GRN 000716, as previously discussed in the FDA meeting held on March 1, 2018: have those memoranda been finalized, such that I can request a FOIA for them?

Please let me know at your earliest convenience.

The client is VERY anxious to move forward with addressing the questions posed by your team, but was indicated during the above-mentioned meeting to utilize the scientific memo as a guide to addressing these questions, and we do not want to deviate from what is expected by your group by moving forward without this information.

Several people at the meeting indicated that the memo was only a "few days" from acceptance… it has now been a month since the meeting.

Is there another person that I should be contacting to move this process forward, in addition to yourself?

Any help you can provide is appreciated.

Sincerely,

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

BURDOCK GROUP
CONSULTANTS

From: Bewry, Nadine [mailto:Nadine.Bewry@fda.hhs.gov]
Sent: Monday, March 26, 2018 1:14 PM
To: Ray Matulka
Cc: Carrie Kennedy
Subject: RE: Memorandum of Meeting on 03/01/2018: bovine osteopontin (bOPN)

Dear Dr. Matulka,

The documents are not available at this time. Thanks for following up.

Best regards,

Nadine Bewry, PhD, MPH

Consumer Safety Officer | Toxicology Reviewer

From: Ray Matulka [mailto:RMatulka@burdockgroup.com]
Sent: Monday, March 26, 2018 11:28 AM
To: Bewry, Nadine <Nadine.Bewry@fda.hhs.gov>
Cc: Carrie Kennedy <CKennedy@burdockgroup.com>
Subject: RE: Memorandum of Meeting on 03/01/2018: bovine osteopontin (bOPN)

Dear Dr. Bewry,

I am inquiring concerning the completion of the scientific and policy memorandum for GRN 000716, as previously discussed in the FDA meeting held on March 1, 2018: have those memoranda been finalized, such that I can request a FOIA for them?

Please let me know at your earliest convenience.

Sincerely,

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

From: Bewry, Nadine [mailto:Nadine.Bewry@fda.hhs.gov]
Sent: Wednesday, March 21, 2018 4:26 PM
To: Ray Matulka
Subject: Memorandum of Meeting on 03/01/2018: bovine osteopontin (bOPN)

Dear Dr. Matulka,

Attached, please find the meeting memorandum. I also included a copy of the draft guidance document, *Best Practices for Convening a GRAS Panel: Guidance for Industry*.

Please let me know if you have any questions.

Best regards,
Nadine Bewry, PhD, MPH
Consumer Safety Officer | Toxicology Reviewer

Office of Food Additive Safety
Center for Food Safety and Applied Nutrition

BURDOCK GROUP
CONSULTANTS

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rmatulka@burdockgroup.com

June 18, 2018

Susan J. Carlson, Ph.D., Director
Division of Biotechnology and GRAS Notice Review
Office of Food Additive Safety (OFAS)
Center for Food Safety and Applied Nutrition
U.S. Food and Drug Administration
4300 River Road, CPK-2 Building Room 2030
College Park, MD 20740
PH: 240-402-1253
E: Susan.Carlson@fda.hhs.gov

RE: General recognition standard for whey-derived osteopontin (GRN000716)

Dear Dr. Carlson,

This letter is concerning the conclusion of GRAS status of whey-derived osteopontin (bOPN) when added to infant formula by Arla Foods Ingredients P/S (AFI), which was notified to FDA by Burdock Group Consultants (BG), and received by, FDA on July 10, 2017 and given the GRAS notification number (GRN) 000716.

During FDA's review of the notification, FDA contacted the notifier and expressed over several conference calls that FDA had concerns that the standard of "general recognition" was not met. An additional in-person meeting was requested by Arla and held at FDA on March 1, 2018 to better understand the information FDA required to address FDA's concerns such that the "general recognition" standard for safety of this whey-derived osteopontin have been addressed. FDA stated during this meeting that no additional studies (toxicological or functional/clinical) were necessary to show safety, but that FDA was concerned that "general recognition" among experts in pediatric immunology was not achieved. FDA attendees also informed AFI during the meeting that a scientific memorandum and policy memorandum on the bOPN that is the subject of GRN000716 were under review for clearance by FDA, and that AFI should obtain these memoranda *via* a FOIA request.

Unfortunately, the scientific and policy memoranda have not yet been released by FDA since the March 1, 2018 conference, with no indication by FDA on a possible time when these memoranda may be completed. Therefore, this letter is to describe the actions that AFI is planning to reiterate that the subject of GRN000716 (bOPN) meets the "generally recognized" standard for GRAS ingredients; that is there is a reasonable certainty of no harm for bOPN when added to infant formula under the intended conditions of use.

The actions to show "general recognition" are detailed below.

1. FDA stated that AFI is to provide evidence from qualified experts (*e.g.*, experts in pediatric physiology and immunology) of the general recognition that bOPN is safe for use in infant formulas for term infants at the proposed use level.
    a. AFI will work with 2 – 3 independent experts in pediatric physiology and immunology (based on the experts' *curricula vitae*) to produce a white paper evaluating whether the potential immunological effects described in the published literature are a safety concern in the intended population, including a discussion that the views of the stated experts would be considered widely accepted.
    b. AFI will present existing data and information on bOPN to the qualified pediatric experts in anticipation that they will agree with the conclusion of GRAS status for bOPN.
    c. The white paper, expressing the opinion of this panel of experts, will be included in a new notification of GRAS status to be submitted to FDA.

We hope that these actions will resolve your questions concerning the "general recognition" standard as having been met among experts in the field of pediatric physiology and immunology for bOPN when added to term infant formula under the intended conditions of use.

Please contact me within fifteen working days with any questions or comments on these actions to address your questions. If input is not received within the stated time period, we will move forward with the actions described above.

Sincerely,

Ray A. Matulka

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

From:	Carlson, Susan
To:	Morissette, Rachel
Subject:	FW: repeat...FW: Burdock checking on scientific memorandum for OPN
Date:	Monday, March 04, 2019 11:43:18 AM
Attachments:	image001.png image003.png image009.png image010.png image016.png image017.png image018.png image019.png

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From: Ray Matulka <RMatulka@burdockgroup.com>
Sent: Thursday, June 21, 2018 5:04 PM
To: Carlson, Susan <Susan.Carlson@fda.hhs.gov>
Cc: Bewry, Nadine <Nadine.Bewry@fda.hhs.gov>; Carrie Kennedy <CKennedy@burdockgroup.com>
Subject: RE: repeat...FW: Burdock checking on scientific memorandum for OPN

Dear Dr. Carlson,

Thank you for sending this email (and acknowledging receipt of the email). As I am somewhat confused on the "many safety questions" concerning the safety of the bovine osteopontin (previous statements both on conference calls and in person focused on meeting the "general recognition" standard), I look forward to the scientific and policy memos.

Sincerely,

Ray A. Matulka, Ph.D.
Director of Toxicology
PH: 407-802-1400, x 164

From: Carlson, Susan [mailto:Susan.Carlson@fda.hhs.gov]
Sent: Thursday, June 21, 2018 4:53 PM
To: Ray Matulka
Cc: Bewry, Nadine; Carrie Kennedy
Subject: repeat...FW: Burdock checking on scientific memorandum for OPN

Dr. Matulka--
I'm sending this a second time as I see that Outlook has automatically inserted quotes in your email address.
Please acknowledge that you have received this email.

Thank you,
Susan

From: Carlson, Susan
Sent: Thursday, June 21, 2018 4:46 PM
To: 'Ray Matulka' <RMatulka@burdockgroup.com>
Cc: Bewry, Nadine <Nadine.Bewry@fda.hhs.gov>; Carrie Kennedy <CKennedy@burdockgroup.com>
Subject: RE: Burdock checking on scientific memorandum for OPN

Dear Dr. Matulka,

Thank you for your message. I would also like to thank you and Arla for your continued patience regarding the finalizing of our memoranda.

I am happy to tell you that the memoranda have undergone their final review and should be available very soon (days). I will let Dr. Bewry inform you when the finalized memoranda are available for request.

I would also like to briefly respond to the proposal from Arla. I would advise that Arla review our memoranda before hiring experts to draft a white paper. I can certainly understand that they are wanting to move along. I realize that we discussed general recognition with you and Arla at length, however, I would caution that after careful review of the literature and further discussion with our colleagues (including FDA scientists outside of CFSAN), we view the science surrounding osteopontin as unsettled. We have identified many safety questions that we elaborate on in our memoranda. These questions do not have obvious answers and will need considerable effort to resolve. Therefore, we do not believe that Arla's proposal to engage two experts to write a white paper will be sufficient. At this point in time, it is unclear how the use of osteopontin in infant formula would be GRAS.

We are happy to discuss our thoughts further.

Regards,
Susan J. Carlson, Ph.D.
Division Director

Center for Food Safety and Applied Nutrition
Office of Food Additive Safety, Division of Biotechnology and GRAS Notice Review
U.S. Food and Drug Administration
Tel: 240-402-1253
Susan.Carlson@fda.hhs.gov

From: Ray Matulka [mailto:RMatulka@burdockgroup.com]
Sent: Monday, June 18, 2018 5:11 PM
To: Carlson, Susan <Susan.Carlson@fda.hhs.gov>
Cc: Bewry, Nadine <Nadine.Bewry@fda.hhs.gov>; Carrie Kennedy <CKennedy@burdockgroup.com>
Subject: RE: Burdock checking on scientific memorandum for OPN

Dear Dr. Carlson,

First, I wanted to thank you for your previous update on the status of the bovine osteopontin memoranda that are being prepared as part of the record for GRN 000716, if I did not previously.

