PRE-SUBMISSION MEETING FDA

Arla wishes to discuss with the FDA how best to address the concerns raised by FDA

Arla Foods Ingredients

Arla Foods Ingredients
Discovering the wonders of whey

AGENDA

1. General recognition
2. Toxicological and safety
3. hOPNin human milk
4. History of consumption
5. Conclusion

GENERAL RECOGNITION

Presentation of OPN in early life nutrition

Year	Conference/event	Presenter	Format	Title
2011	ESPGHAN, Sorrento	Esben Skipper Sørensen, Professor of Bioactive Food Proteins (A University, Denmark)	Oral presentation	*Osteopontin– a bioactive milk protein with implications in infant nutrition?*
2011	ESPGHAN, Sorrento	Sharon Donovan, Professor of Pediatric Nutrition and Health (University of Illinois, USA)	Oral presentation	*Transcriptional responses of the neonatal Rhesus intestine to Osteopontin*
2011	ESPGHAN, Sorrento	Bing Wang, Professor of Physiology and Nutrition (Charles Sturt University, Australia)	Oral presentation	*Osteopontin– a bioactive milk protein with implications in infant nutrition?*
2014	Early Nutrition, Power of Programming, Munich	Bo Lönnerdal, Distinguished Professor Emeritus of Nutrition and Internal Medicine (UC Davis, USA)	Oral presentation	*Growth, nutrition and early programming of immune function in breastfed infants and infants fed formula with added osteopontin (OPN)*
2014	Experimental Biology, San Diego	Sharon Donovan, Professor of Pediatric Nutrition and Health (University of Illinois, USA)	Oral presentation	*Osteopontin supplementation of formula shifts the peripheral blood mononuclear cell transcriptome to be more similar to breastfed infants*
2014	Experimental Biology, San Diego	Bo Lönnerdal, Distinguished Professor Emeritus of Nutrition and Internal Medicine (UC Davis, USA)	Poster	*Growth, nutrition and immune function of breastfed infants and infants fed formula with added osteoponti*
2014	Experimental Biology, San Diego	Sharon Donovan, Professor of Pediatric Nutrition and Health (University of Illinois, USA)	Poster	*Dietary bovine osteopontin increases vaccine response, T-cell phenotype and cytokine secretion in piglets*
2014	International conference of Milk Genomics and Human Health, Aarhus	Esben Skipper Sørensen, Professor of Bioactive Food Proteins (Aarhus University, Denmark)	Oral presentation	*Osteopontin – A bioactive milk protein with immunological properties*
2017	Nestlé 90th Symposium	Sharon Donovan, Professor of Pediatric Nutrition and Health (University of Illinois, USA)	Webcast	*Proteins in human milk composition and biological effects*
2018	Ordesa Symposium, Madrid	Lotte Neergaard Jacobsen, MSc in Molecular Biology (Arla Foods Ingredients, Denmark)	Oral presentation	*Osteopontin– cornerstone in immunology*
2018	ESPGHAN, Geneve	Signe Bruun, MD (Odense University Hospital, Denmark)	Oral presentation	*Osteopontin levels in human milk vary across countries and within lactation period: Data from a multicenter study*
2019	American Society for Nutrition, Baltimore	Bo Lönnerdal, Distinguished Professor Emeritus of Nutrition and Internal Medicine (UC Davis, USA)	Oral presentation	*Robert Suskind and Leslie Lewinter – Suskind Pediatric Lifetime Achievement Award Lecture*
2019	Event Catedra Ordesa, VI International Scientific Symposium, Palma de Mallorca	Lotte Neergaard Jacobsen, MSc in Molecular Biology (Arla Foods Ingredients, Denmark)	Oral presentation	*Osteopontin in human milk and infant nutrition*

GENERAL RECOGNITION
Publication of OPN in early life nutrition

Ren et al. 2019. Gut and immune effects of bioactive milk factors in preterm pigs exposed to prenatal inflammation. Physiol Rep Am J Physiol Gastrointest Liver Physiol. 15 Epub ahead of print] (https://www.ncbi.nlm.nih.gov/pubmed/31091150)

Donovan 2019. Human milk proteins: Composition and physiological significance. Nestle Nutr Inst Workshop Ser. 90: 93-101 (https://www.ncbi.nlm.nih.gov/pubmed/30865978)

Jiang & Lönnerdal 2019. Osteopontin in human milk and infant formula affects infant plasma osteopontin concentrations. Pediatr Res. 85(4): 502-505 (https://www.ncbi.nlm.nih.gov/pubmed/30636771)

Chen et al. 2018. Osteopontin-enriched formula feeding improves the T cell-dependent humoral immune response in infant rats. Int J Food Nutr Sci. 69(8): 969-975 (https://www.ncbi.nlm.nih.gov/pubmed/30001650)

Bruun et al. 2018. Osteopontin levels in human milk vary across countries and within lacta ion period: Data from a multicenter study. J Pediatr Gastroenterol Nutr. 67(2): 252-256 (https://www.ncbi.nlm.nih.gov/pubmed/29668569)

Demmelmair et al. 2017. Benefits of lactoferrin, osteopon in and milk fat globule membranes for infants. Nutrients. 9 (8) (https://www.ncbi.nlm nih.gov/pubmed/28788066)

Lönnerdal 2017. Bioactive protein in human milk: potential benefits for preterm infants. Clin Perinatol 44(1): 179-191 (https://www.ncbi.nlm.nih.gov/pubmed/28159205)

Jiang & Lönnerdal 2017. Biological roles of milk osteopon in. Curr Opin Clin Nutr Metab Care. 19(3): 214-219 (https://www.ncbi.nlm.nih.gov/pubmed/27504516)

Lönnerdal 2016. Human milk: Bioactive proteins/peptides and func ional properties. Nestle Nutr Inst Workshop Ser. 86: 97-107 (https://www.ncbi.nlm.nih.gov/pubmed/27337145)

Christensen & Sørensen 2016 Structure, function and nutri ional potential of milk osteopontin. Int Dairy J. 57: 1-6 (https://www.sciencedirect.com/science/article/pii/S0958694616300437)

Lönnerdal 2016. Bioactive proteins in human milk: Health, nutrition, and implications for infant formulas. J Pediatr. 173: S4-S9 (https://www.ncbi.nlm.nih.gov/pubmed/27234410)

Lönnerdal et al. 2016. Growth, nutrition, and cytokine response of breast-fed infants and infants fed formula with added bovine osteopontin. JPGN. 62(4): 552-557 (https://www.ncbi.nlm.nih.gov/pubmed/26465791)

Donovan et al. 2014. Bovine osteopontin modifies the intestinal transcriptome of formula-fed infant rhesus monkeys to be more similar to those that were breast-fed. J Nutr. 144(12): 1910-1919 (https://www.ncbi.nlm.nih.gov/pubmed/25320184)

Kvistgaard et al. 2014. Pre-clinical in vitro and in vivo safety evaluation of bovine whey derived osteopontin, Lacprodan® OPN-10. Food Chem Toxicol 73: 59-70 (https://www.ncbi.nlm.nih.gov/pubmed/25072164)

Lönnerdal 2014. Infant formula and infant nutrition: bioactive proteins of human milk and implications for composition of infant formulas. Am J Clin Nutr. 99(3): 125-S17S (https://www.ncbi.nlm.nih.gov/pubmed/24452231)

Chatterton et al. 2013. Anti-inflammatory mechanisms of bioac ive milk proteins in the intestine of the newborn. Int J Biochem Cell Biol. 45(8): 1730-1747 (https://www.ncbi.nlm nih.gov/pubmed/23660296)

Lönnerdal 2011. Biological effects of novel bovine milk fractions. Nestle Nutr Workshop Ser Pediatr Program. 67: 41-54 (https://www.ncbi.nlm.nih.gov/pubmed/21335989)

Schacket al. 2009. Considerable varia ion in the concentration of osteopon in in human milk, bovine milk, and infant formulas. J Dairy Sci. 92(11): 5383-5378 (https://www.ncbi.nlm.nih.gov/pubmed/19841198)

GENERAL RECOGNITION

Next step

- Q3 2019 we will submit a Novel Food application in EU to enhance general recognition
  - We will request 138 mg/L as novel food approved dose
- We have discussed in scientific forums and also with individual pediatric MD immunologists on some of the concerns raised by FDA in scientific memo
  - We can arrange a round table discussion of bOPN safety that includes OPN structural experts, pediatric immunologists and nutrition experts together with scientists from FDA for discussion
  - The outcomes and minutes of this meeting could be added to future bOPN submission for no questions on GRAS to FDA

[redacted]