However, Arla is anxious to move forward to address concerns within your group on the safety of this bovine osteopontin ingredient. Therefore, Arla requested that I provide your group the attached letter indicating the planned actions to address the concerns stated in the previous meetings with your group. I will follow this email with a certified mailing of the original letter, for your records.

Please let me know if you have any questions.

Sincerely,

Ray A. Matulka, Ph.D.
Director of Toxicology
PH: 407-802-1400, x 164

From: Carlson, Susan [mailto:Susan.Carlson@fda.hhs.gov]
Sent: Thursday, April 19, 2018 4:57 PM
To: Ray Matulka
Cc: Bewry, Nadine; Carrie Kennedy
Subject: RE: Burdock checking on scientific memorandum for OPN

Dear Dr. Matulka,

I want to give you an update on the status of the bovine osteopontin memoranda that we are preparing as part of the record for GRN 000716.

We are still working on the memoranda. I can assure you that these documents are at the forefront of our work. Indeed, we have been having numerous internal conversations with our colleagues about the issues raised by the proposed use of this substance. We are working to further refine our thoughts and written record in a manner that will be useful to us and your client. I will share with you that there are drafts that are circulating for comment and there will need to be some further work on the documents by the team.

As you are well aware, the GRAS concept offers much in the way of regulatory flexibility; where we must take a pause is when we are asked to assess a substance like osteopontin in that it lacks a counterpart in our program. We believe that this substance warrants our best thinking and we hope that you and your client can be patient for a bit longer. I am sure that you are anxious for a date. I've learned over the years that there are too many variables in our work to accurately project completion dates. However, I invite you to continue to check in with us on a regular basis because it does help to keep the pressure on us. We don't like to leave projects undone—there isn't any satisfaction in that for anyone!

I would also like to take this opportunity to thank you for your continued participation in our GRAS Notification Program. We would not be able to do the work that we do without the dedicated efforts of our stakeholders like you.

Sincerely,

Susan J. Carlson, Ph.D.
Division Director
Center for Food Safety and Applied Nutrition
Office of Food Additive Safety, Division of Biotechnology and GRAS Notice Review
U.S. Food and Drug Administration
Tel: 240-402-1253
Susan.Carlson@fda.hhs.gov

From: Bewry, Nadine
Sent: Wednesday, April 18, 2018 5:13 PM
To: Carlson, Susan <Susan.Carlson@fda.hhs.gov>
Subject: RE: Burdock checking on scientific memorandum for OPN

From: Ray Matulka [mailto:RMatulka@burdockgroup.com]
Sent: Tuesday, April 17, 2018 9:43 AM
To: Bewry, Nadine <Nadine.Bewry@fda.hhs.gov>
Cc: Carrie Kennedy <CKennedy@burdockgroup.com>
Subject: RE: Memorandum of Meeting on 03/01/2018: bovine osteopontin (bOPN)

Dear Dr. Bewry,

I was wondering if there has been any indication on the possible timing of the release of the scientific memorandum for the Arla GRN (see below)?

Any information you can provide would be appreciated.

Sincerely,

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

From: Bewry, Nadine [mailto:Nadine.Bewry@fda.hhs.gov]
Sent: Tuesday, April 3, 2018 11:18 AM
To: Ray Matulka
Cc: Carrie Kennedy
Subject: RE: Memorandum of Meeting on 03/01/2018: bovine osteopontin (bOPN)

Good morning Dr. Matulka,

Thank you for checking-in on the memoranda and for your patience. I understand the notifier's desire to move forward. Please know that I share your correspondences with the review team and I check on the status of both memoranda regularly.

The reference to a "few days" that you mentioned in your email below was pertaining to the completion of the draft scientific memorandum. At the time of our meeting, both memoranda were in different stages of development.

Currently, the scientific and policy memoranda are undergoing the office's internal review and clearance process and the team is working diligently to complete that process.

Best regards,

Nadine Bewry, PhD, MPH
Consumer Safety Officer | Toxicology Reviewer

From: Ray Matulka [mailto:RMatulka@burdockgroup.com]
Sent: Monday, April 2, 2018 1:48 PM
To: Bewry, Nadine <Nadine.Bewry@fda.hhs.gov>
Cc: Carrie Kennedy <CKennedy@burdockgroup.com>
Subject: RE: Memorandum of Meeting on 03/01/2018: bovine osteopontin (bOPN)

Dear Dr. Bewry,

I am inquiring concerning the completion of the scientific and policy memorandum for GRN 000716, as previously discussed in the FDA meeting held on March 1, 2018: have those memoranda been finalized, such that I can request a FOIA for them?

Please let me know at your earliest convenience.

The client is VERY anxious to move forward with addressing the questions posed by your team, but was indicated during the above-mentioned meeting to utilize the scientific memo as a guide to addressing these questions, and we do not want to deviate from what is expected by your group by moving forward without this information.

Several people at the meeting indicated that the memo was only a "few days" from acceptance… it has now been a month since the meeting.

Is there another person that I should be contacting to move this process forward, in addition to yourself?

Any help you can provide is appreciated.

Sincerely,

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

From: Bewry, Nadine [mailto:Nadine.Bewry@fda.hhs.gov]
Sent: Monday, March 26, 2018 1:14 PM
To: Ray Matulka
Cc: Carrie Kennedy
Subject: RE: Memorandum of Meeting on 03/01/2018: bovine osteopontin (bOPN)

Dear Dr. Matulka,

The documents are not available at this time. Thanks for following up.

Best regards,

Nadine Bewry, PhD, MPH
Consumer Safety Officer | Toxicology Reviewer

From: Ray Matulka [mailto:RMatulka@burdockgroup.com]

Sent: Monday, March 26, 2018 11:28 AM
To: Bewry, Nadine <Nadine.Bewry@fda.hhs.gov>
Cc: Carrie Kennedy <CKennedy@burdockgroup.com>
Subject: RE: Memorandum of Meeting on 03/01/2018: bovine osteopontin (bOPN)

Dear Dr. Bewry,

I am inquiring concerning the completion of the scientific and policy memorandum for GRN 000716, as previously discussed in the FDA meeting held on March 1, 2018: have those memoranda been finalized, such that I can request a FOIA for them?

Please let me know at your earliest convenience.

Sincerely,

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

BURDOCK GROUP
CONSULTANTS

From: Bewry, Nadine [mailto:Nadine.Bewry@fda.hhs.gov]
Sent: Wednesday, March 21, 2018 4:26 PM
To: Ray Matulka
Subject: Memorandum of Meeting on 03/01/2018: bovine osteopontin (bOPN)

Dear Dr. Matulka,

Attached, please find the meeting memorandum. I also included a copy of the draft guidance document, *Best Practices for Convening a GRAS Panel: Guidance for Industry*.

Please let me know if you have any questions.

Best regards,
Nadine Bewry, PhD, MPH
Consumer Safety Officer | Toxicology Reviewer

Office of Food Additive Safety
Center for Food Safety and Applied Nutrition

FDA U.S. FOOD & DRUG ADMINISTRATION

Memorandum

Date	June 29, 2018
From	Kotaro J. Kaneko (HFS-255) Through Romina Shah (HFS-255) Romina Shah -S Digitally signed by Romina Shah -S DN: c=US, o=U.S. Government, ou=HHS, ou=FDA, ou=People, cn=Romina Shah -S, 0.9.2342.19200300.100.1.1=2000418924 Date: 2018.07.10 11:55:28 -04'00' Nadine Bewry (HFS-255) Nadine N. Bewry -S Digitally signed by Nadine N. Bewry -S DN: c=US, o=U.S. Government, ou=HHS, ou=FDA, ou=People, 0.9.2342.19200300.100.1.1=0014360008, cn=Nadine N. Bewry -S Date: 2018.07.02 10:13:45 -04'00'
Subject	GRN 000716, scientific memorandum
To	Administrative File, GRN 000716 (bovine osteopontin)

Keywords: osteopontin (OPN), early T-lymphocyte activation-1 (Eta-1), secreted phosphoprotein 1 (SSP1), 44kDa bone phosphoprotein, sialoprotein 1, uropontin, infant formula, immune development, developmental immunotoxicity, immunomodulatory, bioactive

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 nonexempt formulas for term infants is generally recognized as safe at 125 mg/L. According to the notifier, formulations of cow's milk-based infant formula (IF) already contain 5 to 13 mg bOPN/L, thus the final bOPN concentration from added and endogenous bOPN would be ~138 mg/L.

This memorandum discusses unresolved questions about certain properties of partially purified bOPN in the context of its intended use which were raised during the scientific review of GRN 000716.

Introduction:

Osteopontin protein (OPN), encoded by the *osteopontin/SSP1*[1] gene, is a N-glycosylated phosphoprotein with wide ranging biological activities that include biomineralization, bone and tissue remodeling, and immunomodulation (Clemente et al., 2016; Kahles et al., 2014; Rittling and Singh, 2015; Wang and Denhardt, 2008). For this reason, OPN has been referred as "secreted phosphoprotein 1 (SSP 1)," "44kDa bone phosphoprotein," "sialoprotein 1,"

[1] NCBI Gene Name for osteopontin is SSP1

U.S. Food & Drug Administration
Center for Food Safety & Applied Nutrition
5001 Campus Drive
College Park, MD 20740

"uropontin," and "early T-lymphocyte activation-1 (Eta-1)." OPN is expressed *in vivo* as secreted/extracellular and intracellular forms. Thus, OPN is found in many biological fluids and expressed in multiple tissues and multiple cell types including osteoblasts, osteoclasts, endothelial and epithelial cells, as well as in most cells of the immune system. OPN has multiple binding sites for Ca^{+2} and heparin, as well as to different subsets of integrins, in which specificity and affinity are highly dependent on splicing, post-translational modification, and proteolytic cleavage (Christensen and Sorensen, 2014; Clemente et al., 2016; Lund et al., 2009). Therefore, both the primary amino acid sequence and numerous post-translational modifications likely contribute to the observed pleiotropic effects of OPN. As with many milk proteins, the full range of biological functions of OPN in humans, and in particular in infants, are not yet fully understood.

Since OPN's initial discovery in 1985, extensive research[2] has gradually advanced our understanding of the molecular mechanisms of action as well as its functional roles in normal development and physiology and in pathophysiological states (Clemente et al., 2016; Denhardt et al., 2001; Iida et al., 2017; Kahles et al., 2014; Rittling and Singh, 2015; Wang and Denhardt, 2008). Although OPN has been linked to a wide variety of biological functions, this scientific memo focuses on its potential effects on the developing immune system as this would be a safety endpoint of concern for infants. OPN has been reported to have multiple effects on the immune system in adult humans and in animal models [Fig. 1; (Denhardt et al., 2001; Iida et al., 2017; Kahles et al., 2014; Rittling and Singh, 2015)]. Induction of cellular and humoral immune responses is dependent on up-regulation of two sets of cytokines synthesized by their respective T helper cell subclasses (Th1 and Th2). OPN/Eta-1 has a profound effect on the activation of T lymphocytes by directing naïve CD4 T cells towards Th1 commitment and differentiation; this in turn results in up-regulating expression of Th1 cytokines, including IL-12 and IFN-γ, leading to polarization of Th1 response relative to Th2 response (Lund et al., 2009; Renkl et al., 2005; Shinohara et al., 2005). In addition, OPN has been reported to have effects on a number of other immune cell types including macrophages, dendritic cells, and neutrophils (Kahles et al., 2014; Lund et al., 2009; Wang and Denhardt, 2008). Thus OPN appears to have one or more roles as an immunomodulatory bioactive molecule by influencing the responses of numerous immune cell components to various stimuli, not least of which may be to promote the appropriate balance of the activities between the Th1/Th2[3] arms of the developing immune system. Furthermore, OPN's anti-apoptotic properties in activated T cells underline the potential causal relationship between OPN and pro-inflammatory diseases such as multiple sclerosis and diabetes (Clemente et al., 2016; Hur et al., 2007; Kahles et al., 2014; Ma et al., 2014; Rittling and Singh, 2015; Wang and Denhardt, 2008).

The perinatal immune system is significantly different from that of an older child or an adult. It undergoes profound changes and maturation after birth (Basha et al., 2014; Dowling and Levy, 2014; Zhang et al., 2017). Birth to one year of age is considered a critical window of immunological maturation for infants (DeWitt et al., 2012a, b; Simon et al., 2015). As discussed in Neal-Kleuver et al. (2014), "…infant CMI (cell-mediated immunity) is polarized relative to the adult, and can predispose infants to specific immune responses and possible

[2] PubMed search using the terms "osteopontin" and "human" under default settings identified 5374 results (as of 6/20/2018).

[3] OPN has also been associated with influencing the activity of another subset of T helper cells (Th17) distinct from Th1/Th2, which is also associated with pro-inflammatory autoimmune disorders (Du et al., 2017; Kourepini et al., 2014; Santamaria and Corral, 2013).

adverse outcomes." This awareness of possible adverse outcomes and specific vulnerabilities in infants related to CMI is important when interpreting the significance of clinical data and study results. This includes whether the study design, endpoints, and statistical power were sufficient to address these issues. For example, the newborn infant immune system typically shows strong Th2 but attenuated Th1 response (Adkins et al., 2004; Neal-Kluever et al., 2014; Simon et al., 2015). This Th2-Th1 balance shifts with growth and maturity such that in young children it is less skewed towards Th2.

In light of OPN being a known promoter and polarizer of the Th1 arm of the adaptive immune system, one hypothesis consistent with the available information is that a particular level of hOPN in breastmilk may be associated with the maintenance of a genetically and environmentally appropriate Th2/Th1 balance for an individual infant. Variability of hOPN levels may thus reflect the health and/or the genetic makeup of the mother-infant dyad as well as the developmental state of the infant and environmental factors. While evidence to firmly support such hypothesis is currently lacking, what is clear is that we do not have a complete understanding of the consequences of increasing bOPN above the background level currently found in bovine milk-based IF. As such, evidence that rules out the potential adverse effects of exogenous and relatively high exposure to bOPN on maintenance or development of immune status in all infants does not currently exist. Because the assumption that bOPN are quantitatively bioequivalent to hOPN has not been proven, the appropriate levels of exogenously added bOPN which would not adversely alter the developmental trajectory of a given infant's immune system have not been determined. Information provided in the notice and the amendment does not include study endpoints or other data and information that would be relevant to resolution of this potential issue.

Specific questions related to the notifier's GRAS conclusion

Arla Foods provides the following overall rationale, in GRN 000716 as well as in amendments provided in response to our questions (see Appendix 1), for their conclusion that the intended use and use levels are generally recognized as safe:

1) *None of the toxicological studies, which included a rat acute toxicity study, rat 13-week repeat dose toxicity study, rat teratogenicity study, and genotoxicity/mutagenesis studies, nor other safety studies, which included three-month and six-month feeding studies in newborn rhesus monkeys and infants, respectively, showed adverse effects relevant to safety. Estimated dietary intake (EDI) from the intended use at the mean and 90th percentile was calculated to be 24.8 and 39.5 mg/kg body weight (bw)/day, respectively. EDI was below the notifier's deduced Acceptable Daily Intake (ADI) of 50 mg/kg bw/day, which was estimated based on No-Observed-Adverse-Effect-Level (NOAEL) of 2500 mg/kg bw/day from a published rat teratogenicity study and the use of 50X safety factor.*

2) *hOPN and bOPN are substantially similar.*

3) *Human OPN is present in breastmilk.*

4) *bOPN, present in cow's milk, has been safely consumed throughout history.*

FDA's viewpoints regarding each of the above provided assertions

- **1. Absence of adverse effects in toxicological and other safety studies:**

The notifier states (in response to FDA's Question 2):

> *"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)."*

FDA notes:

a. The available evidence indicates that OPN is a bioactive substance with immunomodulatory and potentially pro-inflammatory properties.

b. In a recent study, IF supplemented with 130 mg (OPN-10)/L (same as the intended use) was shown to increase some T-cell subpopulations, although the study did not appear to utilize experimental protocols designed to specifically evaluate activities/proportions of Th1/Th2 subpopulations (West et al., 2017). These observations are consistent with the presumption that bOPN in IF has immunological effects in infants, and do not resolve the question of whether bOPN supplementation of IF could have persistent effects on immune function by influencing the developmental trajectory of the immune system. The consequences of such effects would not necessarily be benign [i.e. susceptibility to pro-inflammatory diseases (Dietert, 2014)].

c. We also note that hOPN is strongly associated with development of immune-mediated and inflammatory diseases (Boggio et al., 2016; Clemente et al., 2016). This association, though not a clear demonstration of causality, is consistent with other data and information on OPN's effects. Furthermore, although clinical significance of the findings is not known, OPN has previously been identified as an autoantigen (Fierabracci et al., 1999; Merl et al., 2013).

d. Safety assessment relies on toxicological studies that examine appropriate endpoints for the substances tested. None of the published safety studies discussed in the notice extensively evaluated its potential effect on immunotoxicity endpoints (DeWitt et al., 2012a, b). Thus a NOAEL from a rat teratogenicity study that was used to derive the notifier's reported ADI may not accurately reflect OPN-10's potential adverse effects on developmental immunotoxicity (DIT). Furthermore, as stated by the notifier, the use of a safety factor less than 100X can be justified based on appropriate scientific reasoning; however, without further context, it is not clear why a 50X safety factor is appropriate when the NOAEL is from a toxicological study that lacks relevant endpoints. Given the lack of these relevant endpoints in the studies cited, a safety factor of greater than 100X is not ruled out and may even be justified.

e. For immunomodulatory substances, it is challenging to determine which study outcomes are pivotal and appropriate in the evaluation for the safety standard of

"reasonable certainty of no harm." Because of the nature of the immune network and its responses, some potential concerns cannot be addressed using standard toxicological endpoints within the currently accepted study designs. For example, a potential adverse effect of an altered immune response and pathology may only manifest itself later in life, upon which its causal link to bOPN exposure early in life would be difficult to prove.

FDA's evaluation of the relevance of the absence of adverse effects in toxicological and other safety studies: Given currently available data on bOPN and hOPN, endpoints from existing toxicology studies are not sufficient to resolve questions about potential adverse consequences of exogenous bOPN in IF on the developing immune system of infants.

➢ *2. Similarities between human and bovine forms of OPN*

The notifier states (in response to FDA's Question 2):

> *"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."*

FDA notes:

a. This statement/conclusion infers that similarities at the amino acid sequence level between bOPN and hOPN indicate that bOPN is also "substantially" bioequivalent to hOPN and thus safe under the intended conditions of use. The existing literature is not consistent with the assertion that OPN from the two species are bioequivalent for the following reasons:

- As stated by the notifier on pg. 25 of the notice, "[t]he diverse functions of OPN may be largely dependent on post-translational modification …" According to Jiang & Lonnerdal, hOPN contains 34 phosphoserines, 2 phosphothreonines, and 5 O-glycosylated threonine, whereas bOPN has 27 phosphoserines, 1 phosphothreonines, and 3 O-glycosylated threonines. Furthermore, glycan structures between bOPN and hOPN are not the same (Christensen et al., 2012; Christensen and Sorensen, 2014). Therefore, it is not clear from the literature that either the specificity and/or the affinity of bOPN and hOPN to their interacting counterparts (i.e. receptors, such as integrins) are the same.