AGENDA

1. General recognition
2. Toxicological and safety
3. hOPNin human milk
4. History of consumption
5. Conclusion

TOXICOLOGY AND SAFETY

-What we know today including new information

- Pre-clinical in vitro and animal safety study (Kvistgaard et al 2014) showed no adverse events on non-immune parameters
- Clinical safety study (Lönnerdal et al 2016) that looked into immune markers, infection rates and vaccination response showed reduction in pyrexia, no changes in vaccination response and showed immune marker levels typically observed infant formulas
- Lymphocyte subsets looked in a subset of population from the clinical study showed (West et al 2017 controlled T-cell activation that is not different from breast fed infants showing bioOPN's role is to bridge the gap in formula fed infants
- New information to be discussed (Jiang and Lönnerdal 2019) would show that even in formula fed infants there is high circulating levels of bioOPN that may contribute to immunological well being of all infants

[redacted]

PLASMA OPN CONCENTRATIONS

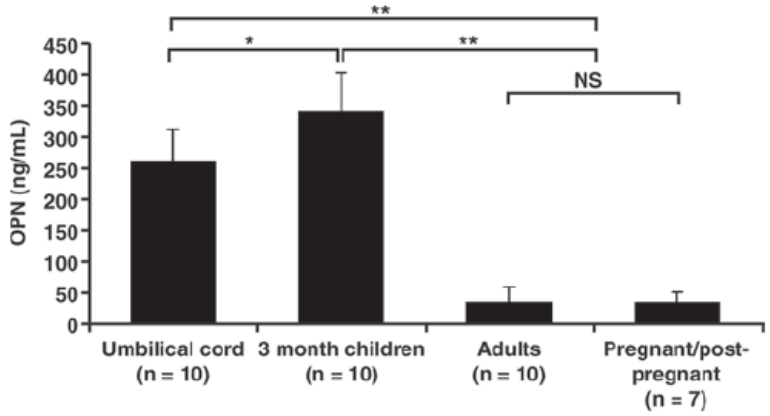


Figure 1. Osteopontin (OPN) concentrations in plasma from umbilical cords, 3-mo-old infants, adults, and pregnant or postpregnant women, measured by ELISA. The number of samples analyzed for each group is indicated and standard errors are represented with vertical bars. *The OPN content in plasma from 3-mo-old infants was significantly higher than that from umbilical cords ($P < 0.05$). **The OPN content in plasma from umbilical cords and from 3-mo-old infants were both significantly higher than that from adults and pregnant or postpregnant women ($P < 0.01$).

MEASURING OPN IN PLASMA - STUDY DESIGN

- Samples:
  - Plasma was isolated from blood samples obtained from 1-, 4-, and 6-month-old infants in a clinical trial in China (Lönnerdal et al 2016)
  - Infants were either exclusively breast-fed (BF group) or fed one of the following formulas: unsupplemented formula (F0 group), formula supplemented with 65 mg/L bOPN (F65 group), or formula supplemented with 130 mg/L bOPN (F130 group) up to 6 months of age
- OPN assays:
  - Human OPN (hOPN) concentrations in breast milk and in all infant plasma samples were measured by an ELISA kit (Human Osteopontin DuoSet ELISA, R&D Systems, Minneapolis, MN) following the manufacturer's instructions
  - Bovine OPN was also measured in plasma samples by an ELISA kit (Bovine Osteopontin ELISA, LifeSpan BioSciences, Seattle, WA) following the manufacturer's instructions
  - There was no cross-reactivity between the two kits; i.e., the hOPN ELISA did not recognize bOPN nor did the bOPN ELISA recognize hOPN