- "Hydrolysis of OPN catalyzed by various proteases may have significant impact on functions of the peptides by changing binding affinities to distinct integrins" (Jiang and Lonnerdal, 2016). Accordingly, Christensen and Sorensen (2014) found several differences in cleavage patterns between human and bovine OPN. For example, whereas cathepsin D and plasmin can cleave hOPN at 7 different sites, only plasmin (without cathepsin D) cleavage was seen with bOPN. Whereas bOPN is cleaved mostly after Phe^{151}, no preferential cleavage sites were identified for hOPN. Christensen and Sorensen conclude that:

    - "[t]hese differences can have significant effect on the ability of bOPN to interact with cells, as single amino acid differences next to the integrin-

binding motifs in the protein can have significant effects on its interaction with integrins …"

- As stated by the notifier, "intrinsically disordered proteins," such as OPN, "exert biological functions by means of motifs and posttranslational modifications in the primary amino acid sequence … and not dependent on any tertiary structure to be functional …" (pg. 24).

b. Rittling et al. (2014) showed that short peptides derived from digested bOPN (the test substance appears to be similar or perhaps identical to the subject of GRN 000716) can enter systemic circulation resulting in unanticipated tumor suppressing activity likely due to regulation of blood vessel size. Furthermore, this activity can be pinpointed to short peptides derived from "SVAYGLK" sequence region (corresponding to "SVVYGLR" in hOPN) that interacts with several integrins. Given that there are two amino acid changes between bOPN and hOPN in this conserved orthologous sequence region, whether similar short peptides derived from hOPN will show a corresponding tumor suppressor/regulation of blood vessel size activity is not known. However, this study indicates the possibility that slight differences in primary sequence between bOPN and hOPN may result in systemic exposure of short peptides (produced upon digestion) that may have different and possibly unexpected or unintended bioactivities. This observation is of particular relevance for infant populations, given that infants have underdeveloped gastrointestinal anatomy and physiology compared to adults (Dallas et al., 2012; Lebenthal and Lee, 1985), increasing the potential for systemic exposure to incompletely metabolized macromolecules via the oral route.

c. Recently, Nielsen et al. (2017) reported that specific peptide fragments from hOPN were present in both foremilk and hindmilk samples from all four mothers tested, of which the majority appears to be derived from the C-terminal half of OPN. Importantly, many of these fragments from hOPN have significant amino acid differences compared to the corresponding orthologous bOPN fragments (see Fig. 2). As discussed above [2(a)], these subtle amino acid differences could result in differential bioactivities between peptide fragments from bOPN and hOPN. Given OPN-10, as stated by the notifier, consists of approximately 20% full-length and 80% N-terminal fragment (pg. 9 of the notice), it is not clear whether the proportion of full-length vs. N-terminal fragment of bOPN reflects similar proportion of specific OPN peptide fragments found in human milk.

While it is likely that bOPN isolated from cow's milk and hOPN in human milk share some common functionalities, there is substantial evidence in the literature that bOPN may have qualitatively and quantitatively different bioactivities compared to hOPN. In fact, Christensen and Sorensen (2014) have shown that "cleaved bOPN binds the important $\alpha_V\beta_3$-integrin more competently than cleaved hOPN from human milk."

FDA's evaluation of the relevance of the similarities between human and bovine forms of OPN: There is ample information in the published literature to indicate bOPN and hOPN undergo different post-translational and peptidic processing in vivo. There is further evidence that these differences in post-

translational processing of OPN likely influence its functionality. Thus, bOPN and hOPN cannot be assumed to be functionally bioequivalent.

➢ *3. Presence of hOPN in human milk*

The notifier states that hOPN is present in human milk. However, the notifier also states in response to FDA's Question 4:

> *"Variation in the OPN content do[es] exist among mothers and among different geographical populations."*

Furthermore, the notifier provides a statement from their expert (in response to FDA's question 5) which states:

> *"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."*

FDA notes:

a. Neither the published nor the unpublished[4] studies discussed in the amendment have quantitatively distinguished the proportions of the various forms of hOPN in the human milk samples. Therefore, given the variability in expression as well as in proteolytic activity in human milk, it is not clear whether the proposed final concentration of 130 mg/L of OPN accurately reflects the "average" activity of hOPN in human milk. For example, high OPN levels detected via antibody-based assays (see below) in some human milk samples may in fact reflect much lower OPN biological activity due to extensive proteolysis in the sample.

b. The published studies tested breastmilk OPN levels in limited samplings from only a handful of geographical locations. For example, estimation of the level of hOPN for the intended use was based on a single study (Schack et al., 2009) of 29 samples of breastmilk from Denmark, a country considered to have a relatively homogeneous population (Athanasiadis et al., 2016). As such, the notifier has not provided an adequate rationale to support the validity of the statistical assumptions needed to make inference from these studies to the general population outside of Denmark. Even if the new published studies with Asian mothers (Bruun et al., in press) are included in the future analyses, a rationale to justify assumptions for statistical inference would still be needed.

c. Several studies report a wide variability of OPN expression (of which the proportion of fragmented and full-length forms are unknown) in human milk samples. Given the identification of several polymorphisms associated with increased OPN expression in humans (Chiocchetti et al., 2004), it may be argued that differences in OPN content in human milk samples simply reflect distinct

[4] Unpublished data mentioned in the amendment is now published online (Bruun et al., in press).

polymorphisms/haplotypes of the mother without biological consequence. Higher levels of hOPN detected in human milk may even conceivably reflect the highest tolerable and/or safe level. However, published studies also strongly suggest that it is premature to conclude that high levels of hOPN are not associated with adverse health outcomes:

- In humans, carriers of haplotypes B and C are associated with higher expression of OPN as well as higher risk for development of inflammatory immune disease (Clemente et al., 2016).
- Expression levels of hOPN are correlated with obesity, diabetes, and inflammation (Kahles et al., 2014).
- Leukocyte levels in breastmilk were found to depend on exposure of the mother and/or infant to pathogens, suggesting that the levels of pro-inflammatory cytokines such as OPN may reflect the health status of the mother-infant dyad (Hassiotou et al., 2013).

d. The biological significance of different OPN levels in human milk is thus unknown. Furthermore, recent unpublished study (now published as Bruun et al., in press) showed that OPN levels decrease during the lactation period; as suggested by the authors, different levels of OPN may reflect the functional level appropriate for the developing infant. The above observation also support the hypothesis that the specific OPN levels may reflect the patho-physiological state of the mother and/or infant, or an adaptively appropriate level based on genetic, dietary, and environmental factors, and not merely due to inconsequential variation. Since it appears that neither the published nor the unpublished (now published as Bruun et al., in press) reports on OPN levels in breastmilk samples determined the potential relationship, if any, between the health-status of the mother/infant dyad and the levels of OPN, it is difficult to conclude that the appropriate levels of OPN for the intended use in the general population in the United States and elsewhere can be inferred from these studies. If the higher OPN levels were observed only in mothers/infants exposed to particular pathogens, which may only be prevalent in certain geographical locations, arithmetic mean from those studies may be skewed towards overestimation. Given that pathogen load/exposure and genetic polymorphisms are just some of potential confounders expected to influence the levels of hOPN in breastmilk, the appropriateness of use level based on arithmetic mean without context is questionable.

e. There are several other sources of variability in hOPN levels. At any specific time point when the milk samples were collected, the measured level of endogenous hOPN may only reflect a level that was dictated by specific genetic, dietary, and environmental factors for an individual mother at that specific time point (Fields et al., 2016; Lee and Kelleher, 2016; Ruiz et al., 2017; Stam et al., 2013). Furthermore, how the temporal variation observed within individual mother-infant dyad accurately reflects temporal variations among populations of mother-infant dyads or vice versa cannot be inferred without further study. Finally, a biological response depends not only on the "dose" (i.e., concentration of OPN) but also on the affinity and the number of receptors/mediators expressed (i.e. integrins), which likely vary among individual infants. Thus, the aggregate mean concentration provides no

information about the appropriate exposure in any particular case. Furthermore, if additional studies indicate that the level and form of hOPN secreted is intimately tied to the status of the individual mother-infant dyad, determining an appropriate level for the general population of bOPN for addition to IF becomes even more challenging. The comment from the notifier's expert on the large variability of the degree of fragmentation observed among mothers suggests that there is a need to quantitatively evaluate the proportion of fragmented and full-length species in samples of human milk via methods other than ELISA assays, since ELISA assays (depending on the epitopes of the antibodies used) usually cannot distinguish between various proteolytic species of OPN. It is not clear from Schack et al. (2009) what the epitope(s) of the antibodies used was for their "in-house" developed ELISA assays; thus, whether their assays quantified all OPN species, including various proteolytic fragments, or only certain species is not clear. As stated above, because specific immunomodulatory bioactivity is influenced by proteolytic cleavage and post-translational modifications, it cannot be assumed that this variability is biologically neutral. There is currently insufficient generally available data to reach the conclusion that ~130 mg/L of OPN-10 (which consists of ~91 and ~39 mg/L of N-terminal fragment and full-length species, respectively) accurately reflects the "average" of levels and/or proportion of N-terminal vs. full-length species found in human milk samples.

f. On Pg. 85 of the notice, the notifier states that *"[b]ovine osteopontin is added to infant formulas in North and South East Asia and China at levels ranging from 21 to 110 mg/L formula."* FDA's view is that previous introduction of bOPN to IF in various parts of the world does not resolve our questions about the current consensus view among experts qualified by training and experience to assess the safety of this substance at the intended use level in IF given the existence of a large inter-individual variations in OPN concentration due to unknown factors (Bruun et al., in press; Nagatomo et al., 2004; Schack et al., 2009).

FDA's evaluation regarding the presence of hOPN in human milk: Although hOPN is present in human milk, there is considerable variation not only in the levels but also in specificity and proportions of various peptide fragments (i.e. full-length vs. various shorter peptides) among samples tested. Thus there is insufficient information in the published literature to conclude that the use of an arithmetic mean of 29 human milk samples from a single study (Schack et al., 2009) is an appropriate intended use level for all infants expected to consume IF.

➢ *4. History of safe consumption of bOPN contained in cow's milk*

The notifier states (in response to FDA's Question 2):

> *"… OPN from bovine sources has been consumed for centuries at low levels when contained in dairy products."*

FDA notes:

a) The relevance of the above statement to safety evaluation of exogenously added bOPN to IF at levels that far exceed those normally found in dairy products relies on the assumption that the intended subpopulation (i.e., infants) have "consumed for centuries" levels of bOPN that equal or exceed exposure from the notifier's intended use of hOPN. According to Schack et al. (2009), the levels of bOPN in cow's milk and cow-derived IF are 18 μg/mL and 5.3-13.0 μg/mL, respectively. However, levels of bOPN from intended use is ~138 μg/mL. There is no evidence provided in the notice, or in the subsequent correspondence, that the intended subpopulation (i.e., infants) has been consistently exposed to bOPN at levels as high as ~138 μg/mL. Thus, occasional and/or lower exposure of bOPN from cow milk-derived foods and IF, as currently consumed during 0-6 months of infant growth, would not be expected to provide the same daily exposure that would result from the intended use (i.e., 39.5 mg/kg bw/day at the 90th percentile as estimated by the notifier). Recognizing that hOPN exposures in human milk are currently poorly characterized but appear highly variable, and that intended bOPN use levels seek to replicate those exposures, significant increases in exposure raise the potential for new consequences not associated with historical exposure patterns.

FDA's evaluation of the history of safe consumption of bOPN contained in cow's milk: Historical information in the published literature does not indicate that infants ages 0-6 months have consistently consumed bOPN at levels comparable to or above the intended use level.

Additional data and observations considered by the FDA

1) hOPN exists in a complex matrix within human milk (D'Alessandro et al., 2010; Munblit et al., 2017), and its overall activity may be modulated by other bioactive molecules, including immunoglobulins, maternal leukocytes and cytokines, present in human milk (Field, 2005). For example, OPN action may be counterbalanced by the modulation of OPN activity either directly (i.e., proteolytic and post-translational modifications) or indirectly (i.e. through other cytokines or other binding partners of OPN). It has been suggested that one of the important functions of human milk is to attenuate or counterbalance the pro-inflammatory response by newborns against pathogen exposure (Walker, 2010). Immune homeostasis is an important determinant in autoimmune and pro-inflammatory disorders (Chevalier et al., 2014; Humrich et al., 2010; Kollmann et al., 2017; Lenardo, 2003), and an adverse effect of an altered immune response and pathology may initially show only a subtle imbalance in immune cell subpopulations. Thus, hOPN in the context of human milk may have altered or attenuated biofunction when compared to bOPN exogenously added to IF for reasons other than species specificity. As Mundblit et al. (2017) states:

> "In view of the large number of potentially immune-active constituents in breast milk, investigation of only a limited range of constituents may well produce conflicting results. There is a lack of studies, attempting to assess [human milk] as a whole, rather than focusing on single components. In other words, the "soup" is likely more important than individual ingredients."

Thus, there is no *a priori* reason to assume that ~138 mg/L hOPN present in human milk has equal immunomodulatory or other bioactivity as ~138 mg/L of bOPN in IF from the intended use.

2) It is well established that altering the levels of proteins that regulate biological response, such as hormones, cytokines and growth factors can cause major unintended biological effects. For example, inflammatory response to foreign pathogens must be kept in check in order to minimize tissue damage and/or sustained response (Murray and Smale, 2012). One mechanism to achieve this is through a complex repression of genes involved in inflammation at the levels of signaling, transcription, post-transcription and translation, and processing. Given that OPN expression and activity is regulated at multiple levels (Christensen and Sorensen, 2014; Clemente et al., 2016; Lund et al., 2009), exogenous exposure to bOPN could alter the appropriate balance and homeostasis of the immune components such as Th1/Th2/Th17 cells or myeloid/lymphoid cells (Kanayama et al., 2017). Relative to a default scenario of infant exposure to dynamically expressed hOPN in maternal milk, the risks and consequences of substantial (new intended use) versus minimal exposure to bOPN (i.e. exposure from current IF formulation) in the infant population are not necessarily equivalent.

3) The notifier states (in response to FDA's question 3):

> "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'."

However, as alluded to by Jiang and Lonnerdahl (2016),

> "Lactoferrin is a pleiotropic whey protein and is present at **an even higher concentration** than OPN in human milk, 1-10g/l … [t]he possibility of a synergistic effect of lactoferrin and OPN has not yet been explored." (emphasis added)

Thus, if lactoferrin (LF) and OPN interactions involve "carrier-like" activities, then the actual concentrations of LF and OPN would be expected to influence the bioavailability of OPN. For example, the actual bioavailability of OPN at ~138 mg/L in IF may be different depending on the amount of LF that is present in IF. Furthermore, if such synergistic effects between LF and OPN do occur (there is no generally available data that suggests that they do not), then the ratio of [LF] to [OPN] in the formulation of IF may impact the immunomodulatory and other bioactive effects of OPN and LF.

4) A recent publication on the safety assessment of food additives in IF proposed a "decision tree" to guide data requirements (Constable et al., 2017). According to this guide, standard toxicological datasets may not adequately address safety of OPN because of the high likelihood that immature immune system is a target organ for its actions. Regardless of the general acceptance of such decision tree for IF ingredient safety assessment, we continue to have questions about the likelihood of adverse unintended consequences resulting from the intended use of OPN which are not addressed by traditional toxicology tests or epidemiological and anthropometric-based

clinical studies. Our concerns are based on the gaps in our understanding of (1) the functional role of OPN in breastmilk, (2) the significance of the inter-individual variability of the levels of OPN in breastmilk, (3) the uniqueness and developmentally dynamic nature of the infant immune system, (4) identifying safety endpoints relevant to assessing long-term effects of altering infants' immune development, and (5) the relevance, if any, to safety assessment of a large body of scientific evidence that links OPN to pro-inflammatory diseases. As stated by Collinge et al. "[t]he difficulty in [endpoint] selection lies primarily in the gaps and limitations of the knowledge base and currently available methodologies for assessing [developmental immunotoxicity]" (Collinge et al., 2012); thus, FDA has not reached any conclusions on what potential toxicological and safety endpoints may be appropriate to conclude "reasonable certainty of no harm" for the intended use of bOPN at this time. However, FDA notes that the burden of proof for providing sufficient evidence that safety is generally recognized lies with the proponent.

Overall conclusion by the FDA

Our evaluation of the available data and information in the notice and in the scientific literature identified several questions about the intended use of bOPN described in GRN 716, given the properties of the substance and the target population. Our questions are based on:

- The vulnerability of the specific population, including the potential to affect the rapidly maturing immune system and the potential for long-term unintended consequences
- The lack of a comprehensive understanding of the modes of action of OPN and their consequences in the infant population
- The absence of metrics or models for establishing the significance of existing bOPN and hOPN exposure to the intended bOPN exposure
- The fundamental lack of clarity about whether or not absence of evidence of toxicity in standard toxicological tests is sufficient, or even relevant, to ensure reasonable certainty of no harm in this population given the known biological activities of bOPN; the notifier has not satisfactorily provided evidence that the "lack of evidence for adverse effects" sufficiently support "evidence of absence for adverse effects"
- The apparent lack of consideration of these issues in the existing secondary literature
- The absence of an evidentiary basis for the assumption that the presence of low levels of bOPN in current IF supports the safe use of bOPN at higher levels (138mg/L)

The safety data discussed in the notice did not identify any adverse effects associated with the intended use of OPN-10 in nonexempt term IF at 138 mg/kg bw/day (final expected concentration of bOPN) at this time. However, given the available data and information about the properties of OPN and the infant immune system, we continue to have questions about whether the existing data is appropriate and sufficient to meet the reasonable certainty of no harm safety standard. These questions warrant further scientific investigation and robust public discussion (such as publication in peer-reviewed literature, scientific sessions at

conferences, etc.) to determine whether scientists with specialized expertise in neonatal immunology would agree with other relevant food ingredient safety experts that the existing safety data presented by the notifier is adequate to establish reasonable certainty of no harm. GRN 000716 does not contain any evidence of the views of this expert community, either through inclusion of representative community members on the GRAS panel or via secondary literature.[5] Until this evidence becomes available, FDA will continue to have questions about the safety of the intended use and the basis for general recognition of the safety data supporting the intended use of OPN-10 presented in GRN 000716.

Kotaro J. Kaneko -S

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Kotaro J. Kaneko

[5] For a discussion of the issues found in GRN 000716 from a general recognition perspective, as well as potential remedies, see GRN 000716 Policy Memorandum.