Jiang and Lönnerdal (2019), Pediatr Res. 85(4), 502-505

CIRCULATING HOPN IN BREAST AND FORMULA FED INFANTS

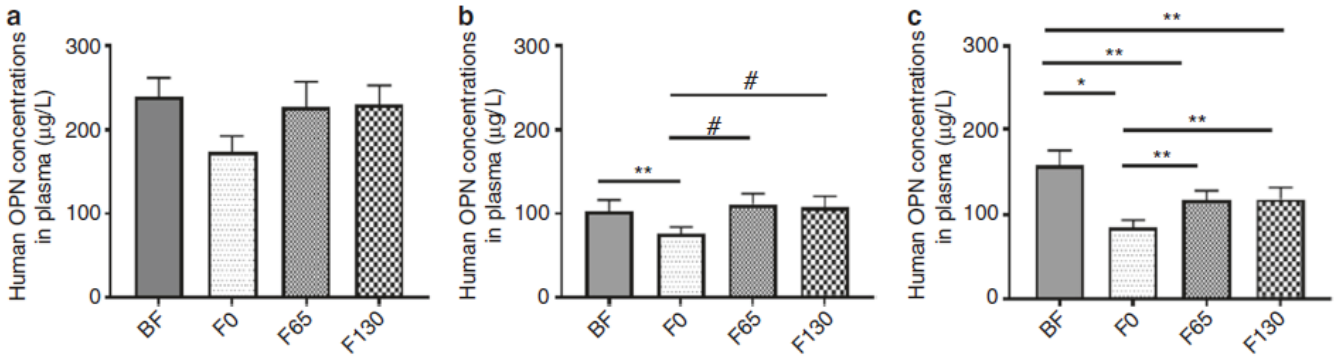


Fig. 2 hOPN concentrations in plasma samples. Human OPN concentrations in all infant plasma samples were measured using a hOPN ELISA kit (R & D Systems) following the manufacturer's instruction. Plasma samples collected from 1-, 4-, and 6 months old infants (BF, F0, F65, and F130) are shown in **a**, **b**, and **c**, respectively. Results are shown as means ± SD, $n = 25$ for each treatment group at each time point, $^{*}p < 0.001$, $^{\#}p < 0.05$, $^{**}p < 0.01$

CIRCULATING BOPN IN BREAST AND FORMULA FED INFANTS

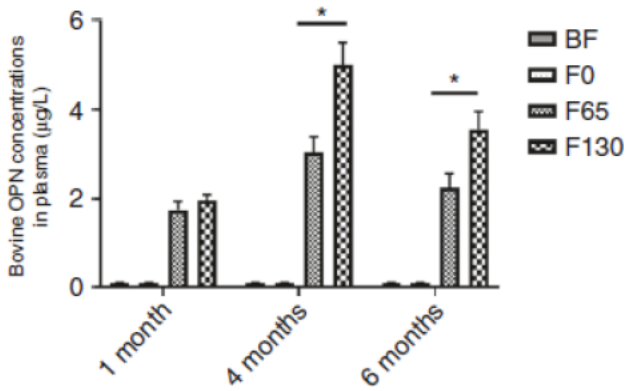


Fig. 3 bOPN concentrations in plasma samples. The bovine OPN concentration in plasma from 1-, 4-, and 6 months old infants (BF, F0, F65, and F130) was measured using a bovine OPN ELISA kit (Lifespan Biosciences) according to the manufacturer's instruction. Results are shown means ± SD, $n = 8$ for each treatment group at each time point. *$p < 0.001$

[redacted]

MEASURING HOPN AND BOPN IN US INFANTS TO ADDRESS LONG TERM CONCERNS

- Need guidance from FDA on what is long term to assess safety of hOPN and bOPN

Study Possibilities:

- Recruit infants and collect random blood sampling from 3 months to 12 months to measure endogenous hOPN and bOPN levels (breast fed at least for first 4 months and exclusively formula fed infants)
- Have a long term follow-up to the just completed Building Block Nutritionals Study (now the infants are 2 year old)
  - In a subset of subjects, Collect health history, one blood sample to measure hOPN and bOPN and possible vaccination response
- Question:
  - Does demonstration of high levels of endogenous hOPN in formula fed infants alleviate FDA concern that hOPN is not the cause for long term immune programming effects?
    - To date, our pre-clinical safety study on bOPN with conventional non-immune end points did not show any toxicity
    - All interventions of exogenous bOPN in animal models of inflammation and cancer have yielded only beneficial outcomes
    - Given the endogenous levels of OPN in circulation in early life, it will be difficult to demonstrate specific bOPN related effects in animal models and human setting

[redacted]

TOXICOLOGICAL AND SAFETY
Conclusion

- High levels of OPN both in breast milk and in circulation indicate that OPN may have a key physiolog role in infants
- High circulating hOPN levels are noted in formula fed infants at 1, 4 and 6 months. bOPN levels measured in plasma at the same time points are at least 20 fold less compared to hOPN
  - Data indicates that any immune programming/ inflammatory / safety concern for infants will predominantly result from high hOPN levels compared to bOPN levels in formula fed infants
  - Immune effects outlined in scientific memo mainly discussed hOPN as the cause for pathological and autoimmune diseases
  - Supplementation of bOPN in infant formula may help infants in two possible ways
    - Local effects in gastrointestinal tract
    - Dietary bOPN promotes endogenous circulating levels of hOPN This phenomena was also noted in a mouse study
- It will be difficult to dissect the endogenous versus exogenous effect of OPN in a clinical model

[redacted]

ADDITIONAL TOXICOLOGY STUDIES

- Two different toxicology models have been explored
  - A mini pig model with dosing and immune assessment
  - A rat model with a virus challenge
- Both models have pros and cons
- Mini Pigs:
  - Post birth dosing, physiologically close to human
  - Questionable if data can be translated to human response
- Rat Model
  - Virus challenge model to measure DIT
  - Rat physiology is not similar to human
    - Immunological response and pathway may differ

[redacted]

AGENDA

1. General recognition
2. Toxicological and safety
3. hOPNin human milk
4. History of consumption
5. Conclusion

OPN IN HUMAN MILK

Structural homology between bovine and human OPN

- OPN is like any other molecule in human milk that varies from early lactation to late lactation on levels and may be on post-translational modifications (Froehlich et al 2011)
- Post-translational modifications are dependent on the enzyme activity in mammary gland and may vary in the same individual mother day to day
- Given the structural homology and conserved phosphorylation sites, the phosphorylation sites are similar between human and bovine OPN (Christensen et al 2005)
- The minor *in vitro* binding differences noted in Christensen and Sorensen 2014 is an undigested bOPN and the difference is not seen upon thrombin cleavage of bOPN (a likely scenario in humans)

Froehlich et al (2011), Anal Biochem **408**, 136-146
Christensen et al (2005) Biochem J **390,** 285-292
Christensen and Sorensen (2014) J Dairy Sci **97**, 136-146

[redacted]

HOPNIN HUMANMILK

Structural homology between bovine and human OPN

- When consulted with experts, it was argued that there are more functional and, to a certain degree, structural differences between hOPN in breast milk compared to bOPN in extracellular secretions (urine) and in organs

- We also looked for FDA guidance targeted for approval of generic biological molecules. FDA acknowledges minor structural differences in post translational modifications exist and the molecules will be considered similar, if these structural variations do not result in adverse functional differences (**"Guidance to Industry: Scientific Considerations in Demonstrating Biosimilarity to a Reference product" was used for guidance)**
  - bOPN has not been shown to have any different biological activity in direct comparison to hOPN

[redacted]

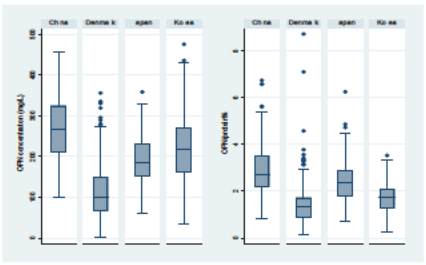
HOPNIN HUMANMILK
CONCLUSIONS ON HOMOLOGY

- hOPNandbOPNare substantially homologous at amino acid levels, functional binding sequences areconserved, and relatively similar post translational modifications
  - The minor differences noted in sequence do not result in adverse physiological consequBittiesg(et al2014)
  - Based on recent observations (Jiang andnerdal2019), in infants at any given time point there20s fold or higherhOPNin circulation compared tobOPN Any physiological consequences during infancy will beattributed to the predominanthOPNin circulation
  - Given the variability in human milk post translational modifications (similartor bioactive proteins likelactoferrin, Bile Salt Stimulated Lipase), it is possible to detect minor variabilities based on modern daytechnology

[redacted]

OSTEOPONTIN LEVELS IN HUMAN MILK

Across countries and within lactation period



Human breast milk samples (829 samples from 629 women) from four different countries – China, Denmark, Japan and Republic of Korea – were collected and OPN content measured

China - 225 samples (infant average age 4 weeks) – mean OPN level 266.2 mg/L

Denmark - 318 samples (infant average age 17 weeks) – mean OPN level 89.7 mg/L

Japan - 169 samples (infant average age 9 weeks) – mean OPN level 185 mg/L

Korea – 117 samples (infant average age 3 weeks) – mean OPN level 216.2 mg/L

The OPN concentration varied across sites, but the mean content was 167.4 mg/L (China, Denmark, Japan, Korea), corresponding to 2.0 % of the total protein content

USA – 10-12 samples (infant age from day 1-12 months) – mean OPN level 178 - 48.3 mg/L

Table 1. hOPN concentrations in milk from different lactation stages

Stage (n=10-12 at each stage)	Concentration (mg/L, means ± SD)
Colostrum (D1–D7)	178.0 ± 17.9
Transitional milk (D8–D14)	134.8 ± 18.5
1 month	65.8 ± 13.7
4 months	48.8 ± 12.0
6 months	55.9 ± 13.8
12 months	48.3 ± 10.2

 Bruun et al. 2018, Jiang et al. 2019

AGENDA

1. General recognition
2. Toxicological and safety
3. hOPNin human milk
4. History of consumption
5. Conclusion

HISTORY OF CONSUMPTION

- Besides the launch in China from 2009-2012, and in Korea in 2008-current, where no safety concerns have been raised, OPN have been launched in several countries since our GRAS were submitted to E
- Launched in stage 1 and stage 2 in EU in Fall 2018
- Launched in stage 1 and stage 2 China in spring 2018

- Potentially exposed to 1.000.000 babies through commercially available products, no safety concerns raised
- Used in a clinical trial in the United States, providing an experimentan infant formula enriched in OPN to 128 babies, no safety concerns observed

[redacted]

AGENDA

1. General recognition
2. Toxicological and safety
3. hOPNin human milk
4. History of consumption
5. Conclusion

CONCLUSION

- We would like to thank FDA for the diligence in looking into our OPN-affirmed GRAS dossier and comments on the scientific aspects and bioactive potential
  - It certainly challenged us to look the molecule differently and discuss with pediatric, immunological and OPN structural experts to further understand the functional and structural similarities/ differences between hOPN and bOPN
- We at Arla Foods Ingredients, and many scientific experts we spoke to believe that OPN at physiologic level would benefit all infants
- We appreciate FDA's continued support and guidance on this topic

[redacted]

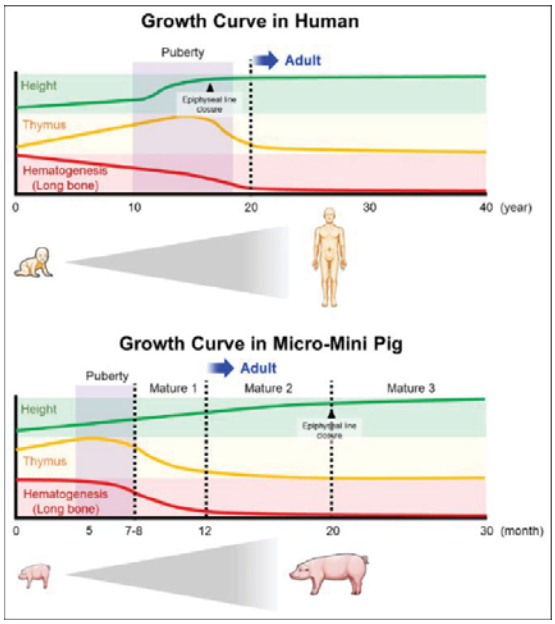
DISCUSSION POINTS AND NEXT STEPS

- ArlaFoods Ingredients would like to have guidance on
- **General Recognition:**
  - *What additional measures would ensure general recognition of safety for bOPN ingredient?*
  - *Can we assemble a round table panel with pediatric, immunology and OPN structural experts to discuss short and long-term safety that would also include FDA scientists?*
- **Toxicology and Safety:**
  - *Given information presented on high levels of endogenous hOPN early in infancy, is there a reason to consider additional animal safety studies ?*
  - *Does measurement of hOPN in USA infants add value to existing knowledge and evaluation of our application?*
- **Homology between hOPN and bOPN and hOPN levels in milk:**
  - Experts acknowledged that differences in post translational modifications are noted within lactation period and between breast milk and other secretions, the minor differences noted may not be functionally consequential. Human milk average for other bioactive ingredients have been used by industry to fortify infant formula
  - *Given this complexity, is there anything we could do that would help FDA's concerns?*

[redacted]

BACKUP SLIDES

DEVELOPMENTAL COMPARISON: HUMAN VS PIG



(b) (4)

(b) (4)

(b) (4)

(b) (4)

(b) (4)

ARLA'S PREFERENCE

- Arla would prefer the mini pig model as it is more physiological, measures all key parameters at various time points, would give us a direction on long term consequences of bOPN on top of endogenous pOPN feeding.
  - It may not accurately reflect human conditions
  - Very expensive to conduct (current estimates 1.5 to 2 million).
  - Still would not dissect the effects between endogenous pOPN versus exogenous bOPN

- Would like to seek guidance from FDA based on March 21st 2018 and June 29th 2018 memos.
  - Do we need additional pre-clinical safety studies?
  - Consensus and general recognition of bOPN safety by pediatric experts to precede this activity?
  - Does it have to be a GLP safety or well controlled animal study that answers the question for eventual FDA approval?

[redacted]