Figure 1: “The role of osteopontin in immune cells focusing on the relationship between inflammation and apoptosis” [figure from (Iida et al., 2017)]

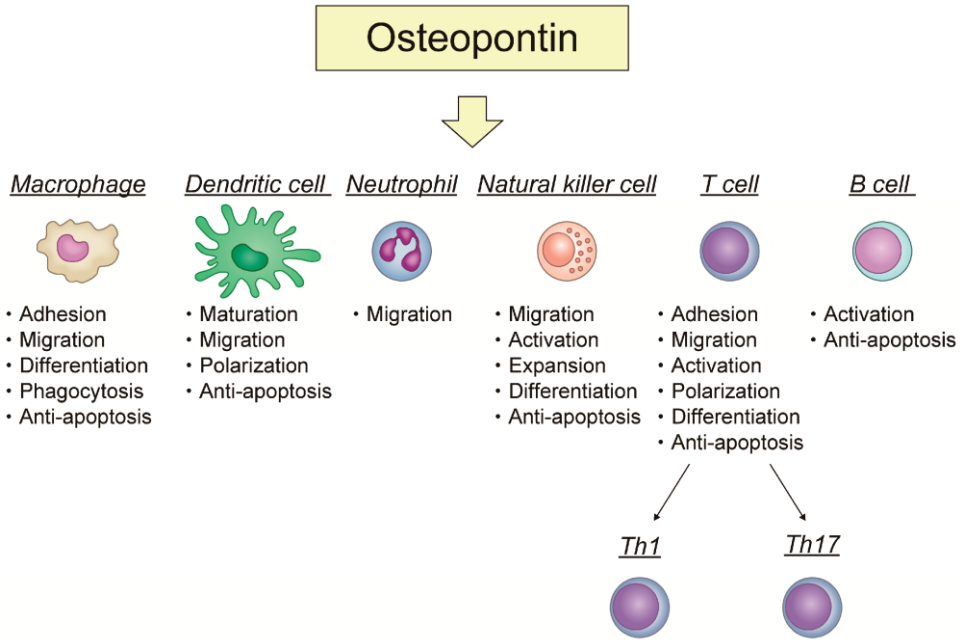
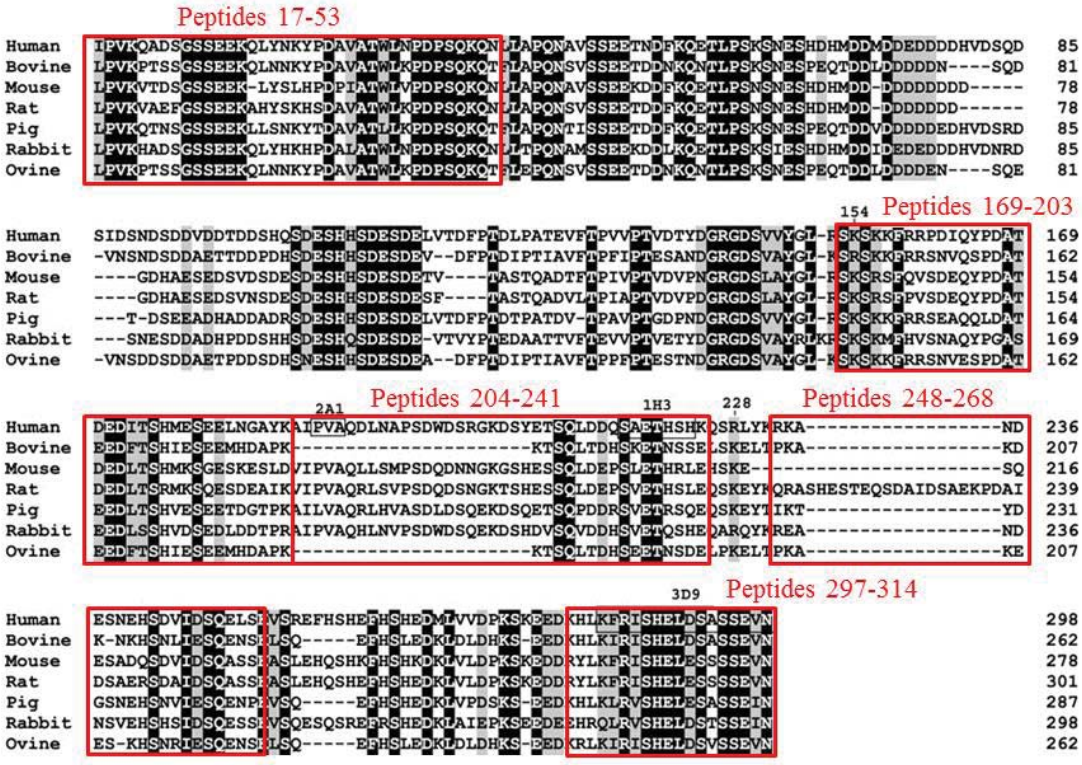


Figure 2: Peptide fragments from hOPN identified in human milk samples and their corresponding locations within mammalian orthologous sequences



Adapted from Brian Christensen et al. J. Biol. Chem. 2012;287:3788-3797 and Nielsen et al. Front. Nutri. 2017; 4: 1-13

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cc: **GRN 000716**
E-mail cc: HFS-255(SCarlson, RChanderbahn, MJDiNovi, RIMerker, SWestBarnette, PMGaynor, RShah, NBrewry, LShepherd)

R/D:HFS-255: KJKaneko:12/15/17; 02/13/18; 02/14/18; 02/28/18; 03/05/18; 03/06/18; 05/16/18; 06/20/18; 06/28/18
Init:HFS-255: JFasano: 02/13/18; 03/05/18; 03/06/18; 05/16/18; 06/20/18; 06/28/18
Init:HFS-255: SChoudhuri: 02/14/18; 03.05.18; 05/02/18; 06/20/18
Init:HFS-255: RChanderbhan : 03/06/18
Init:HFS-255: NBewry: 03/05/18
Init:HFS-255: RShah: 03/05/18
Edits/Init:HFS-255:SCarlson:4/26/2018; 06/19/2018
F/T:HFS-255: KJKaneko: 06/29/18

Appendix 1

(FDA questions sent to the notifier)

GRN000716 (Bovine whey-derived osteopontin)

- FDA Questions and Comments -

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 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.

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

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]knowledge 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:

> "*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.

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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.

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.

U.S. FOOD & DRUG ADMINISTRATION
CENTER FOR FOOD SAFETY & APPLIED NUTRITION

August 3, 2017

Ray A. Matulka, Ph.D.
Director of Toxicology
Burdock Group
859 Outer Road
Orlando, FL 32814

Re: GRAS Notice No. GRN 000716

Dear Dr. Matulka:

The Food and Drug Administration (FDA, we) received Arla Foods Ingredients Group PIS (AFI)'s GRAS notice dated July 6, 2017. We received this notice on July 10, 2017, filed it as of the date of this letter, and designated it as GRN 000716.

The subject of the notice is bovine whey-derived osteopontin for use as a source of protein in milk-based term non-exempt infant formulas and in powdered beverages at levels up to 138 mg/L as consumed. The notice informs us of AFI's view that this use of bovine-whey-derived osteopontin is GRAS through scientific procedures.

In accordance with 21 CFR 170.275(b)(1), the information in this notice described in 21 CFR 170.225(c)(2) through (c)(5) will be accessible to the public at www.fda.gov/grasnoticeinventory. If AFI has any questions about the notice, contact me at 240-402-1007 or by electronic mail at Nadine.Bewry@fda.hhs.gov.

Sincerely,

Nadine N. Bewry -S

Digitally signed by Nadine N. Bewry -S
DN: c=US, o=U.S. Government, ou=HHS, ou=FDA, ou=People, 0.9.2342.19200300.100.1.1=0014360008, cn=Nadine N. Bewry -S
Date: 2017.08.03 18:11:50 -04'00'

Nadine Bewry, Ph.D., MPH
Division of Biotechnology
and GRAS Notice Review
Center for Food Safety
and Applied Nutrition

U.S. Food & Drug Administration
Center for Food Safety & Applied Nutrition
5001 Campus Drive
College Park, MD 20740

From: Cathryn Sacra <csacra@easconsultinggroup.com>
Sent: Monday, November 05, 2018 10:39 AM
To: Morissette, Rachel <Rachel.Morissette@fda.hhs.gov>
Subject: RE: memo from pre-submission meeting on bovine lactoferrin in infant formula

Dear Rachel,

Our client has asked if it is possible to have a meeting to discuss the issues raised in the memo before November 15th.

Best regards,
Cathryn

Cathryn W. Sacra
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From: Morissette, Rachel <Rachel.Morissette@fda.hhs.gov>
Sent: Tuesday, October 23, 2018 8:54 AM
To: Cathryn Sacra <csacra@easconsultinggroup.com>
Subject: memo from pre-submission meeting on bovine lactoferrin in infant formula

Dear Cathryn,

Please see attached our memo of meeting for the bovine lactoferrin pre-submission meeting held on September 19, 2018. After you and your clients have had a chance to consider the points raised in the meeting and clarified in this memo, we would strongly suggest scheduling a follow-up meeting with our team, either in person or over the phone, to discuss the path forward. Please let me know if you have any questions at this time.

Best regards,

Rachel

Rachel Morissette, Ph.D.
Consumer Safety Officer

Center for Food Safety and Applied Nutrition
Office of Food Additive Safety
U.S. Food and Drug Administration
rachel.morissette@fda.hhs.gov

MEMORANDUM OF MEETING (COR2018-6011)

Date: November 29, 2018

Time: 2:00 p.m. – 3:00 p.m. EST

Location: FDA, Center for Food Safety and Applied Nutrition, Office of Food Additive Safety, 5001 Campus Drive, College Park, MD 20740

Participants:

Visitors:	
Cathryn Sacra	EAS Consulting Group, LLC
Angela Walter	Glanbia Nutritionals
Noreen Hobayan (by phone)	Glanbia Nutritionals
Brent Peterson (by phone)	Glanbia Nutritionals
Marian Kruzel, Ph.D.	University of Texas Health Science Center
Robert Martin, Ph.D.	Independent Advisor
Robin Guy	Expert Consultant
CFSAN/OFAS/DBGNR:	
Shayla West-Barnette, Ph.D.	HFS-255
Jeremy Mihalov, M.S.	HFS-255
Kotaro Kaneko, Ph.D.	HFS-255
Jeremiah Fasano, Ph.D.	HFS-255
Perry Wang, Ph.D.	HFS-717
CFSAN/OFAS/DPR	
Mical Honigfort, Ph.D.	HFS-265
CFSAN/ONFL/IFMFS:	
Andrea Lotze, M.D.	HFS-850
Carrie Assar, Pharm.D.	HFS-850
Suzanne Wolcoff, M.S., R.D.	HFS-850

Subject: Pre-submission meeting for the intended use of bovine lactoferrin in infant and toddler formulas

In an electronic mail message dated November 5, 2018, Cathryn Sacra requested a meeting with OFAS/DBGNR to discuss issues that were raised during a meeting held on September 19, 2018. The subject of the previous meeting and the newly requested meeting was the intended use of bovine lactoferrin (bLf) in infant and toddler formulas, with a specific focus on the intended use in infant formulas.

The meeting started with OFAS/DBGNR noting that Ms. Sacra provided a memorandum dated October 10, 2018, summarizing the visitors' perspective of the September 19, 2018 meeting. OFAS/DBGNR noted that statements in that memorandum differed from those summarized in OFAS/DBGNR's memorandum dated October 23, 2018. OFAS/DBGNR offered to clarify their perspective to assist the visitors in planning next steps.

The visitors summarized their conclusion that their intended use of bovine lactoferrin in infant formulas is GRAS. They discussed their expertise with respect to the safety of lactoferrin. They discussed publicly available literature they consider support the safety of bLF consumption by infants, including studies conducted using *in vitro* models and in infants. The visitors provided an overview of bLF's iron-binding, antimicrobial, and immunoregulatory functions; the occurrence of lactoferrin in human milk, and the bioequivalence of bovine and human lactoferrins. The visitors discussed the bioavailability of bLF, stating that after consumption, bLF does not cross the gut barrier or enter the bloodstream.

In response to the visitors' discussion of bLF's antimicrobial properties, we stated that beneficial properties resulting from a substance's consumption are not evaluated under the GRAS Notification Program. We stated that only data related to the safety of a substance's consumption are evaluated under our program.

OFAS/DBGNR summarized our questions regarding limitations of the evidence of safety and the evidence of general recognition of safety supporting the visitors' GRAS conclusion. OFAS/DBGNR noted that previous GRAS conclusions for bLF in infant formula submitted to FDA through our GRAS Notification Program focused on relatively low use levels that were both broadly comparable to existing use levels in bovine milk-based infant formulas and significantly lower than levels of human lactoferrin (hLF) found in human milk. This fact pattern was a significant factor in our responses at the time. OFAS/DBGNR also noted that:

- lactoferrin exhibits complex functionality with respect to immune function,
- human lactoferrin and bLF are structurally related but not identical in properties or functionality,
- lactoferrin appears capable of exerting substantive effects via the oral route,
- the infant immune system is rapidly developing and sensitive to perturbation, and
- the studies presented in support of the notice do not incorporate endpoints relevant to the functionality of bLF.

OFAS/DBGNR stated that we acknowledge the absence of specific adverse effects (blood chemistry changes, body weight changes, tissue pathologies, etc.) following consumption of bLF in the publicly available literature. However, we have questions about 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. Specifically, our questions[1] are:

- 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?
- 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?
- 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?
- 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?

OFAS/DBGNR provided the following recommendations[2] for how the visitors can address our questions:

1) Determine and explicitly discuss Glanbia's basis for concluding that currently available information about the properties and activities of bLF at the intended use level in this population is consistent with a conclusion of safety. This basis could involve one or more of the rationales identified above, supported by publicly available data and information.
2) Determine and explicitly discuss Glanbia's basis for concluding that there is a consensus at this time among experts qualified to assess the safety of the intended use in infant formula that the data and information relied upon by Glanbia is appropriate and sufficient, Given the properties of bLF and the population, expertise in both immunology and pediatric development would be essential considerations. This basis could involve published secondary literature, statements from appropriate experts with views representative of the consensus in their fields, or the conclusions of a GRAS panel[3] that incorporated the appropriate expertise.

[1] 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?

[2] We also recommend that Glanbia determine and explicitly discuss their basis for concluding that increased use levels of bLF will not adversely affect iron homeostasis in infants, given the currently available information in the literature.

[3] Although not currently operative, FDA's draft guidance on best practices for convening a GRAS panel is a useful illustration of the potential considerations involved in designing a GRAS panel capable of providing robust evidence of consensus.

OFAS/DBGNR clarified that we are not recommending that the visitors conduct or request new safety studies; we are recommending that the visitors develop a narrative that explicitly addresses our questions by placing the publicly available literature in the appropriate context.

OFAS/DBGNR concluded the meeting by agreeing to share this memorandum[4] to clarify our questions.

Shayla West-barnette -S

Digitally signed by Shayla West-barnette -S
Date: 2019.02.26 14:05:07 -05'00'

Shayla West-Barnette, Ph.D.

ATTACHMENTS:

1. List of attendees provided by Cathryn Sacra in an email dated November 26, 208.
2. Hard copy material provided by the visitors during the meeting on November 29, 2018.
3. Illustrative list of references

[4] In our view, recent literature suggests that discussion among the relevant scientific communities is beginning to shift from a focus on benefits alone to a broader consideration of the modes of action of LF and potential consequences for large-scale infant consumption of bLF at levels closer to those found in human milk. We have included an illustrative list of references that have informed our thinking on this topic. It is not intended to be comprehensive.

R/D: HFS-255: SWestBarnette:11/30/2018
Edit/Comment: HFS-255:JFasano:12/20/2018
Edit/Comment: HFS-255:KKaneko:12/20/2018
Comment: HFS-255:SCarlson:12/20/2018
Edit: HFS-255:JMFasano:12/20/2018
F/T: HFS-255:SWestBarnette: 2/26/2019

From: Cathryn Sacra
To: West-Barnette, Shayla
Subject: RE: Pre-Submission Request for Bovine Lactoferrin
Date: Monday, November 26, 2018 3:58:45 PM
Attachments: image001.png
image013.png

Dear Shayla,

Below is a list of the attendees for the meeting on Thursday. All who are attending in person are US citizens and will bring their driver licenses for identification. We will have several people who will attend via conference call.

Glanbia Nutritionals

Angela Walter	Senior Product Manager Lactoferrin	Attending
Noreen Hobayan	Director of Quality Assurance Specialties	Conference Call/WebEx
Peter Budde	Senior Director Product Management Bioactive Dairy Fractions	Conference Call/WebEx
Brent Peterson	Senior Director Ingredient/Bioactives R&D	Conference Call/WebEx
Ankur Jhanwar	Senior Technical Services Manager	Conference Call/WebEx

Marian Kruzel, PhD	University of Texas Health Science Center at Houston, Texas	Attending

EAS Consulting Group

Robert Martin, PhD	Independent Advisor	Attending
Robin Guy	Expert Consultant	Attending
Cathryn Sacra	Director, Labeling and Cosmetics	Attending

Cathryn W. Sacra
Director of Labeling and Cosmetic Services
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November 29, 2018

Subject: FDA Pre-submission meeting related to bLf (Bioferrin) for use in infant formula

Statement for Expertise

My name is Marian Kruzel and I am an adjunct faculty member at the department of Integrative Biology and Pharmacology of McGovern Medical School, University of Texas at Houston. Also, I am a founder and scientific director of R&D company – PharmaReview Corporation – for testing the clinical utility of lactoferrin.

I have a Ph.D. in biochemistry with extensive training in molecular biology and applied immunology. For more than 25 years I have focused my research on potential utility of lactoferrin for prevention and/or treatment of inflammatory processes, particularly during an insult-induced metabolic imbalance due to infection or trauma.

I have published extensively on lactoferrin's ability to modulate immune responses *in vitro* and *in vivo.* The most recent review in Frontiers in Immunology entitled "Lactoferrin in a Context of Inflammation-Induced Pathology" summarizes my research on studying the effects of lactoferrin during experimental insults in various animal models. In my latest book contribution (Translational Inflammation), I focused on susceptibility of individuals to various disorders due to single nucleotide polymorphism (spontaneous mutations) in the lactoferrin gene.

Since 2011, I am a member of NIH study section – Center for Scientific Review Special Emphasis Panel, Innovative Immunology Research – recently serving as a chairperson for the latest session.

I am on the editorial board of several journals including Archivum Immunologiae et Therapiae Experimentalis. I am also an *ad hoc* reviewer for many immunological journals worldwide.

I have knowledge and experience in studying the complexity of immune responses in various animal models and humans.

My complete cv will be attached to the Bioferrin GRAS dossier.

Lactoferrin Facts

Lactoferrin is an iron binding glycoprotein found in mucosal secretions of all mammals; it is also present at high concentration in mother's milk.

Lactoferrin is a multifunctional protein and it's immunoregulatory property has been extensively studied over more than five decades with a wealth of scientific reports. It is regarded as a bridge between innate and adaptive immune function by regulating target cell response. Lactoferrin is a first line defense protein involved in protection against a multitude of microbial infections and prevention of systemic inflammation.

Indeed, lactoferrin is well documented as having direct antimicrobial activity, including an iron-dependent bacteriostatic property and non-iron-dependent bactericidal action on LPS-bearing Gram-negative bacteria. While suppressing microbial growth, lactoferrin also directly exerts its activity towards development of adaptive immune responses. Sequestration of iron by lactoferrin reduces insult-induced oxidative stress, thus altering the magnitude and specific production of cytokines.

Lactoferrin has a profound modulatory action on the adaptive immune system 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, lactoferrin augments the delayed type hypersensitivity (DTH) response to antigens, leading to a strong induction of cell-mediated immunity (CMI) in mice model.

The ability of lactoferrin to bind large quantities of iron may also provide protection against pathogens and their metabolites by enhancing phagocytosis and cell adherence and controlling the release of pro-inflammatory cytokines.

The majority of research on lactoferrin has been done on species specific lactoferrin *in vitro* or *in vivo* by using systemic administration (intravenous, intramuscular or intraperitoneal). It has to be emphasized that the mechanism of action for lactoferrin given orally is not the same as with a parenteral administration due to reduced oral bioavailability.

Based on current knowledge there is no evidence that use of bLf at a level of up to 1g/L as fed (770 mg/100g infant formula powder solids) in reconstituted infant formula may alter the developmental trajectory of the infant immune system in potentially adverse ways. Conversely, by virtue of protective effects on infant gut microbiome it may play an important role on the development of healthier babies at later stage of their life.

Oral administration versus systemic – intravenous, intramuscular, intraperitoneal.

The gastrointestinal 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. In this context, immune tolerance is defined as the antigen-specific suppression of cellular or humoral immune responses. **When the initial antigen exposure is mediated through the GI tract, a robust T cell-mediated suppression develops called oral tolerance.**

Infants fed cow's milk will develop tolerance to some "foreign proteins", for example, alpha casein, beta lactoglobulin and bovine serum albumin. Because bLf is not 100% compatible/identical with human LF, it will be recognized as "foreign" as well. Consequently, infants will develop a tolerance to such proteins. This is in accordance with a well-established phenomenon of oral tolerance acquired by immunologically immature mammals (Strobels, NYAS, February 1996, 88-102). The mechanism of natural development of oral tolerance involves a shift from Th2 to Th1 type immune response accompanied with augmentation of T regulatory cell activity (Savilahti, Ped Aller Immunol, 2013, 24, 114-121).

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. 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 subject to this notification (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.

A significant body of evidence supports the safety and beneficial effects of bovine milk-derived lactoferrin (bLf) supplementation of infant formula. Hundreds of infants have been studied and no adverse effects have been reported on bLf fortified formula in their diet. In particular the multi-center, double-blind, paralleled-designed, gender stratified study of 480 infants confirmed no adverse effects for infant consuming up to 1 gram of bLf as supplement to their diet over 1 year (Johnston et al. BMC Pediatrics (2015) 15:173). This study demonstrated 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 through 365 days of age.

Based on the results from the animal studies, it was concluded that bLf is well tolerated with no significant adverse effects at the concentrations tested (Nishimura, as cited in GRN 465; Yamauchi et al., J Toxicol Sci 2000,25:63-66). The No Observed Adverse Effect Level (NOAEL) was determined to be at least 2,000 mg/kg/day in both the rat 4- and 13-week studies. Chronic oral toxicity studies (40- and 65-weeks) also supported the safe consumption of bLF. Bovine milk-derived lactoferrin is also non-genotoxic in an Ames assay (Yamauchi et al., J Toxicol Sci 2000,25:63-66).

There is a general consensus that, based on current knowledge, there is no evidence that use of oral bLf at a level of up to 1g/L may alter the developmental trajectory of the infant immune system in potentially adverse ways.

Final Remarks

Finally, it has to be emphasized that bovine milk is a component of a standard diet for infants from the age of 6 months. Hence, they are exposed to bovine milk proteins (for example bLf) on a daily basis and there are no adverse effects due to consumption of milk. While the amount of bLf consumed by infants varies (40 mg to 202 mg per day per 2 cups a day) and is less than 1 gram/day, it does not raise any health issues due to development of oral tolerance as discussed above.

Additional references

- Vaarala O1, 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.

Development of humoral and cellular immune responses to orally administered antigens in human beings is poorly understood, although antigen administration has been suggested as a treatment for hypersensitivity disorders and autoimmune diseases.

The purpose of the study was to investigate the development of systemic immune response in infants fed with formula containing whole cow's milk proteins or hydrolyzed formula containing casein peptides.

In a double-blind trial, 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.

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.

These results support the view that induction of oral tolerance in human beings is an age-dependent phenomenon.

- Savilahti EM1, Savilahti E. Pediatr Allergy Immunol. Development of natural tolerance and induced desensitization in cow's milk allergy. Pediatric Allergy Immunol. 2013 Mar;24(2):114-21.

Cow's milk allergy (CMA) affects 2-3% of infants. It resolves in the great majority spontaneously during childhood. CMA encompasses a spectrum of clinical and immunologic characteristics. Non-IgE-mediated allergy typically resolves earlier than IgE-mediated allergy. The most documented prognostic characteristic is that intense-specific IgE response predicts persistence of CMA. Low serum levels of cow's milk (CM)-specific IgG4 are also associated with persistent CMA. Natural development of tolerance involves an immunologic shift where Th2 responses diminish, and Th1 as well as T regulatory cell responses strengthen. Accordingly, specific IgE levels decrease and specific IgG4, possibly also IgA, levels increase in serum. Specific oral immunotherapy (OIT) with CM induces desensitization in most cases where spontaneous recovery has not yet occurred. Data on long-term tolerance induction are still scarce. According to current research data, the immunologic changes induced by OIT resemble those seen during natural development of tolerance

- Nowak-Węgrzyn A. Using Food and Nutritional Strategies to Induce Tolerance in Food-Allergic Children. Nestle Nutr Inst Workshop Ser. 2016;85:35-53. doi: 10.1159/000439484. Epub 2016 Apr 18.

Food allergy is an important and increasing public health problem worldwide, affecting predominantly infants and young children. There is an urgent need to develop effective treatment strategies to restore oral tolerance in food-allergic individuals. Among diverse research approaches, those involving native or heat-modified food proteins are most advanced and are currently being evaluated in clinical trials. Extensively heated (baked) milk and egg diets have already been adopted in clinical practice and benefit the majority of milk- and egg-allergic children. Oral, sublingual and epicutaneous immunotherapy with native foods remain in the sphere of clinical research with encouraging data suggesting that they may induce desensitization in a large proportion of treated patients and potentially permanent tolerance following an adequately long period of treatment. Synbiotics appear to have the most beneficial role in the prevention of food allergy; Lactobacillus rhamnosus GG may promote the development of tolerance to milk in allergic infants.

- Lack G. The concept of oral tolerance induction to foods. Nestle Nutr Workshop Ser Pediatr Program. 2007;59:63-8; discussion 68-72.

The conventional wisdom is that early exposure to allergenic food proteins during pregnancy, lactation, or infancy leads to food allergies, and that prevention strategies should therefore aim to eliminate allergenic food proteins during pregnancy, breastfeeding, and early childhood. Prolonged exclusive breastfeeding and delayed weaning onto solid foods is therefore seen as an effective public health policy to prevent allergies. However, there is little epidemiological data to support this belief. Interventional studies on dietary elimination have failed to reduce IgE-mediated food allergies. Conversely, there is preclinical data and some clinical data to suggest that early cutaneous exposure to food protein through inflamed skin leads to allergic sensitization and that early oral exposure results in the induction of tolerance. New strategies to prevent food allergy in infants need to be put to test in randomized controlled interventional studies

Links to relevant Literature

https://www.frontiersin.org/articles/10.3389/fimmu.2017.01438/full

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4645956/

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4636804/

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6165050/

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4510916/

Illustrative References – Bovine Lactoferrin in Infant Formula

Breakey, A.A., Hinde, K., Valeggia, C.R., Sinofsky, A., and Ellison, P.T. (2015). Illness in breastfeeding infants relates to concentration of lactoferrin and secretory Immunoglobulin A in mother's milk. Evol Med Public Health *2015*, 21-31.

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.

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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.

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

From:	Cathryn Sacra
To:	Morissette, Rachel
Subject:	RE: pre-sub meeting memo on bovine lactoferrin
Date:	Wednesday, October 10, 2018 5:13:26 PM
Attachments:	image007.png Glanbia - Memorandum of Meeting with CFSAN FINAL 10-10-2018.docx.pdf

Rachel,

Thank you for the update. In the meantime, we have put together a Memorandum of Meeting with our understanding of the issues that were raised in the meeting, which I have attached. Please let me know if you or the team have any comments or questions.

Best regards,
Cathryn

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From: Morissette, Rachel <Rachel.Morissette@fda.hhs.gov>
Sent: Thursday, October 04, 2018 9:15 AM
To: Cathryn Sacra <csacra@easconsultinggroup.com>
Subject: pre-sub meeting memo on bovine lactoferrin

Hi Cathryn,

I just wanted to let you know that I haven't forgotten about sending you the meeting memo. We're waiting on a staff member to weigh in on the memo before I can finalize and send it to you. Hopefully within the next week or so I should have it to you.

Best,

Rachel

Rachel Morissette, Ph.D.
Consumer Safety Officer

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FDA U.S. FOOD & DRUG ADMINISTRATION

DBGNR Meeting Requests

Information for Logging into FARM/Appian

Requestor: Cathryn Sacra, EAS Consulting Group

Contact Information: 571-447-5505

csacra@easconsultinggroup.com

Date of Request: August 13, 2018 (meeting to be held September 19, 2018)

Subject: GRAS notice for bovine lactoferrin in infant formula

Date Request Received by DBGNR: August 13, 2018

Date Prepared by DBGNR: August 20, 2018

Prepared by: Rachel Morissette

If agent, name of company or individual this is on behalf of: